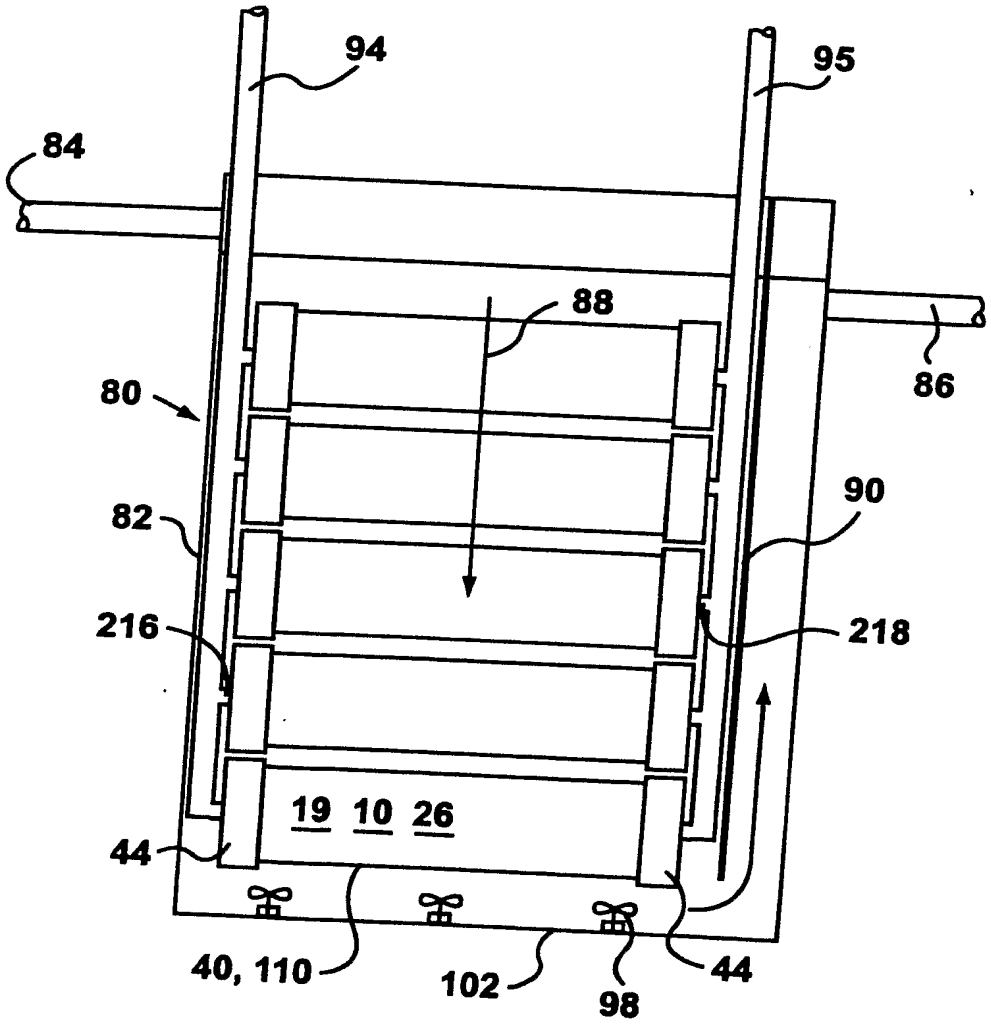


FIG. 16

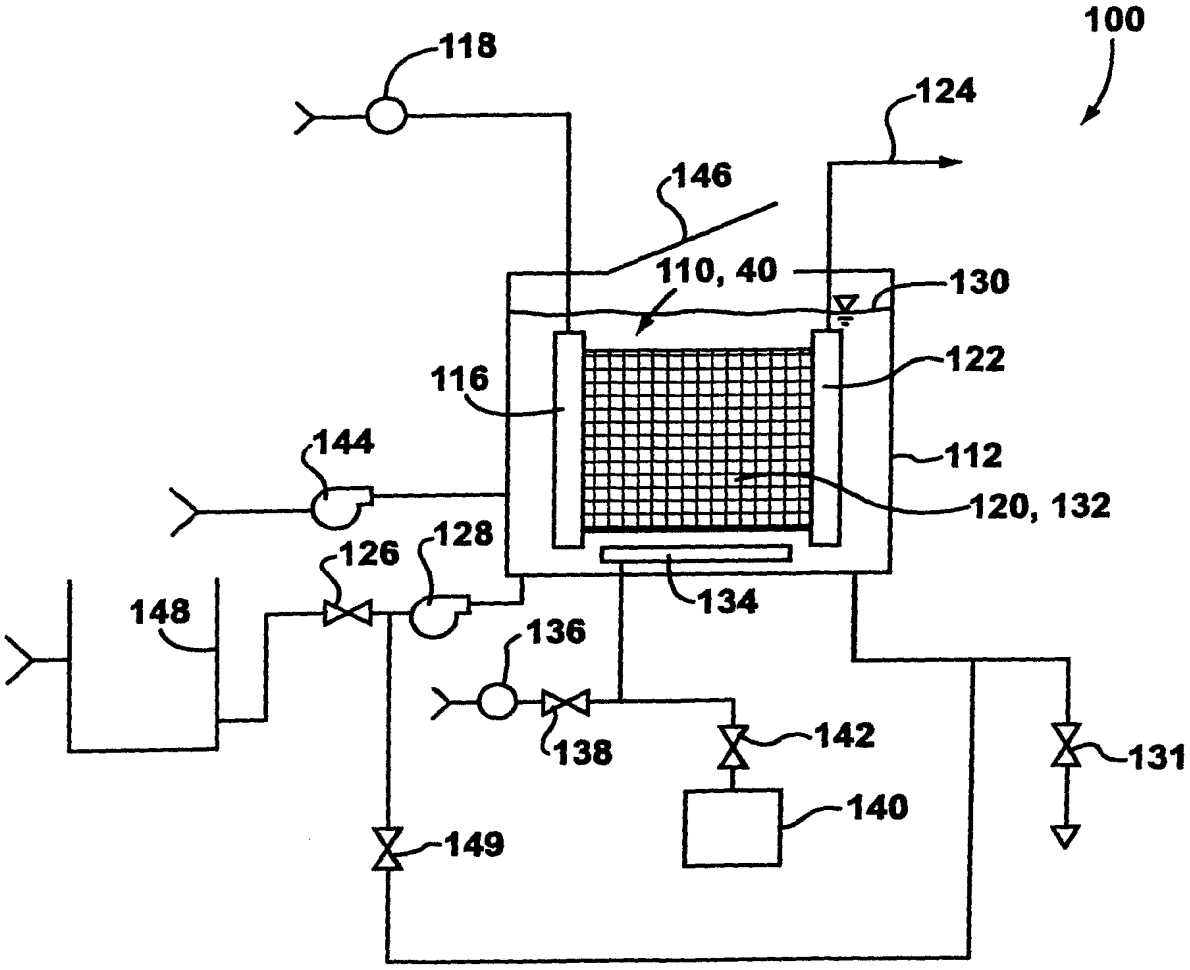

100
118
124
146
110, 40
130
122
116
112
144
120, 132
128
126
134
148
136
138
142
131
140
149

FIG. 17

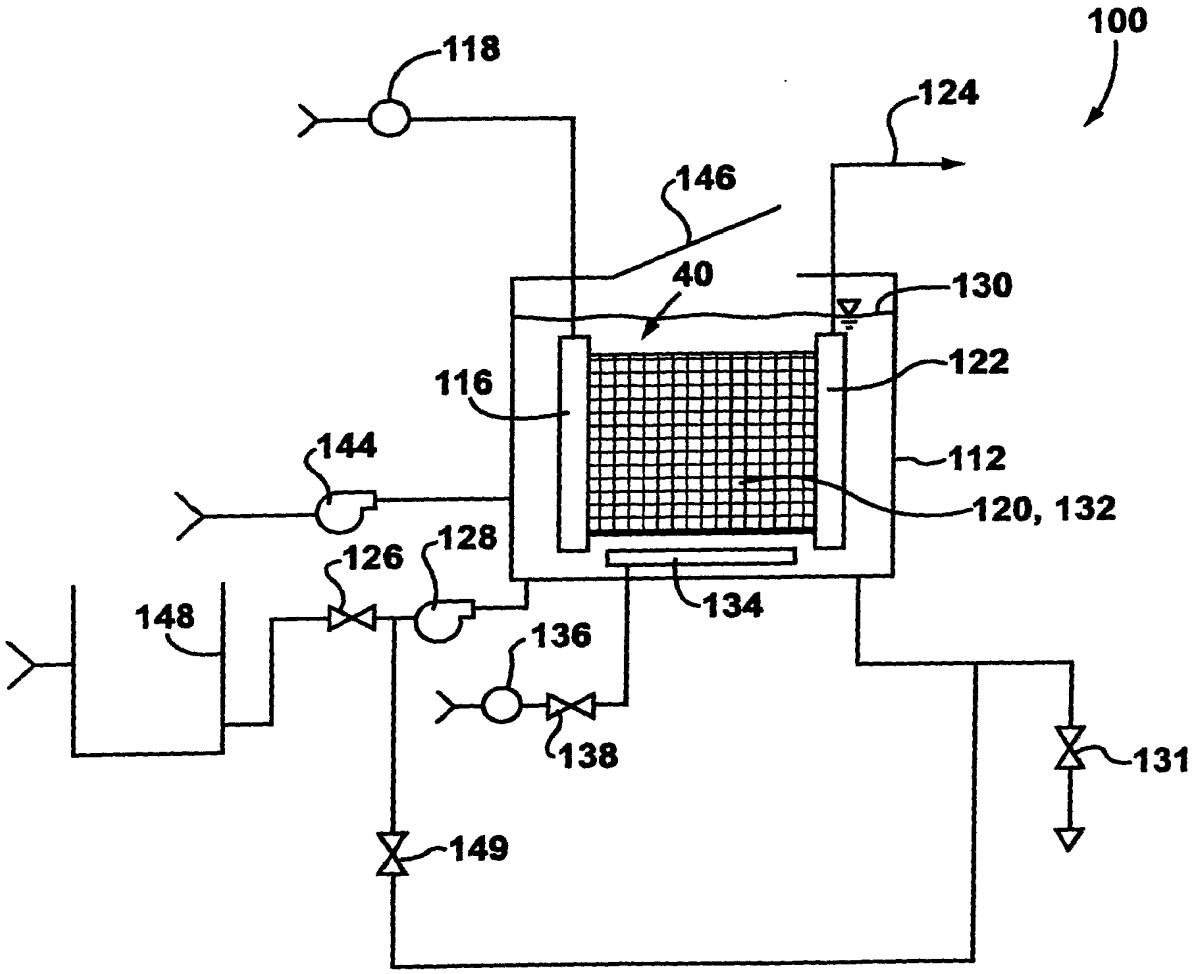

100
118
124
146
40
130
122
116
112
144
120, 132
126
128
148
134
136
138
131
149

FIG. 18

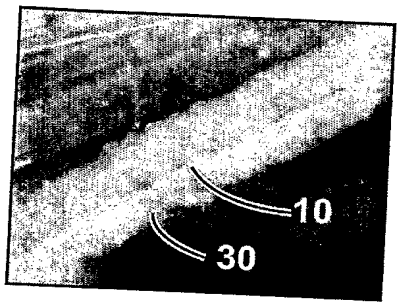


FIG. 19b

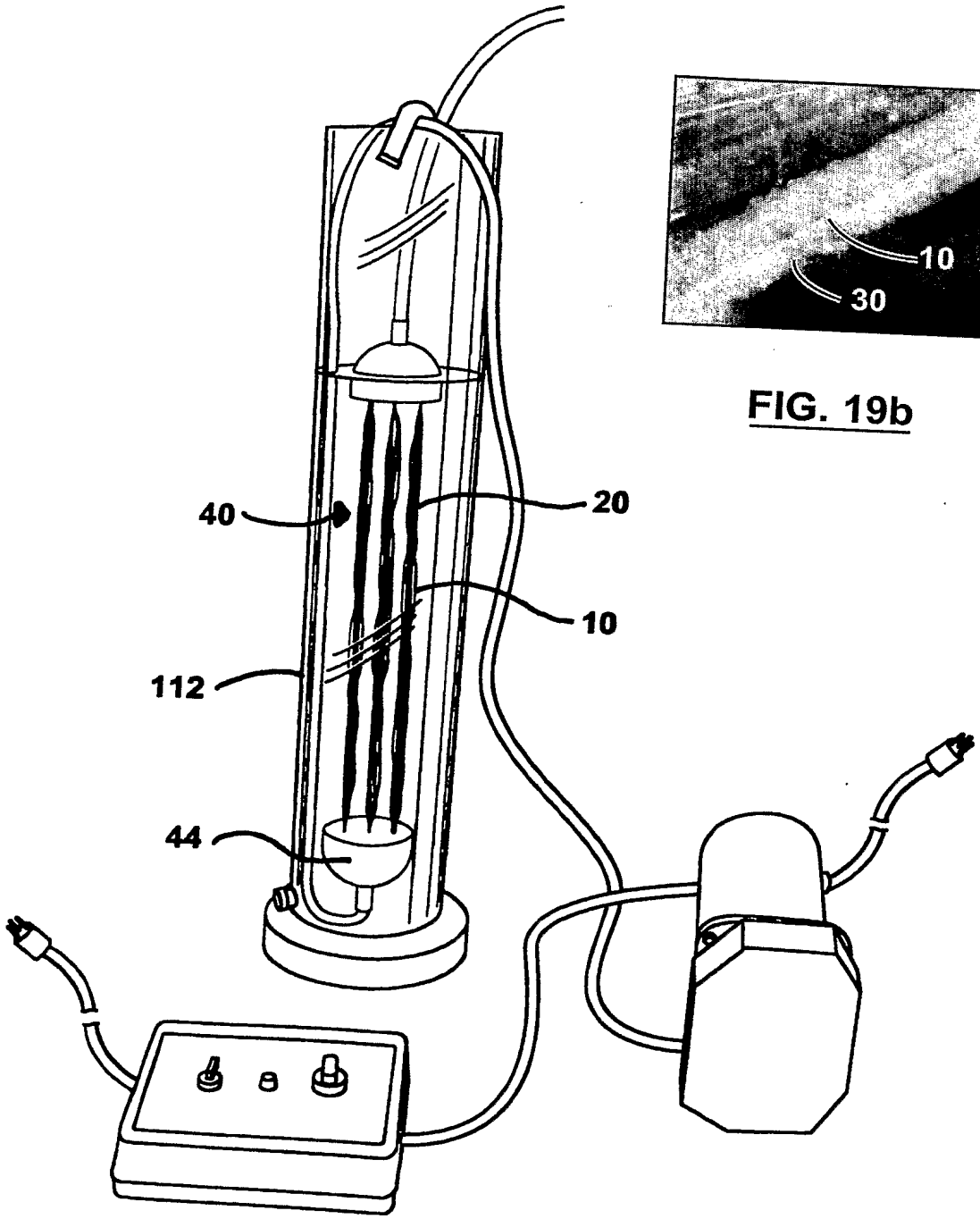


FIG. 19a

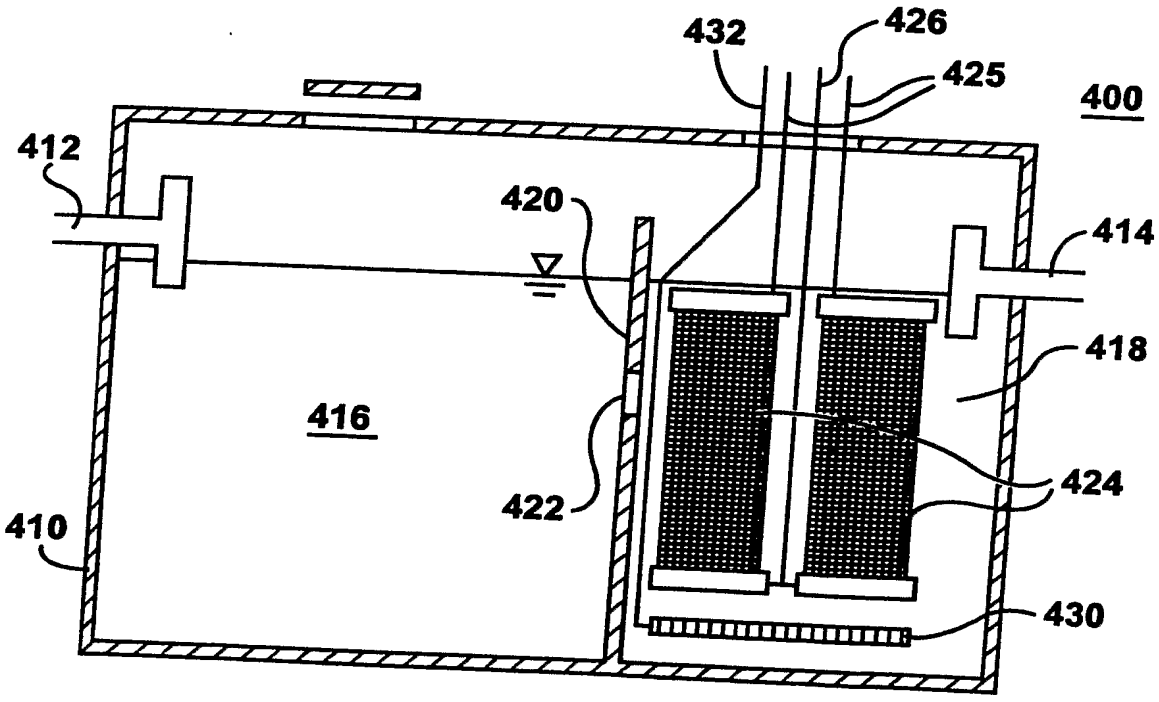


FIG. 20

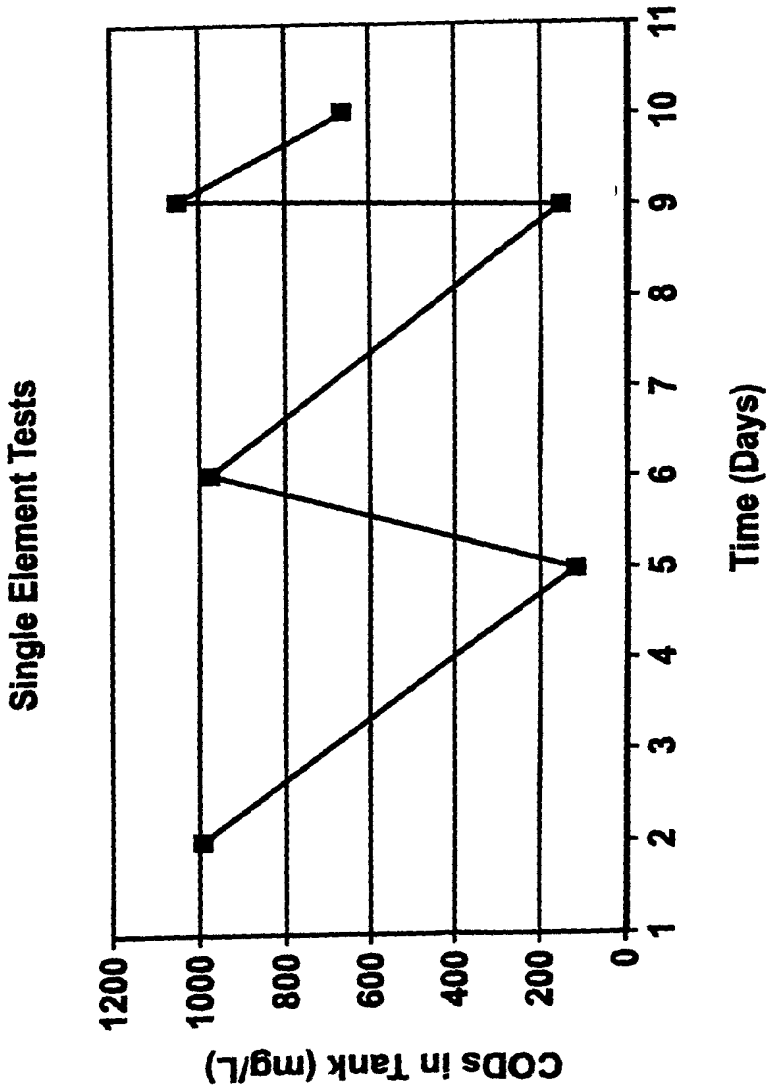

Single Element Tests
CODs in Tank (mg/L)
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
10
11
Time (Days)

FIG. 21

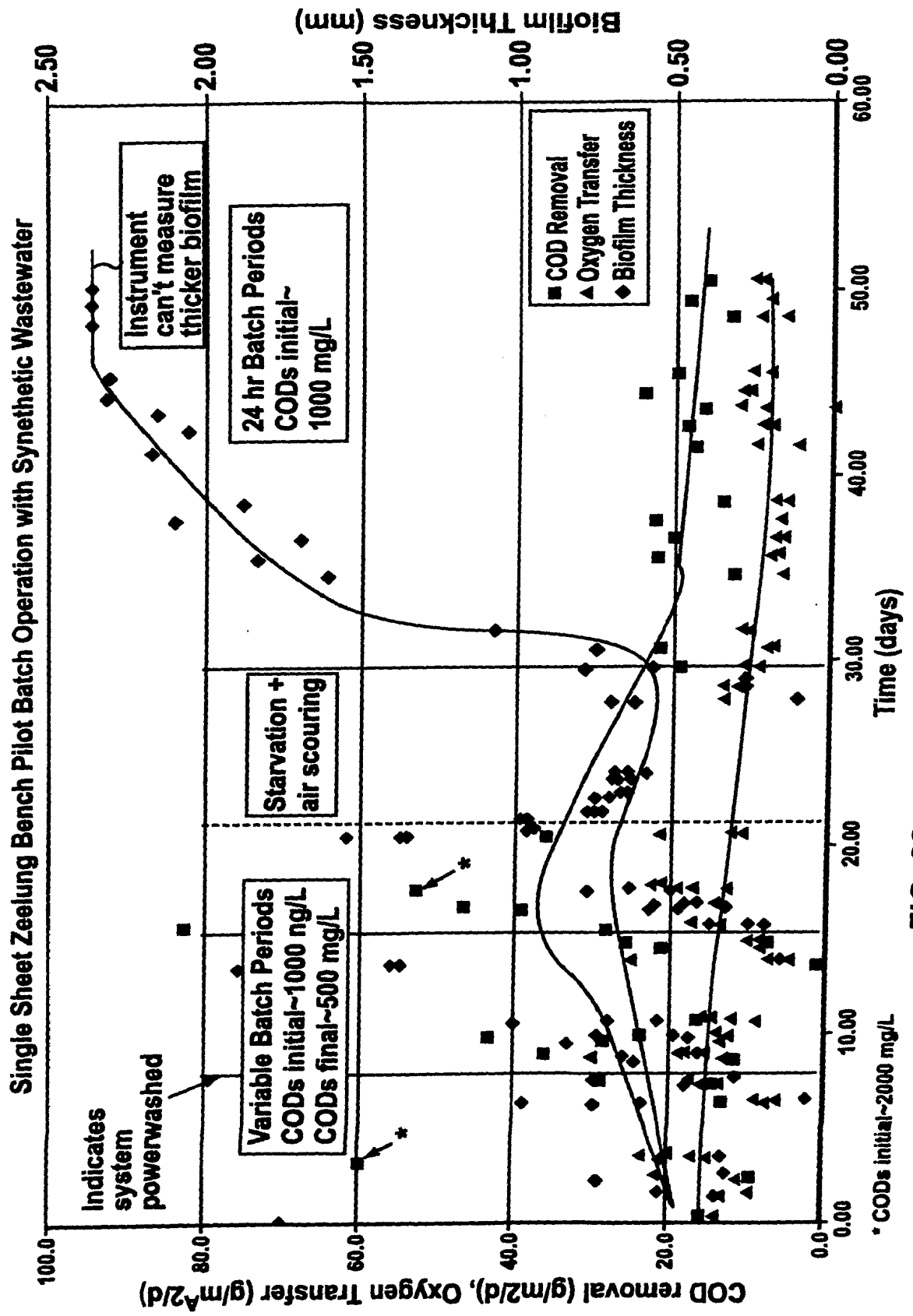

Single Sheet Zeelung Bench Pilot Batch Operation with Synethetic Wastewater
Biofilm Thickness (mm)
2.50
2.00
1.50
1.00
0.50
0.00
Instrument can't measure thicker biofilm
24 hr Batch Periods CODs initial~ 1000 mg/L
Starvation + air scouring
Variable Batch Periods CODs initial~1000 ng/L CODs final~500 mg/L
Indicates system powerwashed
COD Removal
Oxygen Transfer
Biofilm Thickness
COD removal (g/m2/d), Oxygen Transfer (g/m^A2/d)
100.0
80.0
60.0
40.0
20.0
0.0
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Time (days)
* CODs initial~2000 mg/L

FIG. 22

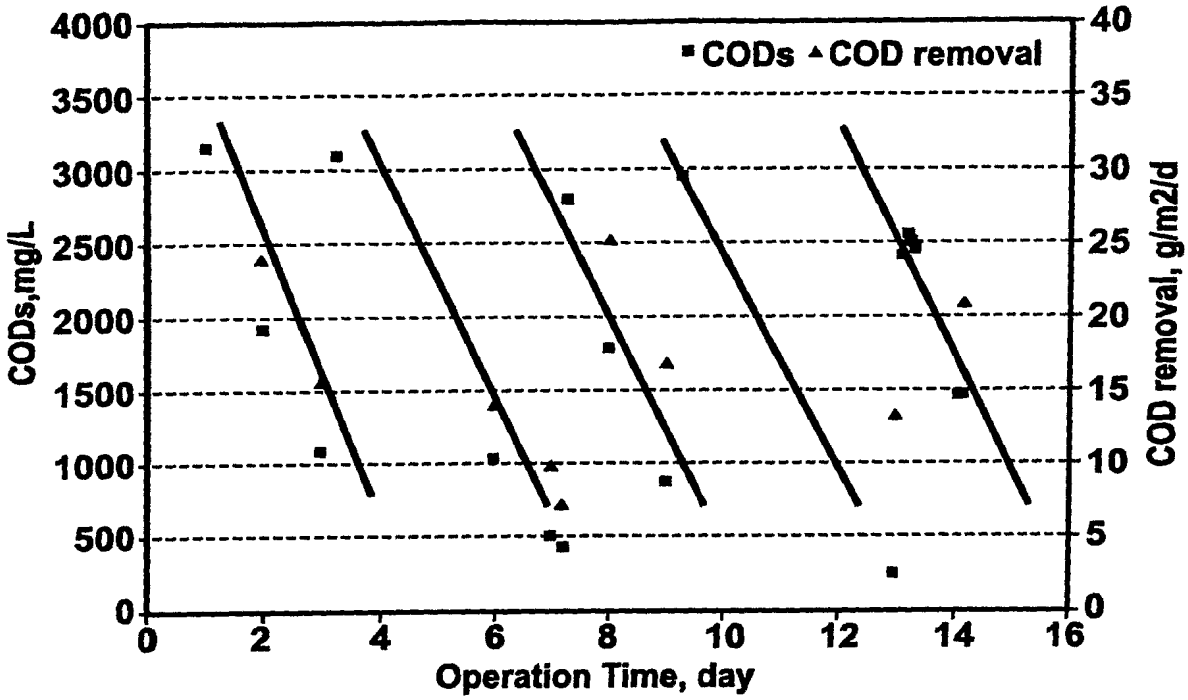

CODs ▲COD removal
CODs,mg/L
COD removal, g/m2/d
Operation Time, day
0
500
1000
1500
2000
2500
3000
3500
4000
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14
16

FIG. 23

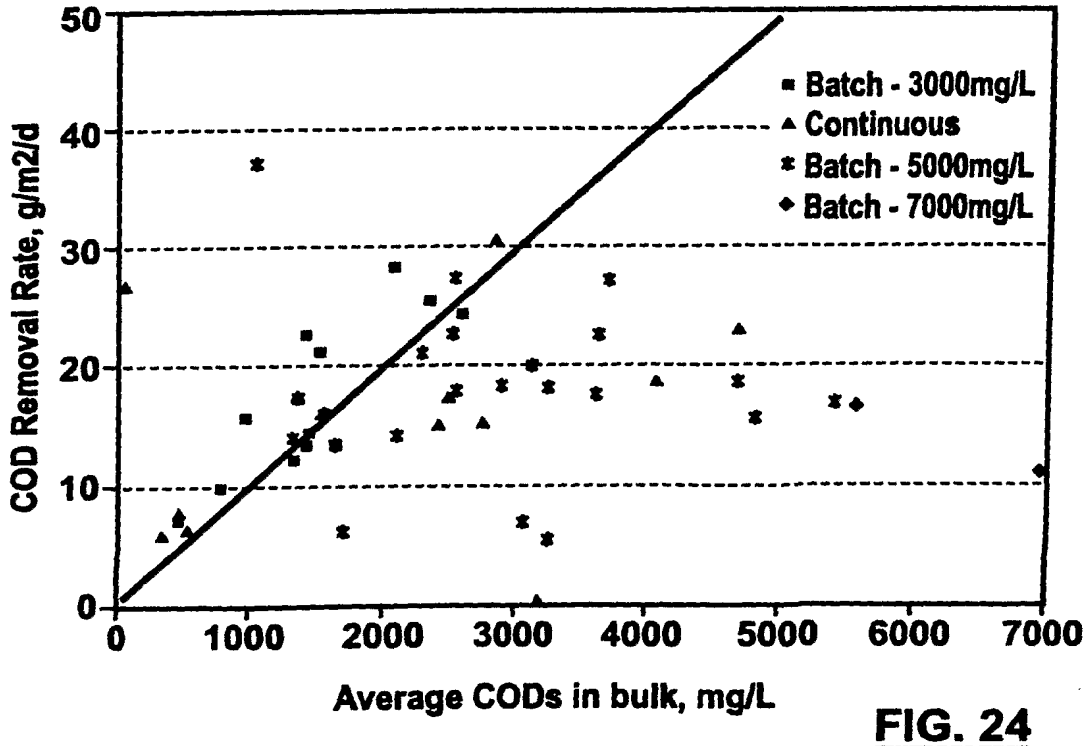

Batch - 3000mg/L
Continuous
Batch - 5000mg/L
Batch - 7000mg/L
COD Removal Rate, g/m2/d
Average CODs in bulk, mg/L
0
10
20
30
40
50
0
1000
2000
3000
4000
5000
6000
7000

FIG. 24

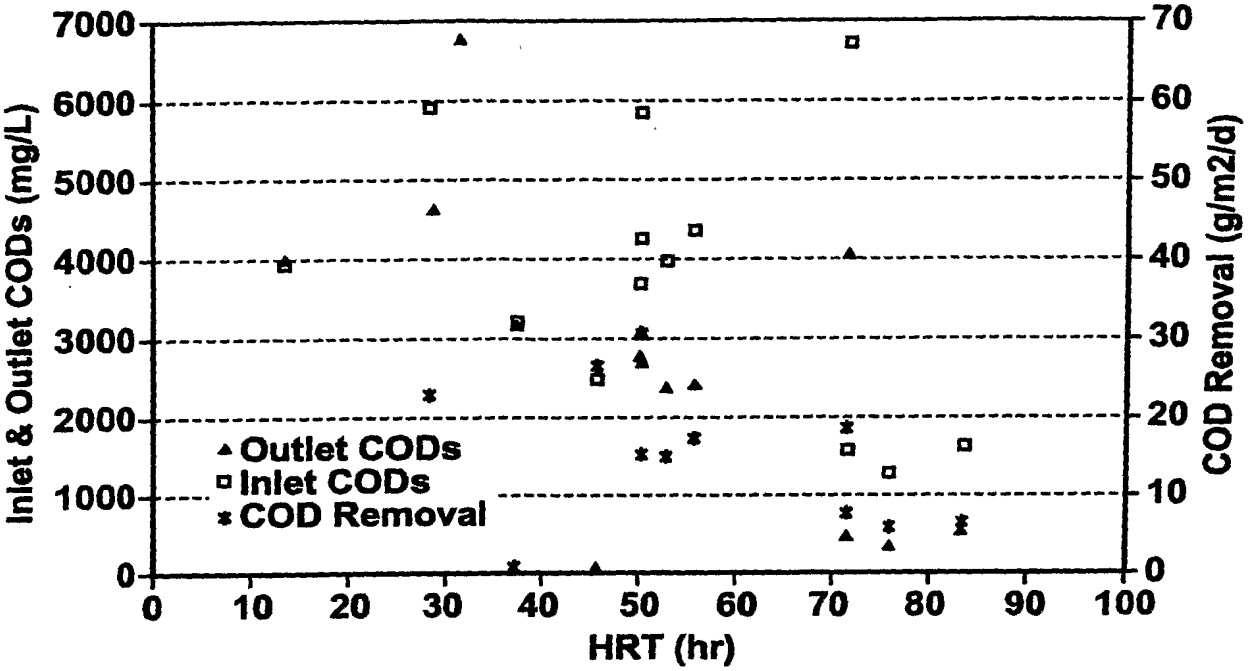

Inlet & Outlet CODs (mg/L)
COD Removal (g/m2/d)
HRT (hr)
Outlet CODs
Inlet CODs
COD Removal
0
1000
2000
3000
4000
5000
6000
7000
0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70
80
90
100

FIG. 25

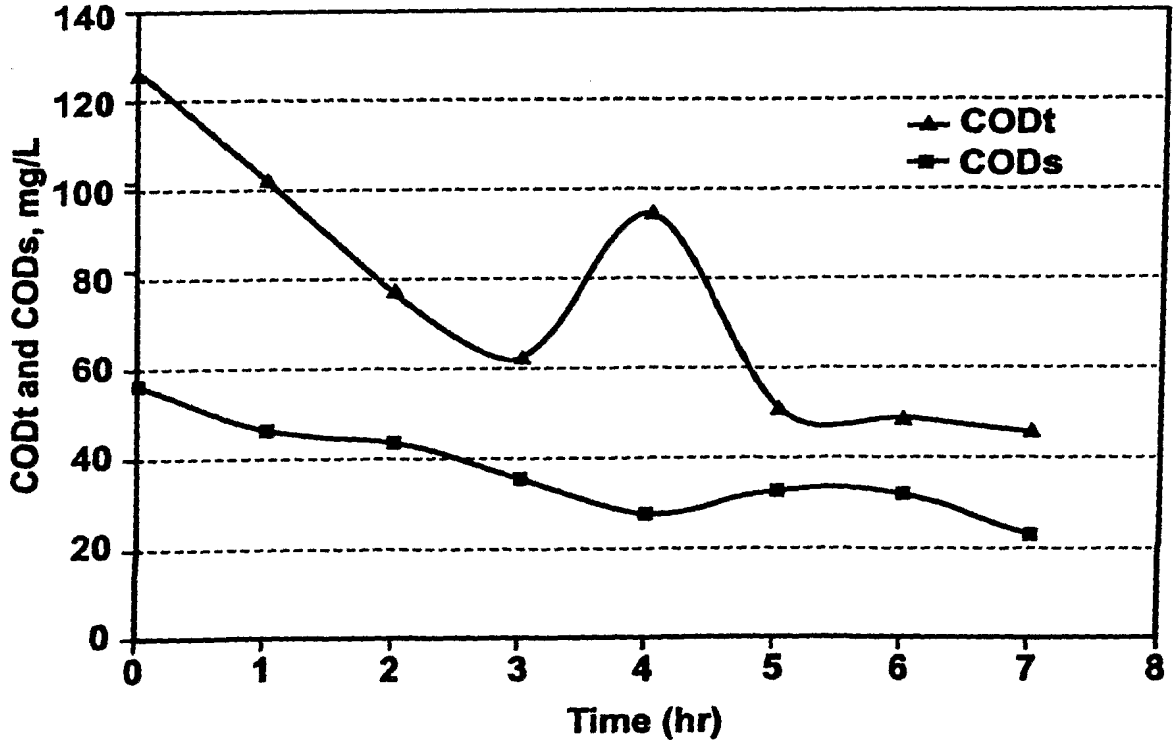

CODt and CODs, mg/L
Time (hr)
CODt
CODs
0
20
40
60
80
100
120
140
0
1
2
3
4
5
6
7
8

FIG. 26

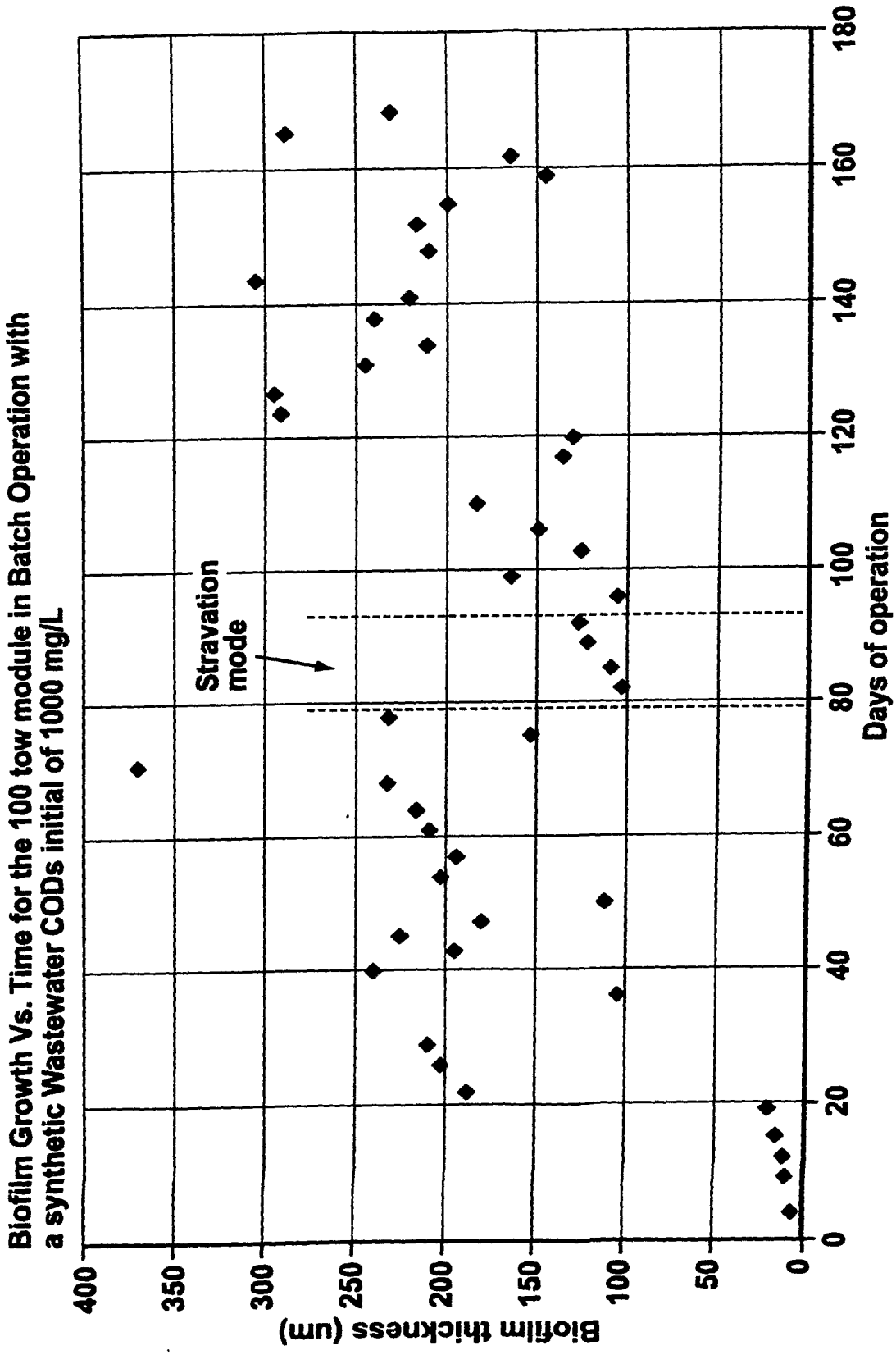

Biofilm Growth Vs. Time for the 100 tow module in Batch Operation with a synthetic Wastewater CODs initial of 1000 mg/L
Stravation mode
Biofilm thickness (um)
Days of operation
0
50
100
150
200
250
300
350
400
0
20
40
60
80
100
120
140
160
180

FIG. 27

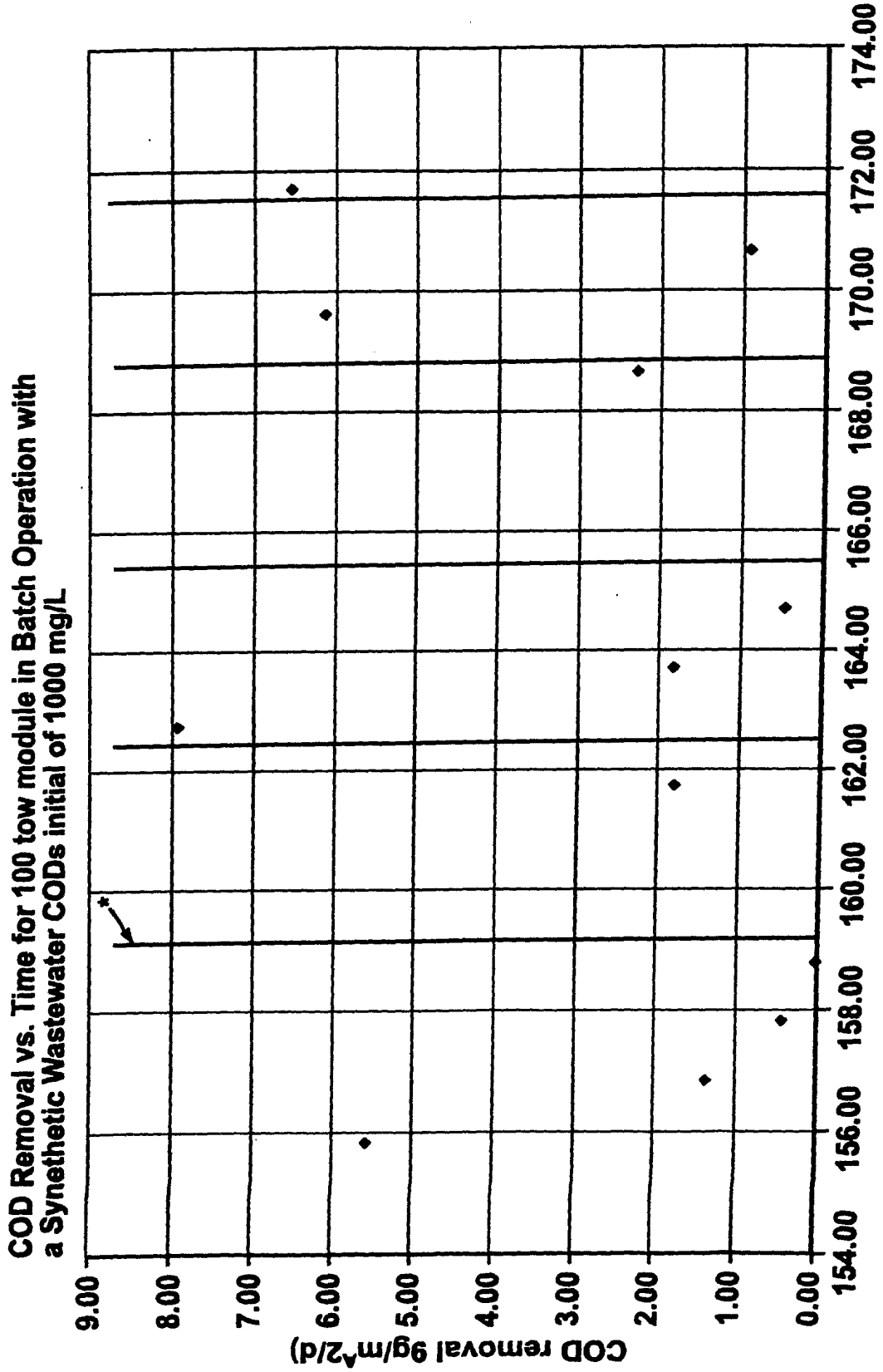

COD Removal vs. Time for 100 tow module in Batch Operation with a Synethetic Wastewater CODs initial of 1000 mg/L
COD removal 9g/m^2/d)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
154.00
156.00
158.00
160.00
162.00
164.00
166.00
168.00
170.00
172.00
174.00
Time (days)
* New batch run started

FIG. 28

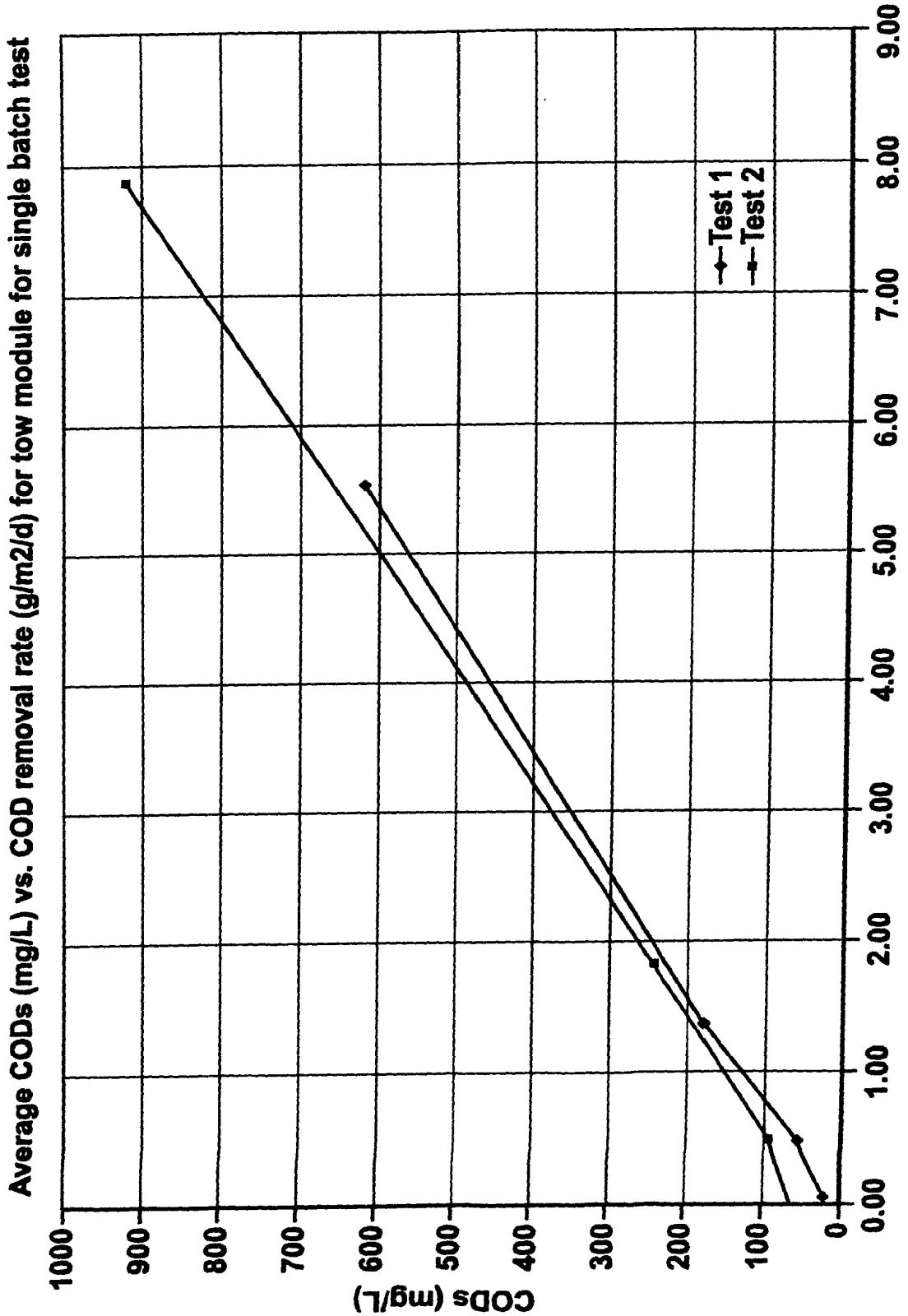

Average CODs (mg/L) vs. COD removal rate (g/m2/d) for tow module for single batch test
CODs (mg/L)
COD removal (g/mA2/d
Test 1
Test 2
0
100
200
300
400
500
600
700
800
900
1000
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00

FIG. 29

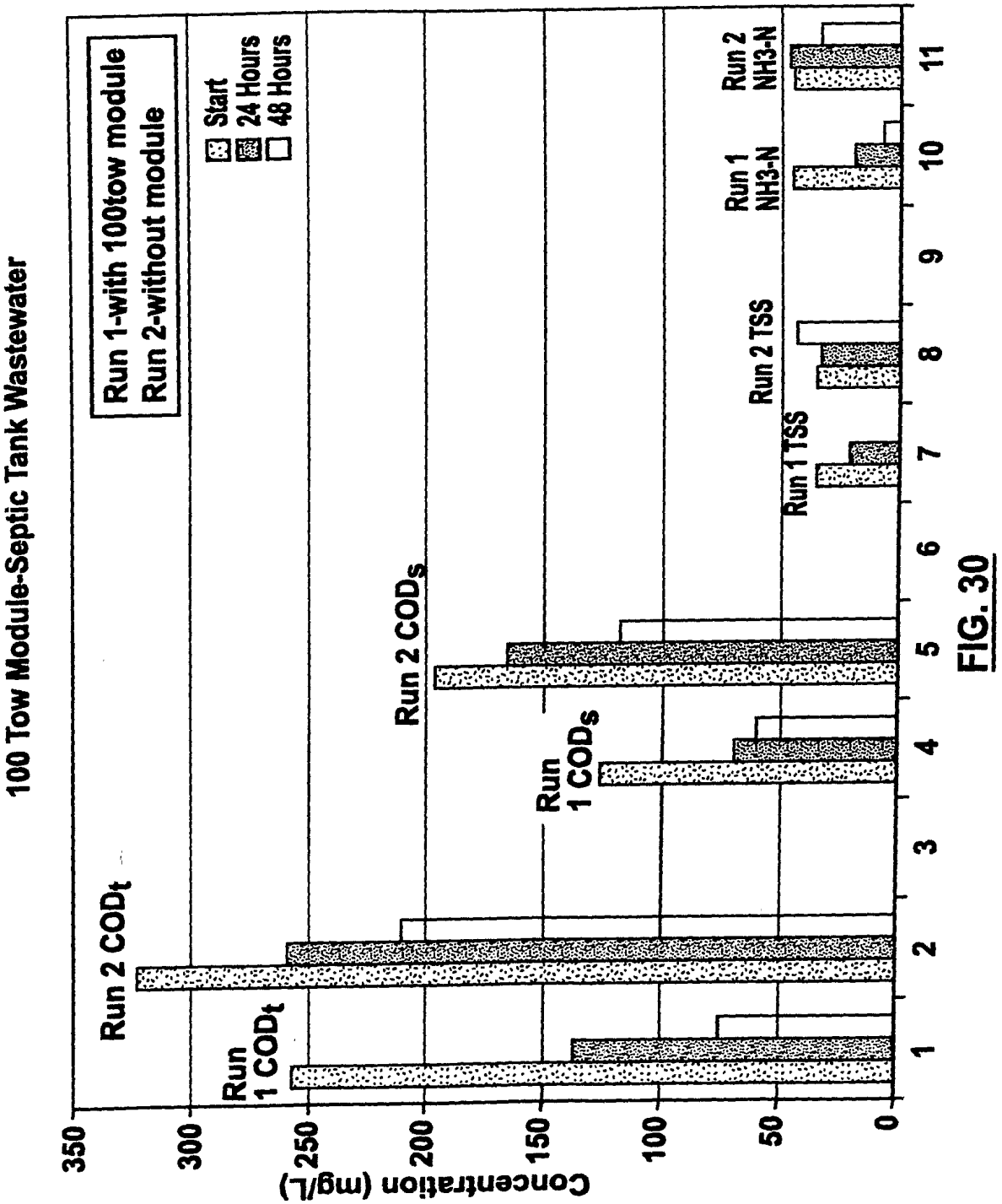

100 Tow Module-Septic Tank Wastewater
Run 1-with 100tow module
Run 2-without module
Start
24 Hours
48 Hours
Run 2 CODt
Run 1 CODt
Run 2 CODs
Run 1 CODs
Run 2 TSS
Run 1 TSS
Run 1 NH3-N
Run 2 NH3-N
Concentration (mg/L)
350
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11

FIG. 30

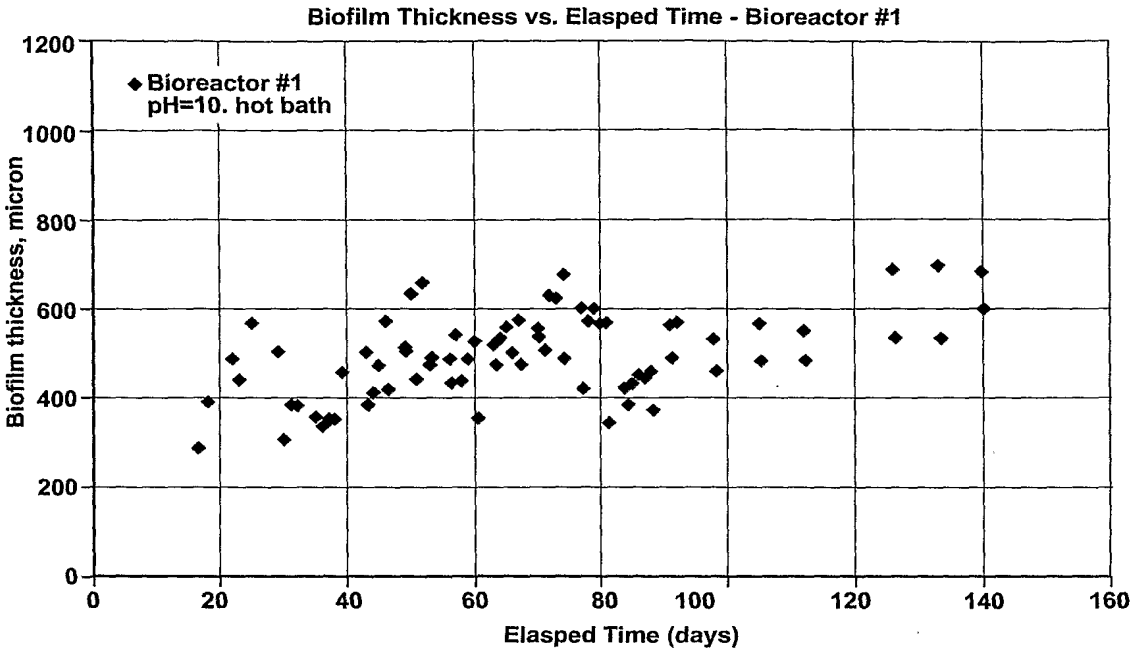

Biofilm Thickness vs. Elasped Time - Bioreactor #1
◆ Bioreactor #1
pH=10. hot bath
Biofilm thickness, micron
Elasped Time (days)
0
200
400
600
800
1000
1200
0
20
40
60
80
100
120
140
160

FIG. 31

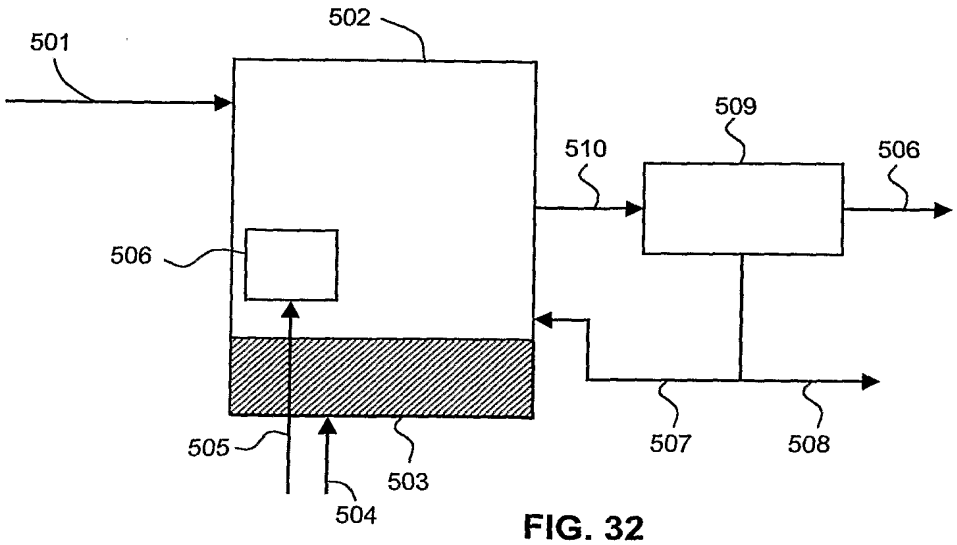

501
502
503
504
505
506
506
507
508
509
510

FIG. 32

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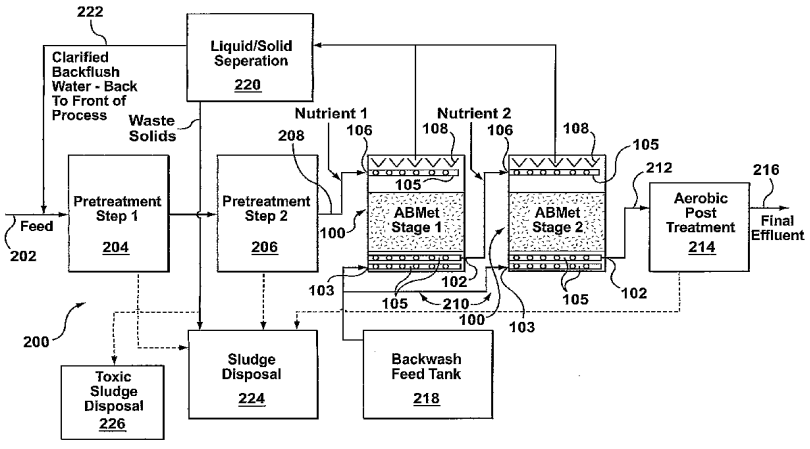
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Published:
— *with international search report*

[Continued on next page]

(54) **Title:** APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS



(57) **Abstract:** A process has steps of one or more of aerobic treatment to remove COD and nitrify a waste stream, anoxic treatment to denitrify a waste stream, anoxic treatment to remove selenium and anaerobic treatment to remove heavy metals and sulphur. The process may be used to treat, for example, FGD blow down water. The process may further include one or more of (a) membrane separation of the waste stream upstream of the anoxic digestion to remove selenium, (b) dilution upstream of the biological treatment step, (c) physical/chemical pretreatment upstream of the biological processes or dilution step to remove TSS and soften the waste stream, or (d) ammonia stripping upstream of the biological treatment steps or dilutions step. These processes may be provided in a variety of suspended growth or fixed film reactors, for example a membrane bioreactor or a fixed film reactor having a GAC bed. Processes for biological treatment of inorganic compounds in a fixed medium reactor is described including steps of one or more of maintaining desired ORP levels, optionally by controlling nutrient addition, and removing solids or gas bubbles from the medium bed.

WO 2007/012181 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE: APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS

[0001] For the United States of America, this is an application claiming the benefit under 35 USC 119(e) of U.S. Application Serial Nos. 60/701,996 filed July 25, 2005; and 60/736,859 filed November 16, 2005; and, this application claims priority to Canadian Patent Application No. 2,517,322 filed August 26, 2005. All applications listed above are incorporated herein, in their entirety, by this reference to them.

FIELD

[0002] This invention relates to water treatment including biological water treatment and treatment of feeds containing inorganic contaminants, for example selenium, nitrates or heavy metals, for further example scrubber blow down water from a flue gas desulfurization (FGD) operation in a coal fired power plant.

BACKGROUND

[0003] The following background discussion does not imply or admit that any process or apparatus described below is prior art or part of the knowledge of people skilled in the art in any country.

[0004] Scrubber blow-down water from a flue gas desulfurization operation in a coal-fired power plant contains a wide range of inorganic contaminants removed from the flue gas. The blow down water may also contain organic contaminants, such as di basic acid (DBA), and ammonia added as part of or to enhance the FGD process. The FGD scrubber blow-down water may have very high total dissolved solids where the main anions are chlorides and the main cations are calcium, magnesium and sodium. The rate of blow-down may be controlled to maintain a desired chloride concentration causing the blow-down water to have a high, but generally stable chloride concentration. The concentration of other contaminants may vary widely as influenced, for example, by burning coal from different sources even in a single power plant. However, the concentration of TDS, TSS, Ca

and Mg hardness, nitrate, ammonia, and sulfur for example as sulphate are all likely to be high, and various heavy metals may be present, making the blow down water very difficult to treat, particularly to achieve very low levels of contaminants. Other wastewaters, such as wastewater discharged from mining operations, agricultural drainage or run off water, other industrial waters or even drinking water, may also have unacceptable concentrations of some or all of these inorganic contaminants.

[0005] Current methods of treating blow down water rely heavily on physical and chemical processes to remove inorganic contaminants. The physical and chemical processes involve costly chemicals and produce large amounts of sludge. Arsenic, mercury and heavy metals may also be present in the blow down water at above regulated levels. Further, some jurisdictions have recently regulated selenium concentrations in effluents discharged to the environment. The permitted concentration of selenium may be 0.5 ppm or less or 200 ppb or less while the blow down water may contain 1-20 or 2-10 ppm of selenium which is not removed in conventional treatment plants.

[0006] In U.S. Patent No. 6,183,644, entitled Method of Selenium Removal and issued on February 6, 2001 to D. Jack Adams and Timothy M. Pickett, dissolved selenium is removed from contaminated water by treating the water in a reactor containing selected endemic and other selenium reducing organisms. Microbes may be isolated from the specific water or imported from other selenium contaminated water. The microbes are then screened for ability to reduce selenium under the site specific environmental conditions. The selected microbes are optimized for selenium reduction, then established in a high density biofilm within a reactor. The selenium contaminated water is passed through the reactor with optimized nutrient mix added as needed. The elemental selenium is precipitated and removed from the water. Products using this or a similar process may be available under the trade mark ABMet® from Applied Biosciences Corp of Salt Lake City, Utah, U.S.A. The entirety of US Patent No. 6,183,644 is incorporated herein by this reference to it.

SUMMARY

[0007] The following summary is intended to introduce the reader to one or more inventions described herein but not to define any of them. Inventions may reside in any combination of one or more of the apparatus elements or process steps described anywhere in this document.

[0008] It is an object of an invention described herein to improve on, or at least provide a useful alternative to, the prior art. It is an object of an invention described herein to provide a wastewater treatment process or apparatus. Other objects of one or more inventions described herein are to provide an apparatus or process for treating FGD blow down water or other wastewaters having selenium or nitrate or both, or a process or apparatus for biologically removing inorganic contaminants, for example nitrogen, selenium, arsenic, mercury or sulphur, from waste water. The wastewater may be a raw wastewater or a wastewater that has been pretreated.

[0009] A process is described herein having steps of anoxic treatment to denitrify a waste stream, anoxic treatment to remove selenium and anaerobic treatment to remove heavy metals or sulphur or both. Removal of heavy metals is possible because SO_4 is present and converted to sulfide by anaerobic SO_4 reducing bacteria. The process may further include one or more of (a) membrane separation of the waste stream upstream of the anoxic digestion to remove selenium, (b) dilution upstream of the biological treatment step, (c) physical/chemical pretreatment upstream of the biological processes or dilution step to remove TSS and soften the waste stream, for example through the addition of lime or sulfides and the removal of precipitates, (d) ammonia stripping upstream of the biological treatment steps or dilution step or (e) aerobic treatment to remove COD and nitrify the waste stream upstream of the anoxic treatment. Some of the biological treatment steps may be performed in a fixed film reactor, for example a granular activated carbon bed. One or more of the biological treatment steps may also be performed in a suspended growth reactor such as a membrane bioreactor. Each biological treatment step may be performed in a distinct reactor optimized to perform a

step or two or more of the biological treatment steps may be performed in a multiple purpose reactor.

[0010] An apparatus is described herein having one or more reactors configured to provide nitrification, denitrification, selenium and heavy metals removal and sulphur removal by biological treatment. The apparatus may further have a reactor to provide aerobic treatment of COD. The reactors may include a membrane bioreactor or a fixed film reactor. The fixed film reactor may comprise a bed of activated carbon or other support materials. The apparatus may further have one or more of an inlet for diluting the feed water to the biological processes, a system for adding lime or sulfides to the wastewater upstream of the biological reactors, one or more physical or chemical pretreatment systems, a precipitate remover, or an ammonia stripper.

[0011] An apparatus or process are also described herein for removing nitrates in a fixed film bioreactor. An apparatus or process are also described herein for providing a fixed film bioreactor having two or more zones. The zones may be adapted to remove different contaminants and may have differing oxygen reduction potential (ORP). An apparatus or process are also described herein for using nutrient location or feed rate to control ORP in a fixed film or media bioreactor. An apparatus or process are also described herein for bumping a fixed film or media bioreactor to release solids or gas bubbles from a media bed.

[0012] A process or apparatus described herein may be used for treating FGD blow down water or pretreated FGD blow down water to produce an effluent with low concentrations of selenium, for example 1 ppm or less or 10 ppb or less, and low concentrations of total nitrogen, for example 1 mg/L or less or 10 ppm or less, in the effluent. However, an apparatus or process described herein may also have applications in treating blow down water when selenium concentration in the effluent is not a concern. An apparatus or process described herein may also be useful for treating other wastewaters having selenium or nitrate, for example mining, or agricultural runoffs or waste

streams, contaminated ground or surface water streams, or petroleum refinery waste streams, particularly where the waste stream also has significant concentrations of one or more of COD, nitrate, ammonia, TDS, TSS, hardness, $CaSO_4$, or sulphate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Examples of embodiments of one or more inventions will be described below with reference to the Figures described below.

[0014] Figure 1 is a schematic process flow diagram of an apparatus and process for treating water.

[0015] Figure 2 is a schematic process flow diagram of another system for treating water.

[0016] Figure 3 is a schematic diagram showing an alternate embodiment for part of the system and process of Figure 1 or 2.

[0017] Figure 4 is a cross section of a bioreactor.

[0018] Figure 5 is a chart of experimental results using a multi-stage bioreactor.

[0019] Figure 6 is a chart of experimental results relating number of selenium reducing organisms to temperature.

[0020] Figure 7 is a chart of selenium reduction in FGD blow down water using DBA as a carbon source.

[0021] Figure 8 is a chart showing ranges of ORP for various reactions.

[0022] Figure 9 is a schematic process flow diagram of another system for treating water.

DETAILED DESCRIPTION

[0023] Various apparatuses or processes will be described below to provide an example of an embodiment of each claimed invention. No embodiment described below limits any claimed invention and any claimed invention may cover processes or apparatuses that are not described below. The claimed inventions are not limited to apparatuses or processes having all

of the features of any one apparatus or process described below or to features common to multiple or all of the apparatuses described below. It is possible that an apparatus or process described below is not an embodiment of any claimed invention. The applicants, inventors and owners reserve all rights in any invention disclosed in an apparatus or process described below that is not claimed in this document and do not abandon, disclaim or dedicate to the public any such invention by its disclosure in this document.

[0024] Table 1 shows the contaminants, and their concentrations, assumed for FGD scrubber blow-down water in the design of an example of an apparatus and process described further below. FGD blow-down water may exist with other contaminants or other concentrations of contaminants. The composition of FGD blow-down can also vary widely over time for a specified coal-fired power plant as influenced, for example, by changes in the source of coal. However, FGD blow-down water is generally characterized by very high total dissolved solids (TDS) where the main anion is chloride and the main cations are calcium, magnesium and sodium. The blow-down water also contains significant concentrations of fine suspended solids, including $CaSO_4$ fines. The blow-down also contains a wide range of inorganic contaminants, including ammonia, which is added for selective catalytic reduction in the scrubbing process. The blow-down water may also contain some organics, particularly DBA (dibasic acid) which may have been added to enhance scrubber efficiency. In the apparatus and process described below, the effluent is intended to have a total nitrogen (TN) content of 10 ppm or less and selenium concentrations of 0.4 ppm or less.

TABLE 1: Typical FGD Blowdown Water

Parameter	Typical Value	Min - Max
Chlorides	30,000 ppm	20-40,000 ppm
pH	>5.0<6.0	
TDS	75,000 mg/L	50,000 – 150,000 mg/L
TSS	2 % dry wt	1 – 5 % dry wt
Aluminum – Total	960 ppm	80-3700 ppm

Antimony	12 ppm	0.03-49.0 ppm
Ammonia – N	31 ppm	0.25-64 ppm
Nitrate – N	350 ppm	200-450 ppm
Total Nitrogen	200 ppm	50-400 ppm
Arsenic – Total	15 ppm	0.27-100 ppm
Barium – Total	100 ppm	2.0-770 ppm
Beryllium	2.1 ppm	0.06-6.9 ppm
Boron	----	20-40 ppm
Cadmium – Total	0.8 ppm	0.12-1.5 ppm
Calcium	18,000 ppm	10,000-30,000 ppm
Chromium – Total	23 ppm	0.5-210 ppm
Chromium VI	----	3-12 ppm
Cobalt	----	0.05-4 ppm
Copper – Total	1.7 ppm	0.3-6.6 ppm
CO_3/HCO_3	1500 ppm	1-3,000 ppm
Fluoride	360 ppm	61-1600 ppm
Iron – Total	1400 ppm	116-6400 ppm
Lead – Total	19 ppm	0.2-140 ppm
Lithium	----	2-3 ppm
Magnesium	15,000 ppm	10,000-20,000 ppm
Manganese	10 ppm	3.6-200 ppm
Mercury – Total	0.38 ppm	0.5-1.4 ppm
Nickel – Total	10 ppm	0.5-74 ppm
Phosphate – Total	1.0 ppm	0-10 ppm
Potassium	6800 ppm	5000-10,000 ppm
Selenium – Total	17 ppm	1.5-100 ppm
Silver – Total	10.0 ppm	0.002-20 ppm
Sodium	15,000 ppm	10,000-20,000 ppm
Sulfate (SO_4)	60,000 ppm	40,000-80,000 ppm
Thallium	0.76 ppm	0.02-2.2 ppm
Vanadium	----	1.0-11.0 ppm
Total Zinc	15.0 ppm	1.7-50.0 ppm

Temperature	130°F	125-130°F

[0025] In greater detail, treating the blow-down raises several challenges which a process or apparatus may address in a variety of ways, as described generally below and then by describing one or more examples of treatment systems and processes. In the following description, pre-treatment refers to treatment occurring upstream of biological process steps.

[0026] High TDS concentrations make it difficult to maintain activity for biological treatment. This issue may be addressed by diluting the waste stream upstream of biological treatment. The high TDS also makes it difficult to flocculate and settle biomass in an activated sludge process. This issue is addressed by using fixed film bioreactors or membrane bioreactors which may operate with TDS concentrations at which settlability is low.

[0027] In general, high hardness causes scaling due to Ca or Mg oversaturation and any pH or temperature shifts may cause precipitation of calcium or magnesium sulfates or carbonates. FGD and other industrial waste water can contain high levels of sulfate, calcium and magnesium resulting in a danger of scaling conditions, which are exacerbated with increasing alkalinity. When nitrate is removed by biological denitrification, alkalinity is produced which tends to encourage scaling in some processes. These issues may be addressed by a softening pre-treatment, for example lime softening, and optionally by adding acid for pH adjustment upstream of the biological process. Scaling may also or additionally be addressed by performing biological denitrification in a fixed film reactor as described below. pH may decrease in the direction of feed flow through such a reactor, thus making such reactors resistant to scaling. In particular, selenium reducing or other microbes populating the reactor may produce organic acids from the fermentation of the nutrients that are fed to the system, thereby neutralizing alkalinity and inhibiting scaling.

[0028] The high TSS, particularly because it is essentially inorganic, causes problems with developing and controlling a suspended biomass and

with bioreactor and membrane plugging. This issue may be addressed by pre-treating the waste stream to coagulate or flocculate and then physically remove (for example by settling or floating) suspended solids.

[0029] High nitrate concentrations are a concern because nitrate is a preferred electron acceptor for biological reduction over selenate. This issue may be addressed by decreasing the nitrate concentration upstream of a selenate reducing step. The nitrate reducing step may occur in an upstream part of a selenate reducing reactor, as part of a multi-step biological process or multi-part fixed film reactor upstream of a selenate reducing process or part, or as part of a distinct process or reactor.

[0030] Ammonia in the blow down water is a concern because concentration in the final effluent may be regulated and because oxidation of ammonia may increase nitrate concentration. This issue is addressed by removing the ammonia, for example, by stripping the ammonia as NH_3 in a pre-treatment process or by removing ammonia biologically by a nitrification/denitrification process either in a series process or with recirculating flows. Denitrification, either as a denitrification step in a nitrification/denitrification process or to remove nitrates otherwise created by oxidizing ammonia may be performed in a fixed film bioreactor which may be part of a single or multipart selenium reducing bioreactor.

[0031] The presence of various heavy metals, for example Cu, As or Hg, or related oxidized contaminants are a concern because they may be regulated in the effluent but are difficult to remove in low concentrations. This issue may be addressed in part by precipitating these elements out in a pre-treatment softening step. The issue may be further addressed by biologically reducing SO_4 and precipitating these contaminants as metal sulfides after removing nitrate and selanate and selenite. This precipitation may occur in a fixed film bioreactor which may be part of a single or multipart selenium reducing bioreactor.

[0032] The presence of selenium, as selenate or selenite, is a concern because of recent regulation of selenium concentrations in the effluent directly

or indirectly, for example through fish tissue concentrations in the receiving body. The selenium is difficult to remove because of its low concentration and its tendency to form selenate or selenite and dissolve in water making physical or chemical removal difficult, costly or inefficient. Selenium is addressed in the process and apparatus by biologically reducing it to elemental selenium and then precipitating it for removal. This may be done in a fixed film bioreactor which may also be used to remove other contaminants.

[0033] Figure 1 shows a treatment system 10 having a pretreatment area 12 upstream of a biological treatment area 14. Feed 16, which may be FGD blow-down water or another feed, flows into pretreatment area 12. In the pretreatment area 12, a large portion of the TSS in the feed is removed and Ca and Mg are removed to soften the feed 16. The pretreatment area 12 uses physical/chemical methods to treat the feed 16. For example, lime or sulfides or both may be added to the feed 16 to precipitate calcium, magnesium and metals. The precipitates may be removed by clarifiers, for example single stage or double stage clarifiers. Settling can be enhanced by the addition of coagulants or polymers.

[0034] Pre-treatment effluent leaves the pretreatment area 12 through a pretreatment effluent line 20. Dilution water 18 is added to the pretreatment effluent. The dilution water 18 reduces the total dissolved solids (TDS) concentration of the pretreatment effluent to make it acceptable for biological treatment downstream. Sufficient dilution water 18 may be added to make the TDS concentration like that of seawater, for example with a TDS of 35 g/L or less. Any low TDS water can be used for dilution water 18, for example cooling circuit blow down water from a power plant. The dilution water 18 also cools the FGD blow-down water, for example from 50 °C or more to about 43 °C or less or 40 °C or less, to further facilitate biological treatment. As shown in Figure 6, the health of selenium reducing organisms declines above about 43 °C. In general, a fixed film reactor may be operated at temperatures of about 40 °F to about 105 °F. An established reactor may also tolerate higher

temperature for short periods of time, for example a temperature to 110°F for up to 24 hours.

[0035] The diluted pretreatment effluent then flows to the biological treatment area 14. The biological treatment area 14 has four zones: an aerobic zone 22; a first anoxic zone 24; a second anoxic zone 26; and, an anaerobic zone 28. These zones 22, 24, 26, 28 are shown connected in series in Figure 1 although one or more of them may alternately be connected with recirculation loops. Further alternately, some of the zones 22, 24, 26, 28 may not be required in some embodiments. The zones 22, 24, 26, 28 may also occur in separate reactors or one or more zones 22, 24, 26, 28 may be combined into a single reactor. One or more of nutrient streams 30, 32, 34 may be used to add nutrients to any zone 24, 26, 28 downstream of a nutrient stream 30, 32, 34, either directly or through intervening zones 24, 26. For example, nutrients may be added in stream 30 or stream 32 to support the growth of bacteria in zones 26 or zone 28 or both.

[0036] The aerobic zone 22 is used to nitrify, assimilate or remove ammonia, to the extent that ammonia has not been stripped in the pretreatment area 12, and to oxidize organic carbon. An optional supplemental aerobic zone may also be added downstream of the anaerobic zone 28 to remove residual nutrients added before or in zones 24, 26, 28 and to oxidize residual compounds from anaerobic zone 28. If there is no TN discharge limit for the effluent, or if ammonia is stripped in the pretreatment area 12 such that TN in the effluent will be acceptable, the aerobic zone 22 may be omitted, or replaced by an aerobic zone downstream of the anaerobic zone 28.

[0037] In the first anoxic zone 24, nitrate acts as a preferred electron acceptor and is removed by denitrification. The nitrate may be removed to a concentration which facilitates the biological reduction of selenium in the second anoxic zone 26, considering that nitrate in high concentration will be used as an electron acceptor over low concentrations of selenate or selenite.

For example, NO_3 may be reduced to 10 mg/L as N or less or 1 mg/L as N or less or 10 ppm as N or less in the stream leaving the first anoxic zone 24.

[0038] In the second anoxic zone 26, selenium is removed by biological reduction and removal, for example by precipitation into flush flow water or waste sludge. These steps may occur, for example, according to the process described in US Patent No. 6,183,644 or in other fixed or suspended bed reactors. The reactors may be seeded with selenium reducing organisms.

[0039] In the anaerobic zone 28, sulfate-reducing bacteria reduce sulfates and produce sulfides in the form of H_2S or HS^-. Part of the HS^- may react with soluble metals to form insoluble metal sulfides which may precipitate out of solution. In this way the anaerobic zone removes heavy metals. The off gas from the anaerobic step 28 can be recycled to the aerobic step 22 or to a downstream aerobic step to reduce the production of odors associated with H_2S.

[0040] In general, the zones 22, 24, 26, 28 may be arranged into one or more reactors. Each zone 22, 24, 26, 28 may occupy its own reactor, for example a CSTR optimized to reduce the primary target contaminant of each zone 22, 24, 26, 28. Alternately, for example, zones 22 and 24 can be combined into a combined nitrification/denitrification reactor which may have 1, 2 or more tanks. Zones 24, 26 and 28 or 26 and 28 may be combined into an ABMet or other fixed film reactor having one or more tanks. Other reactors may also be used. For suspended growth reactors, the limited concentrations of the target contaminants may be low and the presence of other contaminants may make biomass separation difficult and so membrane bioreactors are preferred. Alternately, fixed film reactors may be used, for example Moving Bed Bioreactors, for example as produced by Anox Kaldnes of Norway, fluidized bed reactors, for example as produced by Shaw Envirogen of New Jersey, USA, biofilters as produced by Degremont of France under the trade mark BIOFOR, granular activated carbon reactors, for example as produced by the Applied Biosciences Corp. of Utah, USA under the ABMet trade mark, or in membrane supported biofilm reactors (MSBR) as

described in PCT Publication Nos. WO 2004/071973 or WO 2005/016826. Depending on the zone, the MSBR may operate autotrophically or heterotrophically optionally using a process of heterotrophic denitrification as described in Canadian Patent Application No. CA 2,477,333. Membrane separation may optionally be used with or after any fixed film reactor although there may also be no need for it. The entire disclosures of each of WO 2004/071973; WO 2005/016826 and CA 2,477,333 are incorporated herein by this reference to them.

[0041] Figure 2 shows a process flow diagram of a treatment plant 50 used for, for example, treating FGD blow-down as described in Table 1. In the pretreatment area 12, pretreatment is by lime softening, optionally with sulfide addition, and 1 or 2 stage settling in clarifiers. Such a process may be provided by WesTech of Utah, USA or others. PH is adjusted in the pretreatment effluent 20 when the dilution water 18 is added to a pH of less than 8.5, for example between 6 and 8 to enhance biological treatment. In the aerobic zone 22, a membrane bioreactor having an aeration tank and a membrane tank containing ZeeWeed™ membranes from GE Water and Process Technologies, Zenon Membrane Solutions of Ontario, Canada, may be used for nitrification. The aerobic zone 22 may alternately be omitted. The first and second anoxic and anaerobic zones 24, 26, 28 may be provided in an ABMet® reactor system by Applied Bioscience Corp. of Utah, USA, or a similar or modified system which may consist of a 2 or more stage reactor configuration. This reactor is an up-flow fixed film reactor using a GAC bed operated in plug flow so that 3 biological zones corresponding to the first anoxic, second anoxic and anaerobic zones 24, 26, 28 can be established in sequence. A down flow reactor may alternately be used. A single nutrient stream 30 may be used upstream of the ABMet reactor. Alternately, if a two or more stage reactor is used, an inlet for nutrients may be provided upstream of one or more of, or each, stage although nutrients will not necessarily be provided from each inlet at all times. Precipitates are removed from this reactor by periodically flushing the GAC bed to overflow troughs. Sludge from the pretreatment area 12 and biological treatment area 14 is fed to a sludge

thickener and dewaterer. Thickened and dewatered sludge is sent to waste. Sludge thickening and dewatering effluent is returned to an equalization tank to be mixed with the FGD blow down feed water 16. Further discussion of the use of an ABMet process or apparatus, or media filter generally, which may include modifications or enhancements to such a process or apparatus, are described below.

[0042] An ABMet® reactor, may be described as an upflow or downflow, simple fixed growth or fluidized, activated carbon or other support media based, biological filter system or bioreactor. A sample reactor 100 is shown in cross section in Figure 4. A media bed 101 is provided on which a population of selected microorganisms will grow and be retained within the system. Activated carbon may be employed as the medium and provides a very large surface area available for microbial growth. The activated carbon may be provided, for example, in the form of granular activated carbon or pelletized activated carbon. Other media might be used, for example polymeric fibers, crushed stone, pumice, sand, plastic media or gravel. With activated carbon, much of the surface area is protected in crevices within each carbon particle, thus sheltering biomass from shear and abrasive forces. The media bed 101 may occupy a zone h2 of the reactor 100.

[0043] The specific gravity of an activated carbon particle with attached biomass is slightly greater than 1.0. The particles may have a settling velocity in still water of about 160 ft/hr. The inventors have noticed that that settling rate is similar to that of granular sludge in upflow anaerobic sludge blanket (UASB) systems and so the influent distribution system and hydraulic regime within the carbon bed during normal forward flow conditions may be similar to UASB systems.

[0044] The reactor may have an upper port 106 and a lower port 102 and a backwash port 103, each of which may be connected to a distribution or collection system 105, for example one or more horizontal pipes. Optionally, for some systems, lower port 102 and backwash port 103 may be both connected to the same distribution or collection system 105 or be replaced by

a single port and distribution or collection system 105. Uniform orifices may be located on the underside of these pipes at proper spacing intervals. Orifices may be designed at about a 1.0 psi pressure drop, which renders friction losses along each pipe insignificant and assures even flow distribution. Multiple pipes may be connected together through various headers or manifolds. One or more grades of aggregate 104 may be installed around and above the distribution or collection system or systems 105 in a subfill layer h1. The aggregate 104 will aid in flow distribution while also preventing break through of media to a distribution or collection system 105. System 105 connected to upper port 106 may be covered in a screen or have small holes to prevent media entry if required. Wastewater may enter the media bed 101 from the top or bottom. Wastewater may enter the reactor 100 through upper port 106, flow downwards through the media bed 101 and be withdrawn from lower port 102. Alternately, wastewater may enter through the bottom port 102, flow upwards through the media bed 101 and exit through the upper port 106. Upflow velocity under normal forward flow conditions may be maintained at about 5 ft/hr, well below the activated carbon settling rate of 160 ft/hr.

[0045] During the course of normal operation, solids will accumulate within the media bed 101. These solids may be of one or more of three types, 1) TSS which enters the reactor 100 and is retained, 2) biomass that is fixed to the media that grows and occupies additional space, and 3) inorganic contaminants that are biologically converted to solid forms and retained within a bed.

[0046] As solids accumulate, the pressure drop across the media bed 101 will increase. At a selected time interval or pressure point, the media bed may be flushed. This operation may be accomplished by utilizing backwash port 103 and its associated distribution or collection system 105. In this case, an upflow velocity of about 80 ft/hr of feed or water may be maintained to flush the media bed 101. Other velocities may be used, for example as in a range that would be used in activated carbon fluidized bed systems.

[0047] The upflow velocity applied during flushing may result in an upward expansion of the bed by up to 30% into a bed expansion layer h3. With this velocity, media particles may be fluidized, resulting in dislodging of trapped influent TSS, excessive biomass growth attached to the media and associated inorganic contaminants that have been removed from or precipitated out of the wastewater. The upflow velocity used is still comfortably below the settling rate of the media particles. Thus, unacceptable amounts of media will not be flushed, if any, from the bioreactors during this operation. The flushing water and entrained solids may be removed through troughs 108. Upper port 106 or lower port 102, whichever is used to withdraw treated effluent, may be closed during flushing.

[0048] A headspace layer h4 is provided above the expansion layer h3 and below a reactor cover 109 in layer h5. Gases released by the microorganisms may collect in the headspace layer h5. A vent or gas outlet 110 in the headspace h4 or cover h5 may be used to release these gases to the atmosphere or collect them for further treatment, for example in a downstream branch process or by recycle to an upstream part of the system.

[0049] From a hydraulic design standpoint, the reactor 100 may operate under conditions similar to UASB systems during upward feed flow periods, if any, and similar to biological fluidized beds during flushing. With proper function of up-stream treatment steps, and given the low growth rate of the biomass within the media bed 101, flushing may be required from between once every two weeks to only a few times each year, for example once a month. Flushing may be a batch operation over a 30 minute period. Spent flush water may be returned to the head of the plant from where, for example, the solids will settle in solids contact clarifiers and ultimately be co-mingled with primary solids for dewatering and disposal. Alternately, flush water may be separately treated to separate out solids for separate post-treatment or disposal of the solids since the solids flushed from reactor 100 may contain selenium and heavy metals.

[0050] In addition to, or alternately in place of, flushing as described above, the reactor 100 may be "bumped", or briefly fluidized or backwashed, periodically or from time to time. Bumping may be done by flowing water, for example reactor 100 feed water, cooling water or tap water, or a mixture of gas, for example air, bubbles and water, through the backwash port 103 at a rate greater than the normal upwards flow feed rate. The bumping may be done at a velocity of between about 1 gpm/ft^2 to 14 gpm/ft^2 for a short period of time, for example 10 minutes or less or 5 minutes or less. The bumping may be done at a frequency between, for example, once per hour to once per week, for example between 1 and 4 times per day. The bumping expands the media bed 101, allows some solids to be removed from the system and also releases gas bubbles that accumulate in the media bed 101. Gas bubbles or solids in the media bed 101 can impede flow through the media bed 101. The primary purpose of bumping is thus to control head loss and provide a more even flow of water through the entire media bed 101 particularly by the release of gas bubbles from the media bed 101. The bump may raise the water level in the reactor to above troughs 108. Bump effluent water collected in troughs 108 can be handled as described for flush effluent. This provides for removal of TSS and may reduce or eliminate the need for more rigorous flushes as described above. Alternately, the effluent from some or all of the bumps may be recycled to the lower port 102. While this may place solids in an otherwise undesired part of reactor 100, the bump flows are small and the recycled solids may be removed in full flushes as described further above. Further, a portion of the solids in the bump effluent is biodegradable. Recycling these solids may allow for their biodegradation and so reduce the total amount of sludge that needs to be wasted from the system as a whole. The desired bumping frequency and duration is related to the removal of gases or solids and so is related to the suspended solids concentration or nitrogen level or both in the inlet to the reactor 100.

[0051] Referring to Figure 9, another system 200 is shown for treating a feed 202 which may be FGD blowdown water. Feed 202 is pretreated in a first pretreatment apparatus 204 and, optionally, a second pretreatment

apparatus 206 or more pretreatment steps. These apparatus 204, 206 may be, for example, physical or chemical or physical and chemical treatment steps such as combined mixer and clarifier units used, for example, for lime or sulfide precipitation or any of the pretreatment steps described in this document. Pretreatment effluent 208 flows to a two stage bioreactor 210 having two of the reactors 100 of Figure 4 operated in downflow configuration in series. Pretreatment effluent 206 flows to upper port 106 of the first reactor 100, then from lower port 102 of the first reactor 100 to the upper port 106 of the second reactor and out of the lower port 102 of the second reactor. Treated effluent 212 leaving the second reactor 100 may optionally flow to an aerobic post-treatment apparatus 214 to produce final effluent 216. Reactors 218 may be flushed or bumped by flowing backwash water from backwash feed tank 218 to the backwash port 103 of the reactor 100. Bump or flush water collected in troughs 108 may be treated in a backwash liquid/solid separation apparatus 220, such as a pond or clarifier. Clarified backwash water may be returned to the head of the plant. All sludge may be sent to a common sludge disposal system 224 or the backwash water sludge may be sent to a separate toxic sludge disposal system 226. Nutrients may be added to the pretreatment effluent 208, the pipe between the lower port 102 of the first reactor 100 and the upper port 106 of the second reactor 100 or both.

[0052] The microorganisms that will perform the various functions discussed above will require a food or carbon source. A molasses-based nutrient mixture may be used. The nutrient may be chosen to provide a carbon: nitrogen: phosphorous ratio (CNP) of, for example, 100:10:1, when mixed with the feed. For example, the feed may already contain sufficient phosphorous and so there may be no need for phosphorous in the nutrient solution. Nutrient may be supplied, for example, at a rate of 0.2-0.4 gallons of nutrient per 1000 gallons of feed water. This basic mixture may be supplemented with micronutrients and other components shown to promote stable growth for the target microbial population. The nutrient can be added to the lead bioreactor and/or to the second or later cell in a two or more stage reactor.

[0053] DBA, if used in the FGD system to enhance sulfur removal, will leave a concentration of DBA in the wastewater. Some or all of the DBA will remain in the wastewater after physical or chemical pretreatments and even to some extent after an aerobic biological treatment step, if there is any. DBA is carbon-based and may be used by the microbes as a nutrient. As shown in Figure 7, DBA in sufficient quantities can be used as the sole carbon source for selenium reducing microorganisms. DBA present in lesser amounts may be accounted for in reducing the nutrient carbon amount or feed rate. An excess of DBA, if inhibiting the growth of desired organisms compared to undesired organisms or interfering with ORP gradient control, can be reduced by oxidation upstream of the reactor 100. In general, the extent to which DBA may be utilized in the anoxic or anaerobic sections of the beds can be managed by redox or nutrient control or upstream processes.

[0054] When reactor 100 is used to provide first and second anoxic zones 24, 26 and anaerobic zone 28, there are three primary, biochemical reactions, which may occur within the biomatrix.

- Remaining nitrates are reduced to nitrogen gas, which will be subsequently released to the atmosphere.
- Selenium is reduced from an oxidized state to elemental form. Elemental selenium will form so-called "nanospheres" that will attach to the cell walls of the microbes performing the reductive function. As the microbes are attached to the media, selenium will be likewise retained within the media bed until flushing.
- Sulfates present are reduced to hydrogen sulfide. Further, the sulfides generated in the biomatrix complex as metal sulfide precipitates that are retained. Sulfides are effective at complexing with zinc, copper, nickel, lead and a host of other primary metals. While required metals removal may be accomplished in the primary treatment steps, the reactor 100 may provide further treatment and polishing for metals removal.

[0055] A bioreactor configuration may involve multiple trains of two or more reactors 100 in series or other configurations. A generally plug flow regime may be maintained as wastewater flows through each train of two or more successive reactors 100. This arrangement may facilitate control of redox potential ranges associated with each of the three biochemical reactions discussed above or others. A single bioreactor 100 can also be used and similarly controlled.

[0056] Each of the reactions discussed above occur primarily in different, although possibly overlapping, redox ranges as shown in Figure 8. Wastewater entering the lead reactor 100 in each train will have a positive or slightly negative redox level. As the flow progresses through the bed, the redox level will gradually decrease. As the redox level drops into negative territory, remaining nitrates will be reduced first. As can be seen from Figure 8, nitrates will be reduced at levels down to -150 mV.

[0057] Selenium will then be reduced as the redox proceeds downward to -200 mV. Lastly, sulfate reduction to sulfide will occur down to -300 mV. Although the ORP range for sulfate reduction and selenium reduction largely overlap, selenium reduction may occur first and in preference over sulfate reduction and so sulfate reduction may not reach high levels or be complete until after selenium reduction is nearly complete.

[0058] With a generally plug flow regime, a redox gradient is developed through the bed or beds. This gradient can be controlled by adjusting the rate of nutrient addition or hydraulic retention time (HRT) or both in the bed or beds. In general, HRT may be altered at the design stage by choosing the bed dimensions in relation to the feed either by changing the dimensions of the bed or the number of beds in series or parallel. HRT in one or more beds in series may be, for example, in the range from 2 hours to 12 hours. After a system is built, changes to feed flow rate to the reactor 100 will alter HRT. However, nutrient addition may be easier to vary after a system is built and operating. Higher levels of nutrient addition will drive redox lower; reducing nutrient addition will cause redox level to rise. Optional inter-stage nutrient

addition between reactors 100 in series may provide additional flexibility in redox control. ORP sensors may be provided at the outlet of each of the two reactors 100 in a two stage system. An ORP sensor may also be provided in the feed to a reactor 100. ORP sensors may also be provided at intermediate points in one or more stages of a reactor. An operator or automated system may record the ORP values at a sampling interval and maintain a record of the values. The values may be used to determine an input parameter to a controller or decision table. For example, the ORP values in the outlet of each of the two stages of a two stage reactor may be recorded at a sampling rate, which may vary from, for example, 1 to 24 times per day. A running average of the values over an averaging period, for example 0.5 to 2 days is calculated. The running averages are used and considered in determining, manually or automatically, how the supply of nutrients upstage of either or both of the stages should be altered to produce a more desired redox level.

[0059] By being able to control redox level, the system can be tuned to optimize removal of certain contaminants. Specifically, nutrient addition can be controlled to maintain optimal redox for selenium reduction (-50 to -200 mV) in at least a zone of the reactor. For example, nutrient addition upstream of a first reactor 100 in a two stage system may be calculated to produce an ORP of -50 to -200 mV at the outlet of the first reactor. Nutrient addition upstream of a second reactor 100 may be controlled to provide an ORP of -200 to -350 mV in the outlet of the second reactor 100 to enhance sulfide production in the second reactor 100. As an alternate example, in an application where metals removed in the reactor is less important, nutrient addition may be controlled to provide an ORP of -50 mV or less, for example -50 mV to -100 mV, at the outlet of the first reactor 100 and -200 mV or more, for example, -150 mV to -200 mV at the outlet of the second reactor 100 of a two stage system.

[0060] The generally plug flow regime allows specific populations to reside in somewhat dedicated zones within the carbon beds and so each zone may contain multiple types of organisms although the redox levels for

various functions may overlap. Nonetheless, removal can be achieved for a range of contaminants.

[0061] The influence of ORP gradient on contaminant removal is demonstrated in Figure 5. Water entering a reactor 100 contains dissolved oxygen, and oxidized species of contaminants (i.e., nitrate, selenate, selenite), and has a positive ORP of +100 in this example. Samples were collected at various points across a multi-stage bioreactor and measured for ORP, selenium, sulfate, and nitrate-N. Contaminants were removed in their respective stages along this ORP gradient that had been established in the bioreactor bed.

[0062] ORP can be controlled through the system by inter-stage nutrient delivery, and by controlling the HRT of each stage, which is done by increasing or decreasing the influent flow rate. The ORP can be controlled within the range of +150 mV to – 400 mV.

[0063] In the example of Figure 5, a reactor was divided into six stages. Nitrate-N is removed in the first stage, with only 25% of the selenium being reduced. None of the sulfate is reduced. Removal in later stages is as shown in the Figure. Through controlling the ORP gradient, a system can be configured to remove for, for example, only Nitrate-N removal, Nitrate-N removal with selenium removal, and/or multiple contaminant removal via direct reduction and sulfide precipitation.

[0064] Denitrification may be carried out concurrent with or before sulfate and selenium reduction. As the biomass carrying out these reactions are fixed to the support media (which will be retained within the system), the evolution of nitrogen gas will not materially impact biomass retention. Gases created in a reactor may be removed by bumping as described above as required.

[0065] The biomass that will grow in the bioreactor cells may occur naturally in our environment. Sulfate reducers and denitrifiers may grow naturally with little encouragement required. Given that required metals

removal may be accomplished in the primary treatment steps, control of redox levels may optionally be such that sulfate reduction (and sulfide formation) is minimized.

[0066] Microbes that have demonstrated the ability to reduce oxidized selenium to elemental form have been isolated from various locations in the western U.S.A., for example by Applied Biosciences Corp. of Utah U.S.A. Several species of these microorganisms may be isolated and grown. At plant start-up, an innoculum charge of these microbes may be supplied to seed the bed. For example, the bioreactor 100 may be seeded with an initial charge of microbes. The initial charge may contain a mixture of 2-6 strains of microbes of the genus *Pseudomonas*, *Shewanella, Alcaligenes* or other environmental microbes, which have been selected based on, or in anticipation of, their growth and contaminant reduction in the water of interest. A population large enough to seed the reactor 100 may be grown on site from a supply of centrifuged and freeze dried starter microbes prepared remotely. These microbes quickly attach to activated carbon or other media, and proliferate thereafter in the presence of nutrient material. Following innoculum loading, the bioreactor cells may be operated in a recycle mode for several days to allow the microbes to attach. An alternate seeding procedure is to first soak the bed in a nutrient solution. After this pretreatment, approximately one reactor value of a mixture of seed microorganisms in water, optionally containing some nutrient solution, is placed in the reactor. The reactor is then allowed to sit for a period of time, for example 1 to 3 days, to allow the microorganisms to attach to the media before starting feed flow through the reactor. Thereafter, normal feed flow can be introduced and plant commissioning may proceed.

[0067] Following the completion of commissioning, a periodic bioassay evaluation may be conducted. This evaluation may involve collection of carbon samples at various depths in each bioreactor cell. Each sample may be measured for redox level and the microbes examined to develop a biological community profile. This study may generate an information

baseline to ensure that the proper microbial mix is maintained within the system over time and may be considered, for example in combination with on-line ORP measurements or to adjust target ORP ranges, in controlling nutrient addition.

[0068] Two or more bioassay evaluations may be conducted following commissioning of a plant. In the event that a bioassay evaluation shows that the specific microbes needed for selenium reduction are not present in appropriate numbers, a supplemental inoculum charge may be provided. Additional bioassay evaluations may be performed from time to time to monitor the performance of the reactor and the results may be considered in modifying desired ORP ranges at one or more points in the reactor. A reactor as discussed above may be used to provide one or more of the first and second anoxic and anaerobic zones 24, 26, 28.

[0069] Figure 3 shows a nitrification/denitrification reactor 80 used to provide the aerobic and first anoxic zones 22, 24 of Figure 1. Nitrification/ denitrification reactor 80 may also be used to replace the bioreactor tank and ZeeWeed tank of Figure 2 to provide the aerobic and first anoxic zones 22, 42 and allow the ABMet reactor to be operated with the second anoxic and anaerobic zones 26, 28 with a minimal or no first anoxic zone 24.

CLAIMS:

We claim:

1. A process having steps of anoxic treatment to denitrify a waste stream followed by anoxic treatment to remove selenium.

2. The process of claim 1 further comprising a step of anaerobic treatment to remove heavy metals, for example arsenic, mercury, or sulphur.

3. The process of claim 1 or 2 further comprising a step of aerobic treatment to remove COD from or nitrify the waste stream upstream of the steps of claim 1.

4. The process of any of claims 1 to 3 further comprising one or more steps of:

a) membrane separation of the waste stream upstream of the step of anoxic digestion to remove selenium;

b) dilution upstream of the digestion steps; or

c) physical/chemical pretreatment upstream of the digestion steps or the dilution step to remove TSS and soften the waste stream.

5. The process of claim 1 or 2 wherein ORP of the waste stream upstream of the anoxic treatment step to denitrify is maintained between +100 and 0 mV and ORP of the waste stream downstream of the anoxic treatment step to remove selenium is maintained below 0 mV.

6. The process of any preceding claim wherein ORP of the waste stream leaving the process has an ORP of -50 mV or less.

7. The process of claim 5 or 6 wherein ORP is maintained by controlling the place or rate of a nutrient feed to the process.

8. The process of any preceding claim further comprising accounting for the presence of DBA in the waste stream in determining a rate of carbon nutrient addition.

9. The process of any preceding claim wherein the anoxic treatment steps occur in a fixed film bioreactor.

10. An apparatus having one or more reactors configured to provide denitrification and selenium removal by biological treatment.

11. The apparatus of claim 10 wherein the one or more reactors further provide aerobic treatment of COD by biological treatment.

12. The apparatus of claim 10 or 11 wherein the one or more reactors further provide heavy metal or sulfur removal by biological treatement.

13. The apparatus of any of claims 10 to 12 wherein one of the one or more reactors is a membrane bioreactor.

14. The apparatus of any of claims 10 to 13 wherein one of the reactors is a fixed film reactor

15. The apparatus of claim 14 wherein the fixed film reactor has an activated carbon bed used to support a population of microbes.

16. The apparatus of any of claims 10 to 15 having one or more of an inlet for diluting the feed water to the reactors, a system for adding lime or sulfides to the wastewater upstream of the reactors, a precipitate remover, or an ammonia stripper.

17. A process for operating a media bed reactor comprising a step of considering the oxygen reduction potential of water entering into or exiting from a part of the reactor in determining a nutrient addition amount, location or rate.

18. The process of claim 17 wherein the ORP of an upstream portion of the reactor is maintained at between 100 and 0 mV.

19. The process of claim 17 or 18 wherein the ORP of a downstream portion of the reactor is maintained at an ORP of -50 mV or less.

20. The process of any of claims 17 to 19 used to remove nitrates.

21. The process of any of claim 17 to 20 used to remove selenium.

22. A process for operating a fixed media bioreactor comprising a step of bumping the reactor at least once per week to release solids or gas bubbles.

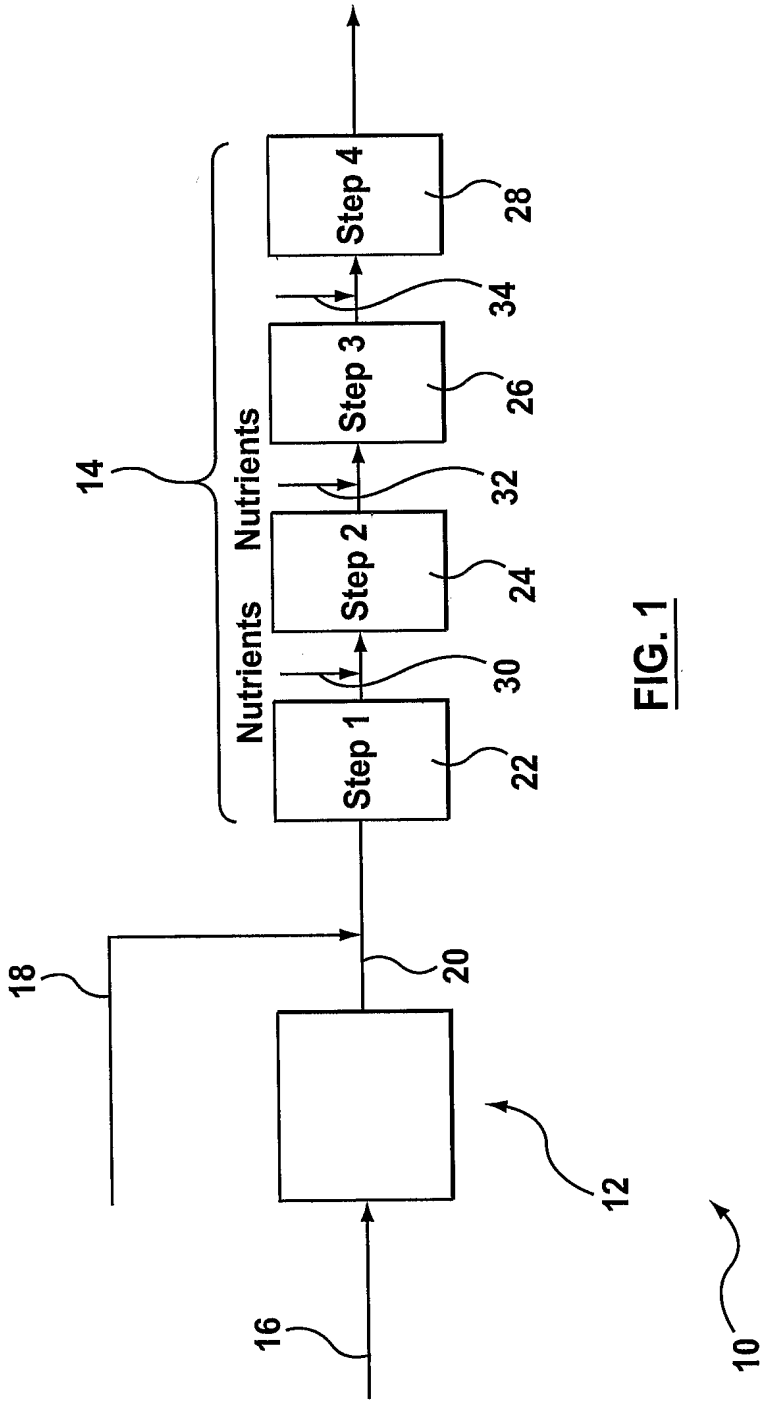

Step 4
28
34
Step 3
26
Nutrients
32
Step 2
24
Nutrients
30
Step 1
22
14
18
20
12
16
10

FIG. 1

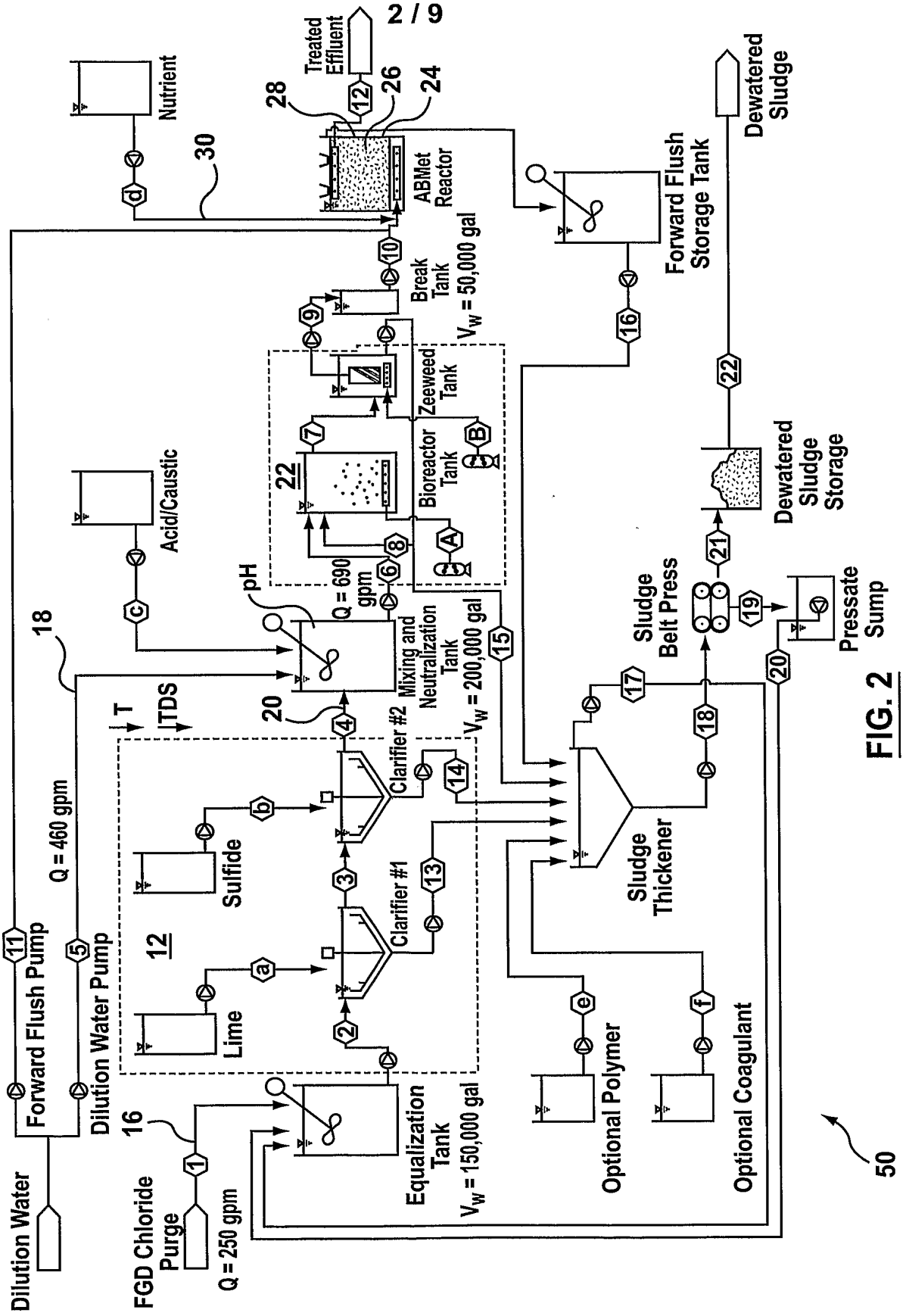

Treated Effluent
Nutrient
ABMet Reactor
Break Tank
V_W = 50,000 gal
Zeeweed Tank
Bioreactor Tank
Acid/Caustic
pH
Q = 690 gpm
Mixing and Neutralization Tank
V_W = 200,000 gal
Forward Flush Storage Tank
Dewatered Sludge
Dewatered Sludge Storage
Sludge Belt Press
Pressate Sump
Sludge Thickener
Clarifier #2
Clarifier #1
Sulfide
Lime
Q = 460 gpm
Forward Flush Pump
Dilution Water Pump
Equalization Tank
V_W = 150,000 gal
Optional Polymer
Optional Coagulant
Dilution Water
FGD Chloride Purge
Q = 250 gpm
T
TDS
12
22
16
18
20
28
26
24
30
50

FIG. 2

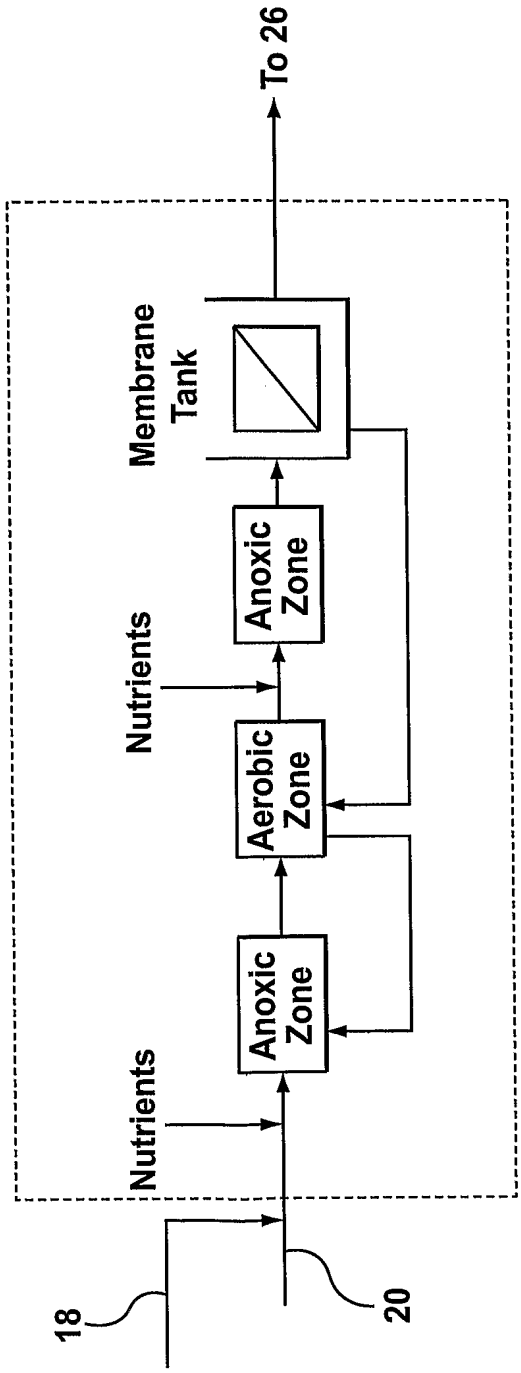

To 26
Membrane Tank
Anoxic Zone
Nutrients
Aerobic Zone
Anoxic Zone
Nutrients
80, 22, 24
18
20

FIG. 3

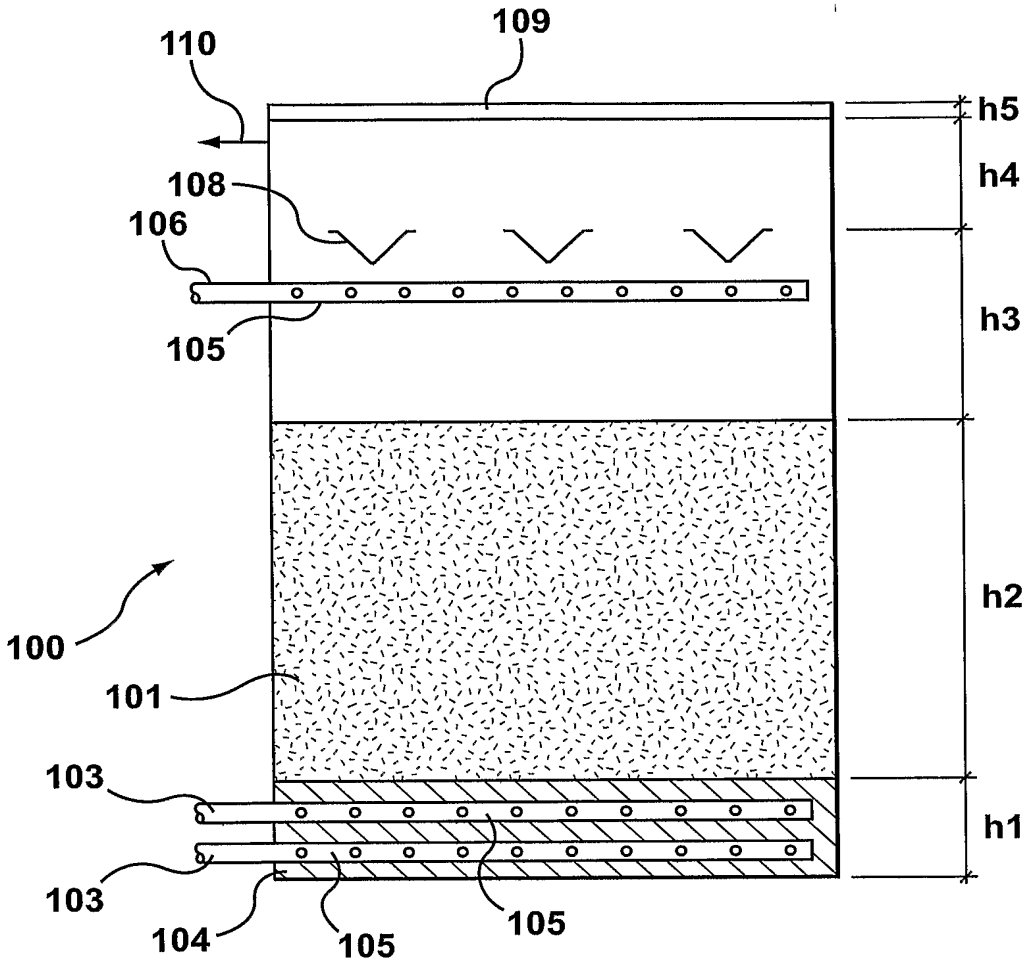


FIG. 4

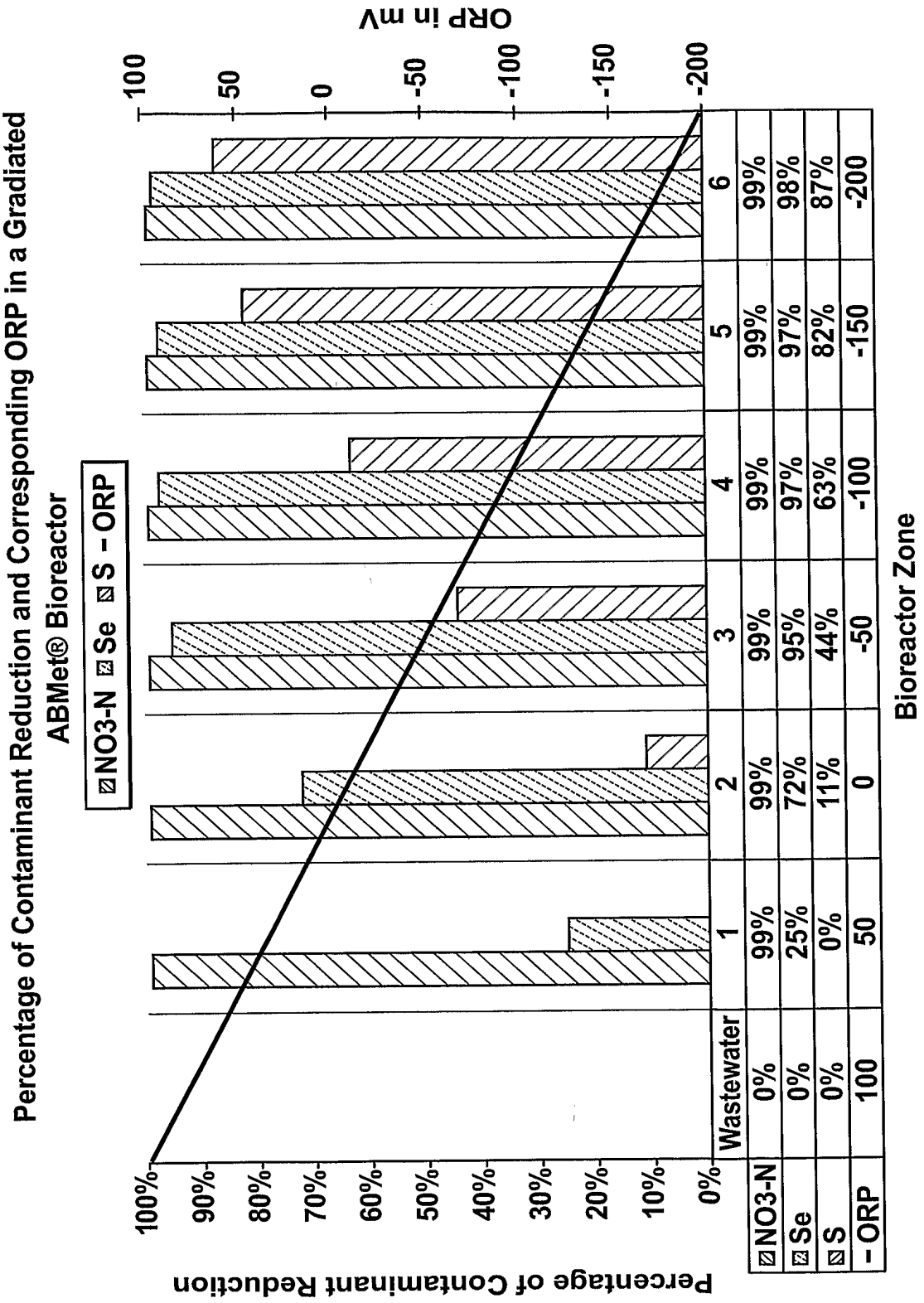

Percentage of Contaminant Reduction and Corresponding ORP in a Gradiated ABMet® Bioreactor
NO3-N Se S - ORP
ORP in mV
100
50
0
-50
-100
-150
-200
Percentage of Contaminant Reduction
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Wastewater
1
2
3
4
5
6
NO3-N 0% 99% 99% 99% 99% 99% 99%
Se 0% 25% 72% 95% 97% 97% 98%
S 0% 0% 11% 44% 63% 82% 87%
- ORP 100 50 0 -50 -100 -150 -200
Bioreactor Zone

FIG. 5

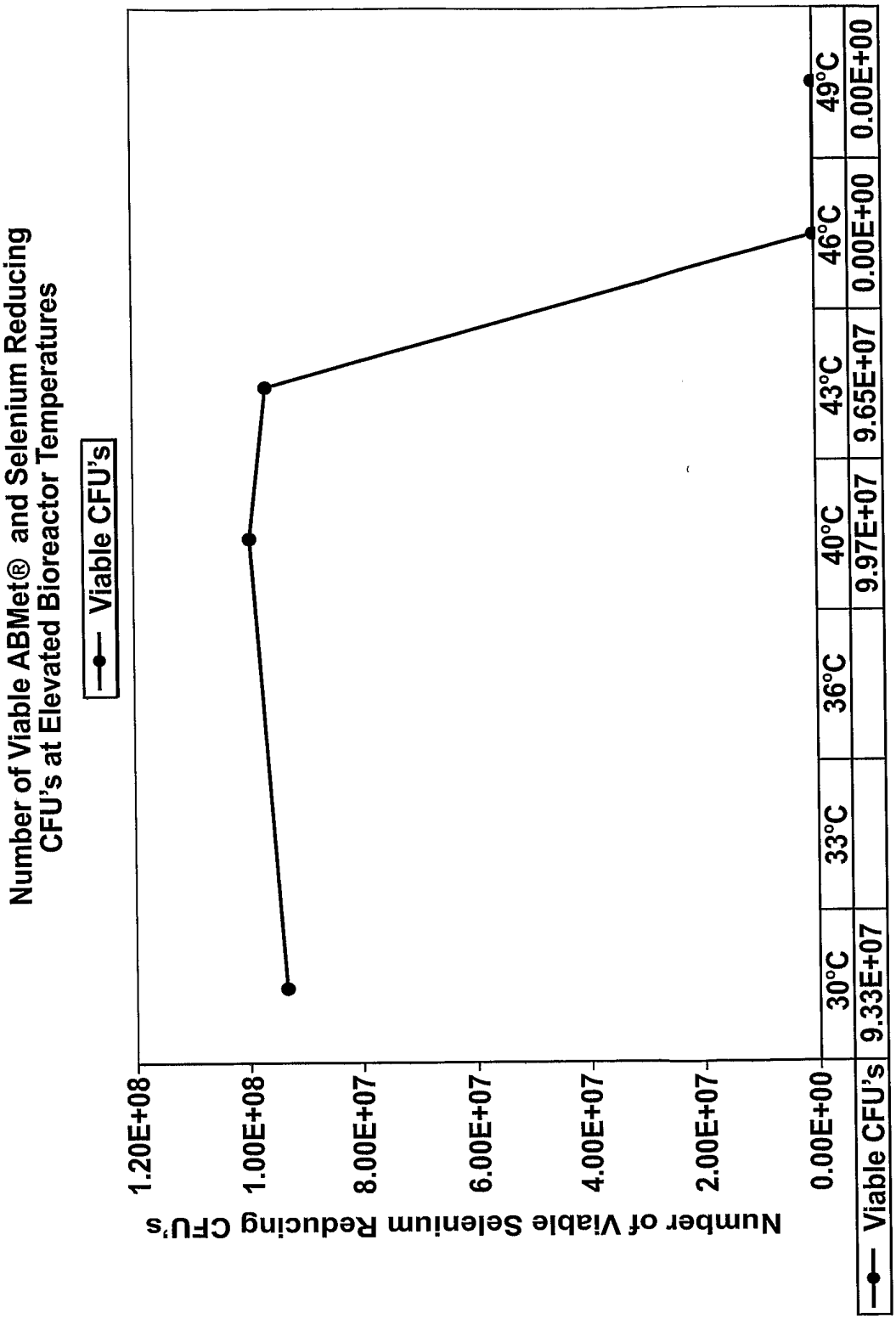

Number of Viable ABMet® and Selenium Reducing CFU's at Elevated Bioreactor Temperatures
Viable CFU's
Number of Viable Selenium Reducing CFU's
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Temperature in Degrees Celcius
30°C 33°C 36°C 40°C 43°C 46°C 49°C
Viable CFU's 9.33E+07 9.97E+07 9.65E+07 0.00E+00 0.00E+00

FIG. 6

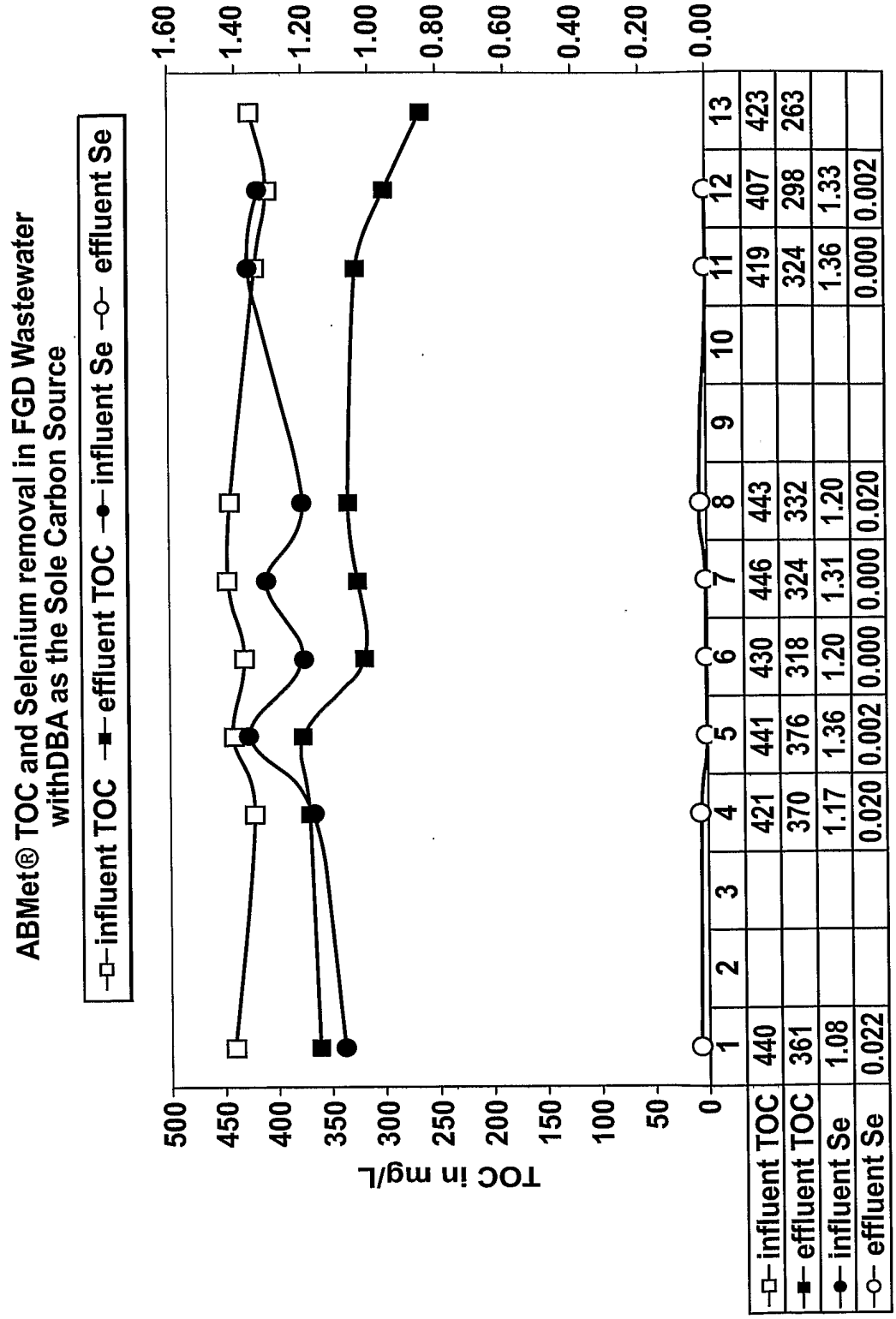
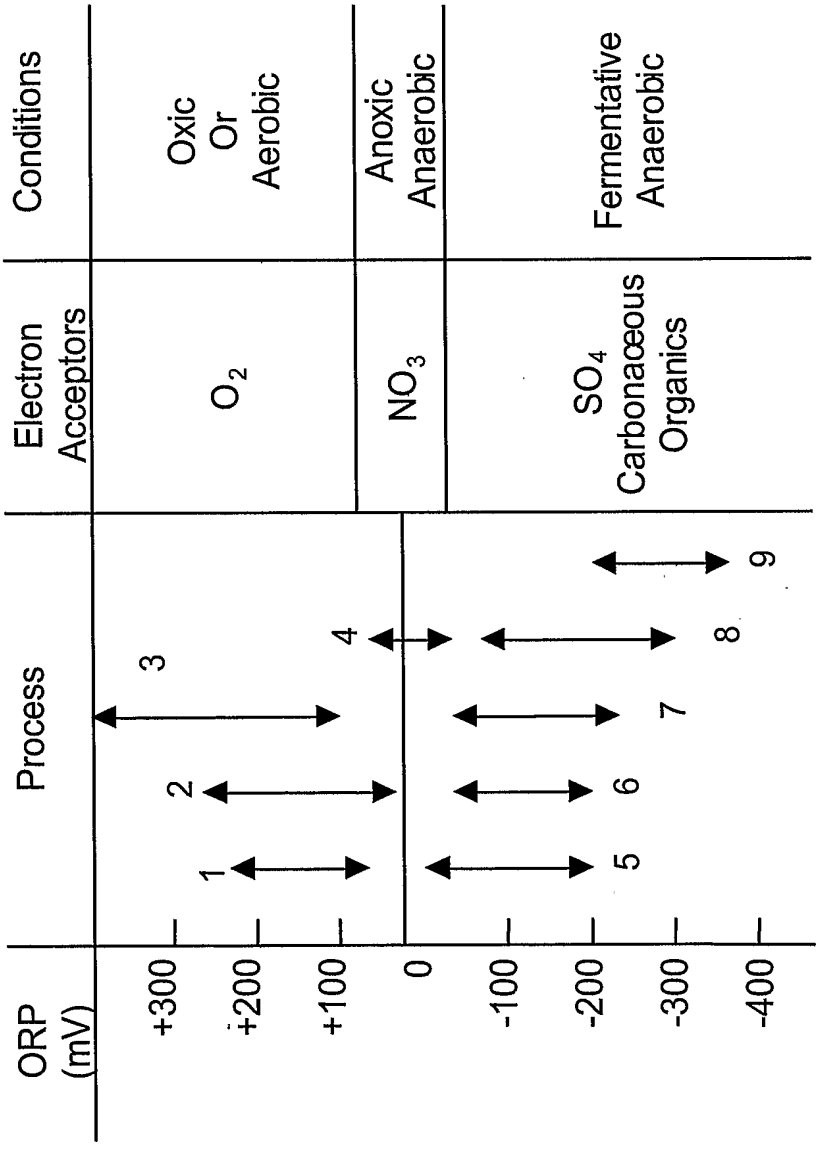

ABMet® TOC and Selenium removal in FGD Wastewater withDBA as the Sole Carbon Source
influent TOC
effluent TOC
influent Se
effluent Se
TOC in mg/L
Selenium Concentration in mg/L
Day
0
50
100
150
200
250
300
350
400
450
500
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1 2 3 4 5 6 7 8 9 10 11 12 13
influent TOC 440 421 441 430 446 443 419 407 423
effluent TOC 361 370 376 318 324 332 324 298 263
influent Se 1.08 1.17 1.36 1.20 1.31 1.20 1.36 1.33
effluent Se 0.022 0.020 0.002 0.000 0.000 0.020 0.000 0.002

FIG. 7



1 – Organic Carbon Oxidation
2 – Polyphosphate Development
3 – Nitrification
4 – Denitrification
5- Polyphosphate Breakdown
6 – Selenium Reduction
7 – Sulfide Formation
8 – Acid Formation
9 – Methane Formation

FIG. 8

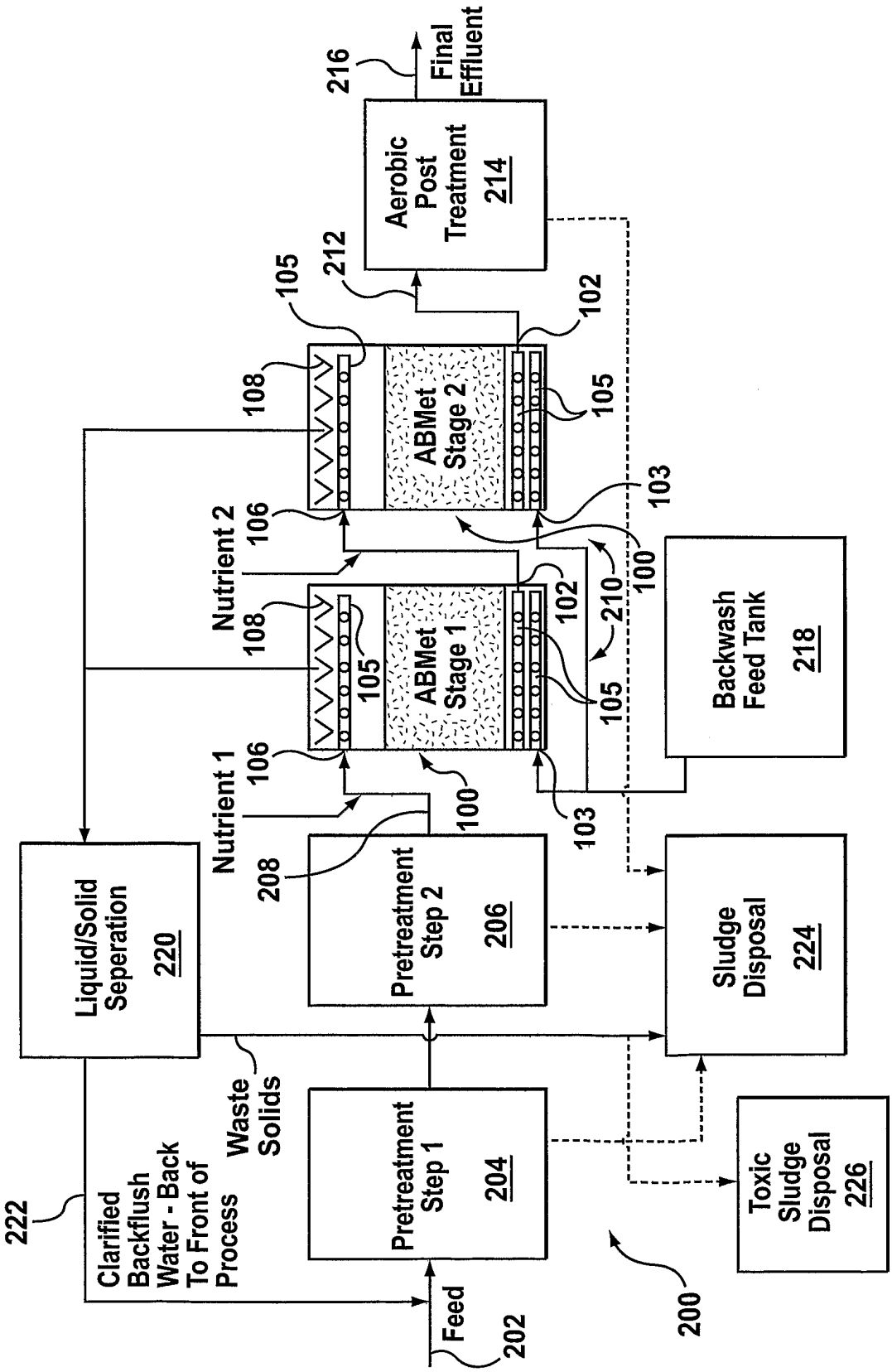

Aerobic Post Treatment 214
Final Effluent
216
212
105
102
108
ABMet Stage 2
105
103
Nutrient 2
106
100
210
102
108
ABMet Stage 1
105
105
Backwash Feed Tank 218
Nutrient 1
106
100
103
208
Pretreatment Step 2 206
Liquid/Solid Seperation 220
Sludge Disposal 224
Waste Solids
Pretreatment Step 1 204
Toxic Sludge Disposal 226
222
Clarified Backflush Water - Back To Front of Process
200
Feed
202

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001220

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C02F 3/30*** (2006.01) , ***B01D 53/22*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C02F 3/30 (2006.01) , **B01D 53/22** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC: C02F

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Esp@cenet, Delphion, QWeb, USPTO, Canadian Patent Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2005/0133443 (Applegate et al.) 23 June 2005 (23-06-2005) abstract paragraphs [0003], [0005]	17 20, 21
X	US 5 651 892 (Pollock) 29 July 1997 (29-07-1997) column 4, lines 63-65	22
Y	US 6 183 644 (Adams et al.) 06 February 2001 (06-02-2001) abstract claim 1	1-4, 9-16, 21
Y	WO 2004/071973 (Cote et al.) 26 August 2004 (26-08-2004) paragraph [0011]	1-4, 9-16, 20

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
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"O" document referring to an oral disclosure, use, exhibition or other means
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2006 (21-11-2006)	23 November 2006 (23-11-2006)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Charles Greenough 819- 994-0243

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001220

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [ ] Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

Claims 1-16: A process and apparatus for denitrifying and removing Selenium from a waste stream using biological treatment.

Claims 17-21: A process for operating a media bed reactor comprising considering the oxygen reduction potential in determining nutrient addition amount, location or rate.

Claim 22: A process for operating a fixed media bioreactor comprising bumping the reactor at least once per week.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	US 4 490 258 (Heijnen et al.) 25 December 1984 (25-12-1984) abstract	3, 11
Y	EP 1 270 513 (Verstraete et al.) 02 January 2003 (02-01-2003) abstract	3, 11
Y	US 5 228 997 (Martin et al.) 20 July 1993 (20-07-1993) abstract	3
Y	US 6 207 047 (Gothreaux) 27 March 2001 (27-03-2001) abstract	3
Y	US 6 896 805 (Austin) 24 May 2005 (24-05-2005) abstract	16
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(21) International Application Number: PCT/GB86/00404

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(31) Priority Application Numbers: 8517531
8601763
8612919

(32) Priority Dates: 11 July 1985 (11.07.85)
24 January 1986 (24.01.86)
28 May 1986 (28.05.86)

(33) Priority Country: GB

(71) Applicant: EPOC LIMITED [GB/GB]; 6 Esterbrooke Street, Hyde Place, Westminster, London SW1 (GB).

(72) Inventors: DOWNING, Anthony, Leighton ; 2 Tewin Close, Tewin Wood, Welwyn, Hertfordshire AL6 0HF (GB). SQUIRES, Rodney, Charles ; Jarretts, Carlton Road, South Park, South Godstone, Surrey RH9 8LE (GB). JAMES, Anthony, Noel ; 82 Downlands, Baldock Road, Royston, Hertfordshire SG8 5BY (GB). HOYLE, Brian, George ; 10 Station Road, Ivinghoe, Leighton Buzzard, Bedfordshire LU7 9EB (GB).

(74) Agent: RUFFLES, Graham, Keith; Marks & Clerk, 57/60 Lincolns Inn Fields, London WC2A 3LS (GB).

(81) Designated States: AT (European patent), AU, BE (European patent), BR, CH (European patent), DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.

(54) Title: REMOVING SELENIUM FROM WATER

(57) Abstract

Dissolved selenium is removed from water by treatment in a reactor containing microbial biomass to cause the hexavalent selenium to be converted to forms of selenium which can be readily removed from the water, and causing or allowing the removable forms of selenium to be removed from the water. Examples of removable forms of selenium include volatile organic selenium compounds, volatile inorganic selenium compounds, elemental selenium, entrainable organically-complexed selenium compounds, entrainable tetravalent selenium compounds, and entrainable bivalent selenium compounds. The volatile selenium compounds can be removed and recovered as a gas, while the entrainable forms of selenium can be entrained by larger particles and separated off, for example by filtration.

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REMOVING SELENIUM FROM WATER

Background of the Invention

The present invention relates to the removal of selenium from water, and more particularly but not exclusively to the treatment of drainage water containing selenium leached from soil.

Selenium is often present in water at concentrations of up to 0.5 mg/l, but a concentration of this order is undesirable for purposes such as drinking water or crop irrigation. For example, in the USA, the permitted maximum selenium level for drinking water is set at 10 µg/l. In some selenium-containing waters such as certain irrigation drainage waters, a substantial proportion of the selenium is present as the selenate anion, $[SeO_4]^{-2}$. These waters are especially difficult to treat because there is also a very high concentration of sulphate.

In the USA, particular attention has focussed on treatment of the agricultural drainage water in the San Joaquin Valley. This Valley has about 1.1 million acres of extremely productive land which is under irrigation. The land is generally low-lying and requires drainage in order to avoid high salinity in crop root zones. A major scheme to drain off subsurface water after use for irrigation has been frustrated by discovery of high levels of contaminants. The drainage water is brackish, and has a relatively high concentration of salts and potentially toxic elements, with selenium representing a particular problem. Environmental concern has led to a suspension

of construction work, a closure order on a large reservoir, and an extensive program of drain plugging. With the plugging of the drains, the water table is beginning to rise, causing concern for growers in the Valley.

Methods are available for the purification of water, but in general the known methods are not suited for use on a large scale with brackish drainage water containing selenium and a range of other contaminants. Reverse osmosis is costly, and so also is ion exchange. In this respect, it is to be borne in mind that the intial aim in treating the drainage water in the San Joaquin Valley and elsewhere is to render it suited for discharge. Hence, an economic process is paramount.

Methods are also described in the literature which are specifically concerned with the removal of selenium from water, but it is not apparent that such methods can be used economically on a large scale.

USA Patent 4405464 describes a process for the removal of selenium by a chemical treatment involving reduction of Se(VI) to Se(IV) using metallic iron which is itself oxidized and forms ferric hydroxide which then entrains the reduced selenium.

An article by Smith and Wiechers in Water SA (1981) 7, 65 describes elimination of toxic metals from waste water by an integrated wastewater treatment/water reclamation system. The system relies upon a combination of biological and chemical processes, including a bacteriological denitrification, clarification through formation of ferric hydroxide, and an anaerobic digestion.

An article by Gersberg and Elkins in Proceedings of Symposium on Selenium in the Environment, California State University, Fresno, June 10-12, 1985, describes selenium removal using an immobilized cell reactor containing Pseudomonas species immobilized in alginate. A selenium medium containing 1975 mg/l of selenium was recirculated through the reactor to give a final concentration of 1180 mg/l selenium. In further experiments with the Pseudomonas, removal efficiencies of 44 and 68 % were achieved.

Objects of the Invention

The primary intention of the present invention is to provide a method for removing selenium from water, in the sense of wholly or significantly reducing the selenium level. Especially desired is the removal of selenate from water containing high levels of sulphate, and in particular a method which may be operated economically on a large scale.

A more specific object is to provide a method of water treatment which might be adopted to remove selenium and other contaminants from drainage water such as occurs in the San Joaquin Valley.

Summary of the Invention

The present invention resides in a method for removing dissolved selenium from water. The method involves treating the water in a reactor containing a microbial biomass in which reducing activity can occur. The method is operated to cause the selenium to be converted to forms of selenium, including elemental selenium, which can be captured or entrained by larger particles. The discharge from the reactor can then be

processed to remove particles with captured selenium. Conversion of the selenium to filterable form is accompanied by conversion to volatile selenium compounds, typically including hydrogen selenide, dimethyl selenide and dimethyl diselenide. Such compounds can also be eliminated from the discharge of the reactor.

Thus, it has unexpectedly been discovered that biological treatment can readily convert dissolved selenate in a way which leads to massive reductions in the selenium level. The present method derives from an empirical discovery, and was not predicted.

By adoption of the present invention, it becomes possible to treat selenium-containing water to eliminate selenium, and other toxic elements, giving a water which is more readily managed for disposal or re-use. Indeed, a preliminary evaluation of a pilot plant installed in the San Joaquin Valley to treat contaminated water indicates that the present invention can yield a water, albeit a salty water, which could be discharged safely either to evaporation ponds or to salt water sinks. The inclusion of a sulphate-removal step, and further processing for desalting, can give a treated water which is acceptable for re-use.

The method of the invention is potentially more economic than alternatives known to the Applicants. The majority of the selenium can be removed and the water purified.

Description of the Preferred Embodiments

The water to be treated by the process of this invention is preferably supplied to the reactor supplemented with a nutrient for the biomass, especially an assimilable

carbon source. The water is suitably supplied in the substantial absence of free oxygen such that biological conversion proceeds anaerobically or anoxically. The reactor can take the form of a single- or multi-stage reactor, with suitable reactor types including fixed-bed reactors, fluidised-bed reactors, sludge-blanket reactors, and stirred reactors.

After carrying out the biological conversion, the selenium is in different forms, including selenium which has become organically bound (probably in the form of a soluble complex compound), selenium which has in some way been captured by larger particles, selenium which has been captured in some way by the biomass retained in the reactor, and typically also selenium in the form of volatile organic and inorganic compounds. The selenium which has been captured by larger particles is removed, for example by filtration, as discussed in more detail below. The volatile selenium compounds are caused or allowed to escape as gas from the water, suitably to a contained environment for safe disposal or recovery.

More specifically, in a preferred aspect, this invention resides in a method of removing dissolved hexavalent selenium from water in order to yield a purified water. The method suitably comprises treating the selenium-containing water in a reactor containing microbial biomass and a nutrient for the biomass, substantially in the absence of free oxygen, to cause at least part of the selenium to be captured by particles having a size of 0.1 micron or greater; and passing the discharge from the reactor through a filter in order to filter out particles which captured the selenium.

The instant method is suited for removing dissolved hexavalent selenium from water which contains a higher

weight concentration of nitrate than of hexavalent selenium (measured as selenium). In such a process, the concentration of nitrate in the water is lowered from say between 25 and 250 mg/l (measured as nitrogen, N) typically between 50 and 200 mg/l nitrate, to 5 mg/l or below, typically 2 mg/l or less; and the water is treated in a reactor containing microbial biomass to remove selenium. The lowering of the nitrate concentration can be effected using biomass, which may be the same biomass which is employed to remove selenium.

Furthermore, the instant method is especially suited for removing dissolved hexavalent selenium from water which contains a high concentration of sulphate, for example 500 to 10000 mg/l (measured as SO_4), typically 2500 to 5000 mg/l sulphate.

The method of the present invention is particularly intended for use in treating water which contains at least 0.05 mg/l selenium. Indeed, the water can contain at least 0.5 mg/l, and typically around 0.2 to 0.5 mg/l of selenium. The method of this invention can readily lower the selenium level to a more environmentally acceptable level. The selenium level is typically lowered to 10% or less of the original value, with 5% or less being preferred. For example, a selenium level of around 0.3 mg/l can be lowered to around 30 µg/l (that is, about 10% of the original value) using a suitably designed and operated reactor. A final treatment, for example with an ion exchange resin, can be employed to achieve the standard of 10 µg/l.

The reaction mechanism of the present process is not known, though it is possible that during fermentation extra-cellular amino acids form organic complexes with

the selenium and these are subsequently assimilated by the microorganisms in the biomass. Precipitation or co-precipitation may also occur. Thus the particles might contain one or more organic complexes, some of which may be in suspension or some in solution, or in general complex molecules or molecules with the selenium compounds absorbed or adsorbed on the surface, or colloidal particles, or rather larger microbial debris. The particles held back in a filter gave no reaction to the normal test for selenate ions, and had to be oxidised with acidic potassium permanganate in order to reform selenate ions. A proportion of the particles appeared to pass through a normal laboratory filter paper, even if the filter paper was first covered with precipitated aluminium hydroxide, special filters being required to remove this fraction of the selenium in reactor effluent.

The term "particles" is used in a general sense and includes particles which are invisible under the optical microscope, for example large molecules, and in this sense the above mentioned soluble complex compounds will also be particles. However, more specifically, as particles having a size of below very roughly 0.1 micron can pass through most practicable physical filters, the particles concerned may have a size of very roughly 0.1 micron or greater so that it is found satisfactory to use a filter of this retention size (though this is very imprecise). More generally, a minimum particle size of 10 microns, especially 1 micron, is desirable.

In the present process, the reactor can be operated so that sulphate is not removed or reduced. This is in spite of the fact that in drainage waters there may be at least 500 mg/l sulphate (measured as sulphate) and up to 12000 times more sulphate than selenate. The

process may be workable because in effect the selenate is attacked before the sulphate. Indeed, without being bound by theory, the presence of selenate in the water might exert a control on the redox reactions likely to occur in the biomass.

Selenate is a known competitive inhibitor of sulphate reduction, having a 40-fold greater affinity for the enzyme uptake system than the natural substrate, sulphate (Postgate J.R. (1984) The Sulphate-Reducing Bacteria, Cambridge University Press). This inhibition can confer certain advantageous features on the process as operated. If significant sulphate reduction is prevented then it is unlikely that the redox potential will drop sufficiently to make it advantageous for the bacteria to use Se(VI) as a hydrogen dump (Huang et al. (1982) Can. Tech. Rep. Fish. Aquat. Sci. 1163; and see also Jones et al. (1984) FEMS Microbiol Lett. 21, 133-36). The more mobile Se(IV) will not, therefore, be produced, and any interactive mechanism with bacteria is more likely to involve organic matter complexation and mobilization.

If desired, a sulphate removal step can be included in the process, after removal of selenium. Typically such sulphate removal can be achieved through anaerobic digestion to give insoluble sulphides and hydrogen sulphide.

The growth of the biomass needs to be promoted, typically by supplementing the selenium-containing water with nutrient. The nutrient feed can be incorporated in the water to be treated, or can be in a separate feed to the reactor. The nutrient can include an assimilable carbon source, such as a suitable readily biodegradable organic compound, for example methanol,

ethanol, sodium lactate, or a type of mixture of materials that may be present in many strong organic wastes. Some assimilable nitrogen and phosphorous may have to be added in order to generate and sustain the necessary biochemical activity, if they are not already sufficiently present in the water to be treated. The method ideally should be operated so that there is practically no nitrate-nitrogen nor organic nutrient left at the outlet. In the case of nitrate this appears to be desirable both for promoting removal of selenium, as well as for reducing the polluting potential of the final effluent due to nitrate per se. Remaining organic nutrient can be removed by well known methods, such as aerobic fermentation or, in the case of volatile nutrients, air-stripping.

In general terms, the bacterial biomass will be heterotrophic but will not be of a specific strain and is unlikely to be a pure culture; it will merely contain organisms growing from natural contamination, at least initially. Suitable bacteria are likely to include strains belonging to the genera Hyphomicrobium, Corynebacterium, Salmonella, Pseudomonas and Bacillus. There is no absolute need for special seeding of the reactor (owing to the presence of microbial organisms), but in practice it is possible to save time by seeding with sludge from a sewage treatment plant, preferably from an anoxic nitrate-removing reactor or an ordinary activated sludge reactor.

The bacteria will normally be tolerant of at least 1 mg/l of selenate, and probably be facultative anaerobes. If desired, suitable strains of bacteria can be selected by obtaining them from selenium-rich environments, such as selenium-polluted waters, but in practice this selection is not necessary. Thus, a

naturally occuring mixed flora such as occurs in sewage sludge provides an adequate source of bacteria: operation of the present method with supply of a selenium-containing water will lead to natural selection and growth within the reactor.

The reactor is preferably operated with no free or dissolved oxygen being present, oxygen only being available in combined form. There may be some oxygen initially, dissolved in the water. No coagulants or floculants, such as ferric chloride, need be added. A reducing agent, such as ferrous sulphate, hydrazine, sodium sulphide or sodium sulphite, could be introduced, preferably after the initial zone or for instance between two separate vessels defining different stages.

The throughput of the reactor will normally be selected to give loadings which are low enough to ensure that little nitrate penetrates through the reactor and also that there is a sufficient growth of faculative biomasss to convert the selenium. Since the maximum rate of growth of biomass is limited by the nitrate content of the feed, it may occasionally be necessary to add nitrate (and a corresponding complement of organic nutrient) to maximise the biomass. This can be facilitated by operating in two stages, the performance of the first stage giving guidance for "tuning" the second.

Although it is preferred to remove nitrates in an anaerobic or anoxic reactor, the nitrates can be removed in any suitable way prior to removing the selenium. It seems that the nitrate concentration must be relatively low (say 5mg/l or less) before the selenium is converted. Nonetheless, selenium removal is possible before all the nitrate has been removed. There appears

to be no definite upper limit to the content of nitrate that is acceptable in the feed water but it is advantageous to balance flows so that this concentration is at a reasonable uniform average of those likely to be encountered.

When operating a single-stage fixed-bed reactor in which the support medium is inert (such as gravel or crushed rock) it appears that unless there is less than around 1 mg/l of nitrate (measured as nitrogen) at the outlet from the reactor, there will be little conversion of the selenium to removable forms. The limit of 1 mg/l nitrate concentration has not been accurately determined, and can vary greatly according to the materials and conditions in the reactor. For instance, when using steel wool as the support, a nitrate concentration of 5 mg/l (measured as nitrogen) at the outlet can be tolerated (the total dissolved selenium concentration in the effluent being acceptable, say 0.014 mg/l (14 µg/l)) - the acceptable nitrate concentration may be even higher. If the nitrate concentration at the outlet is too high but there is adequate organic nutrient present, reduction in the rate of flow through the reactor normally allows the conversion of selenium to proceed.

In the preferred arrangements, one, two or three reactor stages are used for the selenium removal, or nitrate removal and selenium removal.

The preferred form of reactor has a fixed bed of support material. Initially it was found that when steel wool was used as the support in the reactor, the reaction rates appeared to be faster, the overall removal greater, and nitrate tolerance greater. Consumption of the steel wool was observed, and so the method may have

to be operated using reactors in parallel, one of which is being used and the other of which is being reloaded. Some of the iron thus goes into solution, but can be removed by for example a cross-flow filter. The effect of the iron remains uncertain, but it may act as a catalyst or may decrease the redox potential. Thus it may be possible to use other reducing agents, either in the form of solids or possibly as solutions injected into the feed to the first or second reactor stages.

The physical arrangement of the iron need not be that of steel wool, so long as the shape provides a high specific surface (high surface/volume ratio) - thus steel scrap or swarf may be usable. Relative to conventional universal media (e.g. gravel, rock, slag), some improvement would be expected from substrates such as plastic filaments which provided a higher specific surface to which the biomass would adhere.

For a fixed-bed reactor, in place of steel the support medium can be a substantially inert, inorganic wool such as glass wool or rock mineral fibre wool. The wool fibre diameter is preferably from 3 to 20 μm and a preferred packing density is from 70 to 300 kg/m^2. To date, the preferred diameter and packing density were about 10 μm and about 230 kg/m^2 bed volume.

Materials other than steel and glass wool or rock mineral fibre wool can be used for the support of the biomass in fixed-bed reactors; in general, it is merely desired that a combination of large specific surface and adequate void space should be provided. The void space suitably amounts to 20 to 40%, for example 25 to 35%, though higher amounts up to almost 100% can be used successfully. Further examples of currently preferred

support materials include stones, for example stones of 0.5 to 4, especially 1 to 3 inches (say, 1.25 to 10, especially 2.5 to 7.5 cm).

It is possible that the selenium removal occurs efficiently because the preferred bed is not homogeneous. In a packed bed, which is not homogeneous, there will be various levels of reducing activity (redox potential) within the bed. In other words, there is probably a plurality of zones in which different reactions take place in the reactor. For reasons already indicated it is most convenient to use a two- or more stage reactor (with separate vessels defining the stages). With an inert-bed reactor, the nitrate apparently has to be removed first in order for selenium to be converted, and the initial stage can be operated for the most efficient nitrate removal to allow selenium conversion then to take place. The subsequent stages can be manipulated to grow further biomass to maximise conversion, especially entrainment or capturing in the form of suspended particles. Additional feed can be provided to the second stage. This feed can include nitrate, which does not reverse previous reactions in which selenium was captured but allows further growth of biomass, and thus allows improved capturing to take place.

Fluidised-bed reactors, sludge-blanket reactors, stirred reactors and other reactor types can be used to carry out the process, as alternatives to fixed-bed reactors.

Any of the reactor stages may be in the form of fluidised beds using a particulate material for example on which the biomass forms a coating. Normally, upward-flow beds would be used (particulate material heavier than water), though it is possible to use

downward-flow beds (particulate material lighter or heavier than water). In experimental work, a six inch diameter (150 mm) fluidised bed was used for fluidising, with sand as the particulate material. In order to recover the particulate material which is carried over, the arrangement used at the Coleshill, England, experimental plant may be used, as reported in the Paper "Sand/Biomass Separation with Production of a Concentrated Sludge" by Cooper et al., presented at a Water Research Centre/UMIST conference entitled "Biological Fluidised Bed Treatment of Water and Wastewater" at Manchester, April 14-17, 1980. However, fluidising has advantages and disadvantages, one disadvantage being the extra power required. Continuously-stirred reactors may be just as economic in practice.

Cross-flow or other filtration can be used to produce concentrated biomass after selenium removal. A preferred type of filter is a cross-flow microfilter, preferably with an aluminium hydroxide or zirconium hydroxide membrane, for instance as described in PCT specification PCT/GB86/00143 and corresponding patent applications claiming priority of GB Patent Application 8506350. It has been noted that selenium included in larger particles such as microbial debris is retained on a sand filter. It therefore appears possible to operate the method so that the major part of the non-volatile selenium is included in such debris or in general in suspended particles, in which case a simple sand filter or filter of equivalent size can be used.

The concentrate from the filter can be passed back into the selenium-removal reactor. This enables one to raise the concentration of biomass (expressed as a percentage weight per unit volume, the biomass being weighed dry)

in the reactor. For example, in a reactor in which biomass is separated by settlement, the concentration is usually limited to about 0.06%, and using cross-flow microfiltration for the return of concentrate in a third reactor, the concentration may be 4% and probably higher; in the latter case, large proportions of sludge are recirculated in the concentrate.

It has been noted that nitrogen bubbles in the reactor, and in theory the nitrogen may contain some hydrogen selenide. It may be possible (especially by addition of suitable reducing agents to the feed) to operate the method so that most of the selenium comes off as hydrogen selenide. In such circumstances, none of the selenium, or only a small proportion of the selenium, would be included in larger particles, and the filtration or equivalent separation step might be eliminated. Equally, elimination of the separation step might apply to the production of volatile organic selenium compounds.

It is believed that the filtered outflow can be further treated by aeration with activated-sludge fed with a sugar such as glucose (or any suitable biodegradable organic compound) as a nutrient, to further reduce the dissolved selenium.

Accordingly, the invention also provides a method of removing selenium from water by treating the selenium-containing water in a single- or multi-stage reactor containing microbial biomass and a nutrient for the biomass, in the substantial absence of oxygen, and then treating the water in a single- or multi-stage reactor containing microbial biomass and a nutrient for the biomass, in the presence of oxygen. As above, the discharge from the end reactor can be filtered, and the

discharge from the each preceding reactor will normally be filtered.

Subsequent to selenium removal, further steps are possible, particularly if other contaminants are present. Ion-exchange can be used for a polishing, for instance for boron removal. It is also possible to arrange for boron to be removed in cross-flow filtration using suitable membranes.

The selenium itself (elemental or compounds thereof) is a useful byproduct of the invention.

Applying the principle of the invention, it is also possible to reclaim selenium compounds from organically-complexed mixtures in sludge from treatment plants or from reservoirs; specifically in the latter case, this enables mud on the bottom of the reservoir to be purified of selenium, and the selenium itself can be re-complexed using the invention and recovered as a selenium-rich sludge or as selenium compound.

The Examples

The present invention will now be illustrated by reference to some non-limiting Examples, in which reference is made to the accompanying drawings.

Description of the Drawings

Figure 1 is a schematic diagram of a plant for carrying out a method in accordance with the invention.

Figure 2 is a schematic diagram of a another plant for carrying out a method in accordance with the invention, where removal of sulphate and boron is desired as well

as removal of selenium.

Referring to Figure 1, selenium-containing water from conduit 1 supplemented with methanol-based nutrient from conduit 2 pass through two identical reactor stages 3, 4 (same cross-section and same contents), preferably with no recyling of feed water, and thence through a small reactor 5, a pump 6 and a cross-flow microfilter 7. Purified water is discharged through conduit 8 and concentrate for waste disposal is discharged conduit 9, part of which can be returned as active biomass through a return line 10 to the reactor 5.

The reactor stages 3, 4 are fixed film reactors and are filled near to the top with approximately 1 inch (2.5 cm) gravel (40% void space). The up-flow mode is preferred, as shown. The stages 3, 4 and the reactor 5 are seeded with activated sludge prior to start-up. The stages 3 and 4 are operated anaerobically or anoxically. The reactor 5 has a sparge tube 11 for the addition of air in order to strip off surplus methanol supplied in the nutrient. The reactor 5 thus contains aerobic activated sludge.

As indicated by the valving, the stage 4 may be by-passed if the selenium content is considered to be sufficiently low at the outlet from the stage 3. Alternatively, the stage 4 may be omitted altogether.

Referring now to Figure 2, like references are used for like parts. Pumps are indicated throughout as plain circles. Effluent is abstracted from a main drain 21 and is passed through biological reactor stages 3, 4 in series. These can be fixed film reactors as in Figure 1, or one or both of them can be a stirred reactor or a fluidised bed reactor. The outflow from the stage 4 is

filtered in a cross-flow microfilter 7. The selenium concentrate is fed through conduit 9 and the liquid passing through the filter 7 is pumped into a reactor 22. At some point between the effluent feed and the inlet to the reactor 22, a carbon dose is added as a biomass nutrient. The reactor 22 operates anaerobically to destroy sulphates and to convert heavy metals to insoluble sulphides for collection in a second cross-flow microfilter 23. Sulphates destroyed in the reactor 22 generate hydrogen sulphide gas which is passed into a photosynthetic reactor 24, and elemental sulphur is produced. From the microfilter 23, the heavy metal sulphides are removed through conduit 25 and the permeate passes through a collection tank 26 to an ion exchange unit 27 for boron removal. Treated water flows out at pipe 28.

Example 1

A synthetic water, similar in composition to the average for farm drainage waters in the San Joaquin Valley, California, but with added phosphate, was treated using the plant illustated in Figure 1. In this and the following Examples, the pH of the feed water varied, but was always between 7.8 and 8.4. Relevant parts of the chemical analysis for this Example 1 were:

30 mg/l nitrate (expressed as nitrogen);
0.36 mg/l selenium (as the selenate ion, expressed as selenium);
4000 mg/l sulphate (expressed as sulphate);
20 mg/l phosphate (expressed as phosphorus).

The flow rate was 3 l/hr. The nutrient feed for the biomass was 5% methanol at a flow rate of 0.03 l/hr. For this Example, and Examples 2 and 3, the initial biomass was anaerobically digested sludge taken from the

sewage works of the Thames Water Authority at Beddington, England. The reactor stages 3, 4 were operated at ambient temperature, about 20°C. The residence time in each of the reactor stages 3, 4 was about 3.0 hours. Each reactor stage 3, 4 had a voids volume of 10 litres.

The outflow was sampled at the exit of each of the reactor stages 3, 4. The liquid was filtered on a laboratory filter of about 5 micron pore size, the filtrate being dissolved or colloidal organic material with selenium and also dissolved inorganic selenium. The amount of dissolved inorganic selenium was determined. Further selenium was either retained in the reactor stages 3, 4 with the biomass (not measured) or evolved as gaseous selenium compounds (not measured).

With the flow rates indicated above, which are believed to be close to the optimum flow rates, there was a period during which biomass was generated and the efficiency of the reaction improved. At the end of the period, the relevant contents of the water at the outlet from each reactor stage 3, 4 were (mg/l):

	Reactor stage 3	Reactor stage 4
Selenium in filtrate	0.06	0.05
inorganic	0.005	0.005
organic complexed	0.055	0.045
Held back on filter	not measured	0.06
Total Se in outflow	not measured	0.11
Nitrate (as N)	0.6	0.4
Sulphate (as SO_4)	4000	4000, unchanged

The total held back and hence the total selenium in the outlet of reactor stage 3 were not measured.

Example 2

Treated water from a two-stage reactor as in Example 1 was filtered through glass-fibre filter paper in order to remove suspended particles containing selenium (which in practice could be done on cross-flow filter 7 or by sand filtration). The water was aerated for an hour with activated sludge fed with glucose. By appropriate variation in the operating conditions, the supernatant after settling the sludge contained only 6 µg Se/l compared with 30 µg/l dissolved Se in the effluent from the two-stage reactor.

Example 3

One laboratory reactor column was used in the up-flow mode, filled half-way with steel wool. The column cross-section was 2000 mm^2 and the volume filled with steel wool was 0.25 litres. The steel wool was a commercially available material. The threads of the wool were of finishing grade 4 or 5, having a diameter of 0.1 to 0.15 mm. The wool was made from a low carbon steel of the rimming variety, not silicon-killed, the chemical composition being stated to be with the limits (% by weight): C 0.8-1.5; Mn 0.3-0.5; S 0.05 or more; P 0.05 or more

The steel wool was inserted into the columns so as to fill the barrel of the column reasonably uniformly but without undue compaction of the wool. This gives a medium having enough void space to allow water to pass through readily but at the same time did not contain large passages which would permit short-circuiting and thus poor contact with the wool surfaces. The bulk density when in place is 60 ± 10 kg/m^3.

A feed of 250 ml/hr was passed through the vessel (i.e. retention time one hour), the feed having the following composition (mg/l):

SO_4	4000
Cl	1500
Alkalinity (as $CaCO_3$)	180
Nitrate (as N)	30
Phosphate (as P)	4
Borate (as B)	10
Selenate (as Se)	0.32
70% lactic acid (nutrient)	5

Samples of the effluent water were taken over a period of 14 days. Results of analyses are as follows (concentrations in mg/l):

Time after start up (days)	Selenium (as Se) Inorganic	Total Soluble	Nitrate (as N)
1	0.22	0.26	-
9	0.005	0.025	-
10	0.006	0.016	7.0 (anomalous)
13	0.004	0.030	-
14	0.006	0.017	-

Example 4

Subsurface drainage water at Murietta Farms, Westlands Water District, near Mendota, California, USA, was treated using the plant of Figure 2. The biomass was obtained from a local sewage works.

Before treatment, the water analysis showed 109 ppm NO_3 (measured as nitrogen), 3000-4500 ppm SO_4, 1800 Cl, 6 ppm B, 0.06 ppm Cr, and 0.35-0.45 mg/l Se, among other contaminants. After treatment, the selenium

level was down to 3 to 5 ppb, along with impressive reductions in the level of nitrate, sulphate, heavy metals and boron.

Example 5

A plant was constructed at Murietta Farms with three reactor stages and a cross-flow microfilter. The design capacity was 40 m^3/day. Each reactor had a volume of about 7 m^3. The first and second reactors were filled with stones of diameter 1.5 to 2 inches (say 3.8 to 5 cm), giving 35 % porosity, while steel wool was used to fill the third reactor.

The drainage water as in Example 4 was fed at 30 l/m and supplemented with a nutrient comprising concentrated Steffen's waste liqour (a waste liqour from a sugar beet factory). Phosphate was added to the concentrated Steffen's waste liquor at the level of 2 mg/l, and the waste liquor was fed at a rate corresponding to 187 mg carbon per litre of drainage water.

Redox potentials were measured in order to assess the operating modes of the three reactors. The drainage water itself typically showed a redox potential in the range -20 to +20 mV, while the outflow from the first, second and third reactors were respectively in the ranges -100 to -140 mV, -140 to -175 mV, and -200 to -300 mV.

The treated water from the third reactor showed less than 1 ppm nitrate and less than 30 ppb selenium. The water from the cross-flow microfilter contained less than 15 ppb selenium, and was passed through a resin exchange column for a final polishing, giving an effluent containing less than 10 ppb selenium.

Operation was sustained for more than 3 months.

Example 6

Further modification of the plant employed for Example 5 is envisaged, particularly in view of the recent realisation that some of the selenium in the drainage water is being converted in the reactors to volatile organic selenium compounds such as dimethyl selenide or dimethyl diselenide. Such compounds represent a useful source of selenium, and can readily be recovered by appropriate containment design of the plant. More generally, the aim is to construct a plant capable of treating 1 million US gallons per day.

We Claim:

1. A method of removing dissolved hexavalent selenium from water which contains a higher weight concentration of nitrate than of hexavalent selenium, comprising:

reducing the concentration of nitrate in the water to 5 mg/l or below (measured as nitrogen);

then treating the water in a reactor containing microbial biomass and a nutrient for the biomass, in the substantial absence of free oxygen, to cause the selenium to be captured as particles larger than the forms of selenium originally present; and

passing the discharge from the reactor through a filter in order to filter out particles with captured selenium.

2. The method of Claim 1, wherein said water contains a high concentration of sulphate which is substantially greater than the concentration of selenium, and in which the method is operated so that the sulphate concentration is essentially maintained.

3. The method of Claim 1, wherein nitrate is added to said water, the nitrate concentration subsequently being reduced prior to causing the selenium to be captured by said particles.

4. A method of removing dissolved selenium from water, comprising treating the selenium in a reactor containing microbial biomass in a non-homogeneous bed in which various differing levels of reducing activity occur, and supplying a nutrient for the biomass, in the absence of free oxygen, to cause the selenium to be captured by particles larger than the forms of selenium originally

present; and

passing the discharge from the reactor through a filter in order to filter out particles with captured selenium.

5. A method of removing dissolved hexavalent selenium from water, comprising:

treating the hexavalent selenium-containing water in a reactor containing microbial biomass to cause the hexavalent selenium to be reduced to forms of selenium which can be readily removed from the water; and

causing or allowing the removable forms of selenium to be removed from the water.

6. The method of Claim 5, wherein said reactor comprises at least two stages.

7. The method of Claim 5, wherein said hexavalent selenium is converted in to a removable form of selenium selected from the group consisting of recoverable volatile organic selenium compounds, recoverable volatile inorganic selenium compounds, elemental selenium, entrainable organically-complexed selenium compounds, entrainable tetravalent selenium compounds, entrainable bivalent selenium compounds, and mixtures thereof.

8. The method of claim 5, wherein said water containing hexavalent selenium also contains nitrate in the range from 25 to 250 mg/l (measured as nitrogen), and said step of treating said water with said biomass gives water containing 2 mg/l or less nitrate.

9. The method of claim 8, wherein said water containing

hexavalent selenium also contains from 500 to 10000 mg/l sulphate.

10. The method of claim 8, wherein said water containing hexavalent selenium contains 0.2 to 0.5 mg/l selenium.

11. The method of claim 10, wherein the step of treating said water with said biomass gives water containing selenium at 10 % or less of the original concentration.

12. The method of claim 5, wherein the treated water contains 30 µg/l or less of selenium.

13. A method of removing dissolved selenium from water, comprising:

treating the selenium-containing water in a single- or multi-stage reactor containing microbial biomass and a nutrient for the biomass, in the absence of free oxygen;

treating the discharge from the reactor in a single- or multi-stage reactor containing microbial biomass and a nutrient for the biomass, in the presence of free oxygen; and

filtering the discharge from the latter said reactor.

14. A method of removing dissolved selenium from water, said method comprising the steps of:

providing a reactor containing microbial biomass capable of converting dissolved selenium to a removable form of selenium;

rectifying any nutritional deficiencies in said water to render it nutritionally adequate and capable of

sustaining said biomass;

treating said nutritionally adequate water with said biomass in said reactor, said treating step causing said selenium to be converted to at least one removable form, and

removing said removable selenium from said water.

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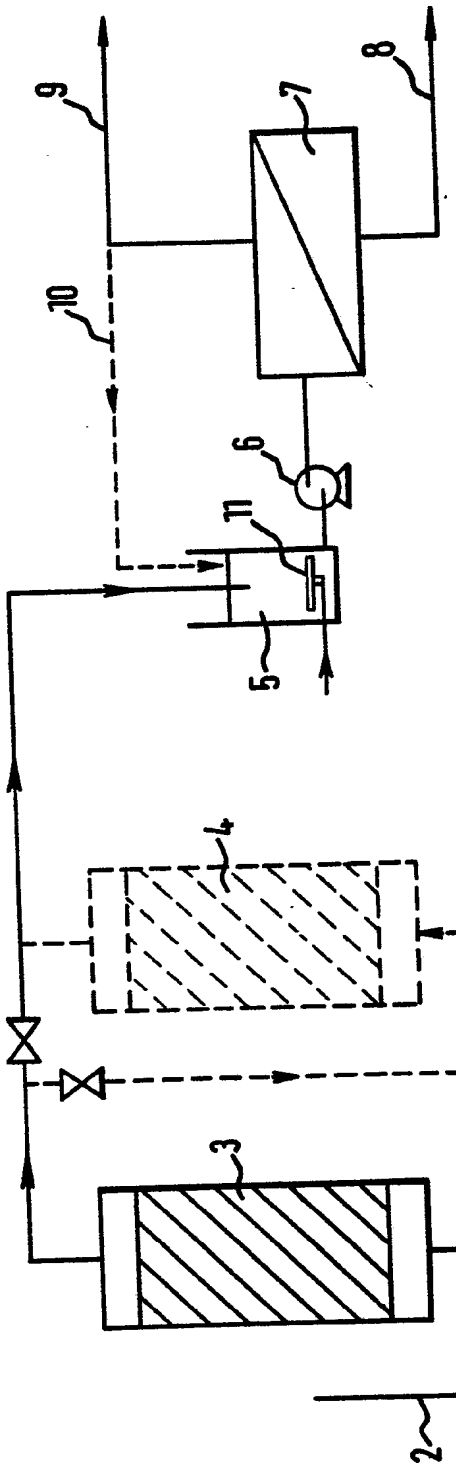


Fig.1

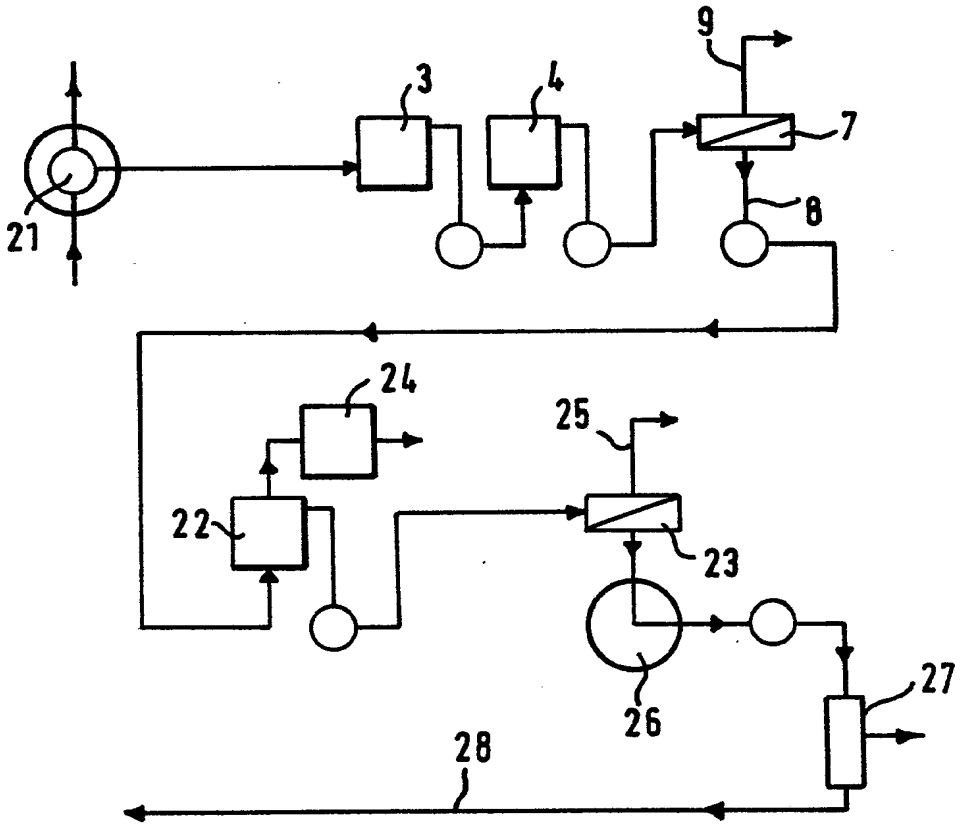


Fig. 2.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00404

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 02 F 1/58; C 02 F 3/28; C 01 B 19/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 02 F C 01 B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	GB, A, 1438041 (PER STAFFAN DELIN) 3 June 1976 see page 5, claims 1-3; page 2, lines 95-117	1,2,5,7,13
A	Chemical Abstracts, volume 99, no. 6, 8 August 1983, (Columbus, Ohio, US), C.L. Brierley et al.: "Biological processes for concentrating trace elements from uranium mine waters", see page 433, abstract no. 45215j, & Report 1981, WRRI-140, 99 pp.	1
A	EP, A, 0110240 (LINDE) 13 June 1984 see claims 1,2,7	1,4
A	DE, C, 666060 (H. DICKMANN) 22 September 1938 see page 2, claims 1,3	1

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
9th October 1986	31 OCT 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 86/00404 (SA 13885)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 15/10/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A- 1438041	03/06/76	FR-A,B	2207871	21/06/74
		DE-A-	2357735	12/06/74
		JP-A-	50158145	20/12/75
		SE-B-	373116	27/01/75
EP-A- 0110240	13/06/84	DE-A-	3244483	07/06/84
DE-C- 666060		None		

For more details about this annex :
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2,438,432	22 August 2003 (22.08.2003)	CA
2,438,050	22 August 2003 (22.08.2003)	CA
2,438,101	22 August 2003 (22.08.2003)	CA

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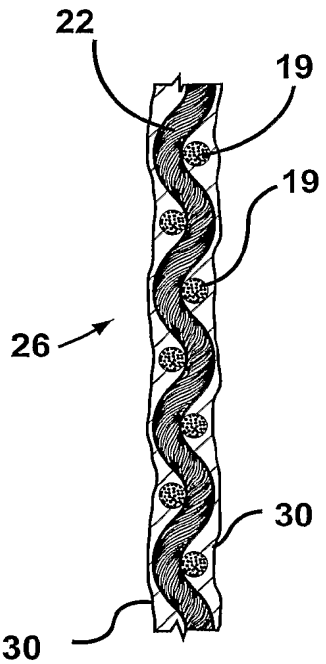
(74) Agent: **BERESKIN & PARR**; 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK,

[Continued on next page]

(54) Title: SUPPORTED BIOFILM APPARATUS AND PROCESS



(57) Abstract: A membrane supported biofilm reactor uses modules having fine, hollow fibres (19), for example, made from dense wall Poly methylpentene (PMP) used in tows or formed into a fabric (26). In one module, one or more sheets of the fabric are potted into a module to enable oxygen containing gas to be supplied to the lumens of the hollow fibres. Various reactors and processes, for example to treat wastewater, using such modules are described. Mechanical, chemical and biological methods are used to control the thickness of the biofilm (30).

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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Title: SUPPORTED BIOFILM APPARATUS AND PROCESS

[0001] For the United States of America, this application is an application claiming the benefit under 35 USC 119(e) of U.S. Provisional Patent Application Serial No. 60/447,025 filed February 13, 2003 and U.S Provisional Application Serial No. 60/496,178 filed August 18, 2003. For other countries, this application claims priority from those two applications. This application also claims priority from Canadian Patent Application Nos. 2,438,441; 2,438,432; 2,438,050; and, 2,438,101 all filed August 22, 2003.

Field of the invention

[0002] This invention relates to a gas transfer apparatus and process, for example to support a biofilm in a liquid, as in a water or wastewater treatment process or apparatus.

Background of the invention

[0003] Currently, most wastewater treatment plants use an activated sludge process, based on biological oxidation of organic contaminants in a suspended growth medium. Oxygen is supplied from air using bubble type aerators. Efficiency of these systems is poor resulting in very high energy use. Tank size is large since oxygen demand loadings are low. The result is high capital and operating cost.

[0004] A second type of established biological oxidation process uses biofilms grown on a solid media. For example, the wastewater may be circulated to the top of the reactor and trickles down. Air is supplied at the bottom. The rate of oxygen transfer is limited by the biofilm surface area, and the operating cost is high because of wastewater pumping requirements.

[0005] Recently, development work has been done on a membrane supported bioreactor concept. For example, US Patent Nos. 4,181,604 and 4,746,435 describe a process for treating wastewater by supplying oxygen from one side of a gas-permeable membrane to micro-organisms growing on the other side of the membrane. Hollow fibers with porous walls were used as the membrane. In US Patent No. 5,116,506, a gas permeable membrane

divides a reactor vessel into a liquid compartment and a gas compartment. A biofilm is grown on the gas permeable membrane on the liquid side of the membrane. Oxygen and alternate gases pass through the membrane to the bacteria growing on the liquid side of the membrane.

Summary of the invention

[0006] It is an object of this invention to improve on the prior art. It is another object of this invention to provide methods and apparatus suitable for treating water, for example industrial and municipal wastewater, using membrane supported bioreactor technology. It is another object of this invention to provide a hollow fibre gas transfer membrane and module which is, for example, suitable for supporting a biofilm. These aspects and others are met by the invention described and claimed herein. The following summary will introduce the reader to various aspects of the invention but is not intended to define the invention which may reside in a combination or sub-combination of various elements or steps found in the following summary or other parts of this document.

[0007] In one aspect, the invention provides a membrane and module with a reasonably high gas transfer rate and adequate surface area, for oxygen transfer, biofilm support or both, to allow a membrane supported biofilm reactor to provide an operating cost advantage over other processes used in the art. The membrane and module may have an oxygen transfer efficiency (OTE) of over 50% or in the range of 50% to 70% or more. The module may be made of non-porous or dense walled hollow fibre membranes to provide a large surface area while avoiding the tendency of porous fibers to wet over time which results in a drastic drop in their oxygen transfer rates.

[0008] In another aspect, the invention provides a very fine dense hollow fibre made from poly methylpentene (PMP), which has a high selectivity and diffusion coefficient for oxygen. In particular, PMP has a gas permeability of about 70,000 cc•mm/m^2•24hr•Bar in dense wall, non-wetting form. While this is significantly less than silicone, which has an extremely high gas permeability, PMP may be melt spun into a hollow fibre. The fiber can

have an outside diameter of 500 microns or less or 100 microns or less. Use of such a small diameter fibre helps reduce module cost as textile fine fibre technology can be used to create modules. A very large surface area can be provided to achieve high OTE. The non-porous wall avoids wetting problems as described above.

[0009] In another aspect, the invention provides a fabric with a very large number of hollow fibres, for example of PMP, providing sufficient surface area so that oxygen transfer does not become a limiting factor in controlling biological kinetics. The fabric may be made, for example, with the hollow fibres, optionally collected into units, woven as weft and an inert fibre as warp to minimize the damage to the transfer fibre while weaving. Other methods of preparing a fabric may also be used. The fabric provides strength to the fine fibre to permit biofilm growth on its surface with minimal fibre breakage.

[0010] In another aspect, the invention provides a module built from fabric sheets with very high packing density to permit good substrate velocities across the surface without recirculation of large volume of liquid. The modules enable a supply of oxygen containing gas, such as air, to be supplied to the lumens of the hollow fibres without exposing the lumens to the wastewater. Long fibre elements, for example between 1 and 3 metres or between 1.5 and 2.5 metres are used and potted in the module header to provide a low cost configuration.

[0011] In another aspect, a biofilm is grown on a fabric made from a gas permeable hollow fibre, for example PMP dense wall hollow fibre. Oxygen bearing gas is introduced into the lumen of the fibre. Aerobic reactions take place near the surface of the fibre, where the highest levels of oxygen exists. These reactions include conversion of organic carbon compounds to carbon dioxide and water, and ammonia to nitrates. The surface of the biofilm is maintained under anoxic conditions such that conversion of nitrates to nitrogen can take place. The result is simultaneous reduction of organic carbon, ammonia and total nitrogen.

[0012] In another aspect, the invention uses oxygen enrichment as a means of dealing with peak flows. Need for such oxygen enrichment may be determined by on-line COD monitors, or set according to time of day for, for example, municipal applications where diurnal flow and strength variations are well known.

[0013] In another aspect, the invention uses the module and bioreactor design to conduct other biological reactions on the surface of the fabric. An example is biological reduction of compounds such as nitrates in water using hydrogen gas supplied to the lumen of the hollow fibre.

[0014] In another aspect, the invention uses either air or enriched air to supply oxygen. Selection of enriched air and level of oxygen present in such air may be determined by the wastewater strength.

[0015] In another aspect, the invention may be used to digest primary and/or secondary sludge.

[0016] In another aspect, the fibres may have a small outside diameter, such as 100 μm or less, and substantial hollow area, for example 30% or more or 40% or more, so as to have a thin wall. The fibres can be woven, knitted, stitched or otherwise made into a fabric. The use of fine hollow fibres allows the thickness of the fibre wall to be low, for example 20 μm or less, which is several times less than what would be required to make a film handleable. The fine fibres may themselves be difficult to handle on their own, but may be combined into units such as threads or tows for handling which may include forming textile sheets. The fabric, having a large number of hollow fibres, provides sufficient surface area for oxygen transfer capability such that air can be used as a feed gas without limiting the growth of the biofilm or other biological kinetics and with acceptable pressure loss due to air flow through the module.

[0017] In another aspect, plug flow or multistage continuous stirred or batch tank reactors may be used to conduct biological reactions at the highest possible substrate concentrations for a given feed. This maximizes mass

transfer of organic carbon compounds and ammonia in the biofilm, eliminating these processes as potential limitations to reaction rates. In multi-stage reactors, module designs with lower surface areas for oxygen transfer to biofilm surface area ratios may be used in downstream stages. The total surface area for oxygen transfer, for example per unit of tank volume or flow rate of feed, may increase or decrease in the downstream reactor since the lower ratio may result from an increase in biofilm surface area rather than a decrease in surface area for oxygen transfer.

[0018] In another aspect, the invention provides a membrane supported batch biofilm reactor (MSBBR). The reactor includes one or more membrane modules which are fed an oxygen containing gas and support a biofilm layer. The modules are located inside of a tank that is cyclically filled and drained to provide a batch treatment process. In an embodiment, the modules are made of a hollow fibre fabric and are used to reduce the COD, ammonia, total nitrogen and suspended solids in an industrial wastewater to concentrations suitable for discharge into a municipal sewer system or for direct discharge to a receiving stream. In another embodiment, the modules are used to reduce COD, ammonia, total nitrogen and suspended solids in a municipal wastewater stream for direct discharge to a receiving stream. In another embodiment, the modules are used to reduce COD, ammonia, total nitrogen and suspended solids in a septic tank to reduce the size of the septic field or to use simpler, lower cost disposal techniques or for direct discharge to a receiving stream.

[0019] In another aspect, the invention provides one or more methods of controlling the growth or thickness of a biofilm layer growing on the modules. Some method(s) involve applying one or more substances to the biofilm from the tank side while the tank is drained of feed. These substances may include gases, such as ozone or chlorine, or liquid such as heated water or basic or acidic solutions. During the application of the control substance, conditions in the biofilm may be cycled from aerobic to anaerobic by turning the supply of oxygen to the inside of the module on and off. The biofilm may

also be starved prior to the application of the control substance by removing the feed water, replacing the feed water with clean water or replacing the feed water with feed at a loading of 0.1 kg COD per kg MLSS per day or less. After the application of the control substance, mechanical biofilm control methods may also be used on the weakened biofilm.

[0020] In another aspect, this invention uses scouring air provided on the outsides of the fibres as a means of controlling the biofilm thickness to an optimum level. Air may be used as a means of controlling the biofilm thickness to a desired level. Treatment with acid, alkali, oxidant, or enzyme, or anaerobic treatment may be used periodically prior to air scouring to weaken the biofilm and to improve the efficacy of air in completely or partially removing the biofilm. Other methods of biofilm control include in-situ digestion, periodic ozonation followed by digestion, periodic alkali or acid treatment followed by digestion, periodic enzyme treatment followed by digestion, and use of a higher life form, such as worms, to digest the biofilm periodically. To speed up the biological digestion reactions, the air supplied to inside of the module may be preheated to raise the temperature of the bioreactor.

[0021] In another aspect, the invention provides a tow of hollow fibers, for example with an outside diameter (OD) of 500 microns or less or 100 microns or less. To facilitate building modules with minimal reduction in the effective surface area of the fibres, the fibres are processed or used as tows over a significant portion, for example one half or more, of their length. Modules may be made directly from the tows without first making a fabric. The tows may also be made into open fabrics to facilitate potting, for example along the edges of the fabric, while leaving significant portions of the fibres as tows, for example a portion between the edges of the fabric. The modules made from tows may be potted at both ends, or potted at one end only with the other end left unpotted with fibre ends open to permit exhaust gas to escape. A single header module may have lower cost than a double header module. A single header module may be inserted in a vertical configuration

with the header at the bottom and the fibres floating upwards. Such a module may be aerated from outside the module to remove accumulations of trash and solids. Feed may also be screened, for example through a 0.5 mm screen, to reduce trash in the feed before it enters the reactor. Where the tow module is used in a downstream stage of a multi-stage reactor, the upstream stage may also reduce the amount of trash fed to the tow module reactor.

[0022] In another aspect, reactors for treating wastewaters of different strength are provided with modules having different ratios of surface area for gas transfer to surface area of the attached biofilm. The surface area for gas transfer is the area of the outer surface of the module that is in contact with the supported biofilm. The surface area of the biofilm is the area of the outer surface of the biofilm that contacts the wastewater. Is some cases, the surface area of the biofilm depends on the thickness of the biofilm which, for calculations or for comparing modules, may be the actual thickness or time average of thicknesses of a biofilm in a rector or a nominal or design thickness or average thickness, for example 250 microns. A reactor for treating wastewater with a COD of over 1000 mg/L may have a module with a surface area for gas transfer to surface area of attached biofilm ratio of more than 1, more than 1.6, or between 1.6 and 10. A reactor for treating wastewater with a COD of less than 1000 mg/L may have a module with a surface area for gas transfer to surface area of attached biofilm ratio of less than 2.5 or between 0.2 and 2.5. A reactor for treating wastewater with a COD of less than 300 mg/L may have a module with a surface area for gas transfer to surface area of attached biofilm ratio less than 1 or between 0.1 and 10. In a multi-stage process, two or more reactors may be connected in series with the outlet of an upstream reactor connected to the inlet of a downstream reactor. The COD of the wastewater to be treated decreases through each reactor and the surface area for gas transfer to surface area of attached biofilm ratio for modules in a downstream reactor is less than for modules in an upstream reactor.

[0023] Other aspects of the invention are described in the claims or in the following drawings or description.

Brief description of the drawings

[0024] Embodiments of the invention will be described below with reference to the following figures.

[0025] Figure 1 is a picture of a group of hollow fibres.

[0026] Figure 1a is a cross-section of a hollow fiber.

[0027] Figure 1b shows a group of hollow fibers and inert fibers collected into a unit.

[0028] Figures 2a through 2d and 2e show slot arrangements and a spinneret for melt spinning fibers.

[0029] Figures 3a and 3b show a plan view and cross-section of a woven fabric respectively.

[0030] Figure 3c shows steps in weaving a fabric.

[0031] Figures 3d shows a warp knitted fabric.

[0032] Figure 4a shows a sheet of hollow fibres with a central portion of the sheet having the fibres in tows. Figure 4b shows details of a part of the sheet of Figure 4a.

[0033] Figure 5 is a cross-section of a loose tow module.

[0034] Figure 6 shows a top view of a module having sheets of fibres.

[0035] Figure 7 is a partial section, in elevation view, of the module of Figure 6.

[0036] Figure 8 is a cross-section of another part of the module of Figure 6 in plan view.

[0037] Figure 9 is an elevation view of a module according to Figures 6 and 7.

[0038] Figures 10a, 10b and 10c are elevation, plan and partial section views of another module having sheets of fibres.

[0039] Figures 11 and 12 are plan and elevation views of a tank having cassettes of modules of sheets of hollow fibres.

[0040] Figure 13 is a drawing of the details of a tensioning mechanism in the apparatus of Figures 11 and 12.

[0041] Figure 14 is an elevation view of the mechanism of Figure 13.

[0042] Figures 15 and 16 are schematic elevation drawings of reactors.

[0043] Figures 17 and 18 are schematic drawings of other reactors.

[0044] Figure 19a is a bench scale batch reactor using a tow module.

[0045] Figure 19b is a photograph of a biofilm on a tow of fibres growing in the reactor of Figure 19a taken through a microscope.

[0046] Figure 20 is a schematic elevation drawing of a septic tank modified to use a supported biofilm module.

[0047] Figures 21 to 31 are results of tests conducted with various sample modules or reactors.

Description of Embodiments

1.0 Module Elements

1.1 Fiber

[0048] Figures 1 and 1a show a Poly (4-methylpentene-1) (PMP) fiber 10 that is hollow inside but non-porous with dense walls. In a group of fibers 10, the fibers 10 may have various diameters, and may be fine fibers having outside diameters of less than 500 microns or less than 100 microns, for example, between 30 and 100 microns, or between 50 and 60 microns. The hollow fibres 10 shown are non-porous, or dense walled, and water does not flow through the fiber walls by advective flow. However, oxygen or other gases may permeate or travel through the fiber walls, for example by molecular diffusion or dissolution-diffusion.

[0049] The hollow fiber 10 can be prepared by melt spinning, alternately called melt extrusion. In melt spinning a polymer granulate, for example of PMP, is fed to the hopper of an extruder. The polymer granulate is heated and melted in the extruder and continuously extruded to a spinning head under a pressure of several tens of bars. The spinning head consists of a heated in-line filter and spinneret. The spinneret is essentially a steel plate with thin arc shaped slots in circular arrangements. Examples of suitable slot arrangements for the formation of a hollow fiber are shown in Figures 2a to 2d. As shown in Figure 2e, the spinneret may have multiple groups of slots so that many fibers, 8 in the spinneret shown, can be extruded simultaneously. The molten polymer is extruded through the spinneret, leaves the slots and closes into a hollow fiber in a cooling zone. The gaps caused by the segment dividers allow air into the fibre to prevent collapse before the fibre sections fuse to form the annulus. In the cooling zone, the polymer fiber form is solidified and cooled by a controlled cross flow of air and the end is collected on a take up winder. Suitable fibers 10 may also be formed by other melt spinning methods. For example, in pipe in hole spinning the polymer is melted and drawn through an annular spinneret while passing a gas into the lumen of the extruded fibers through another hole in the spinneret to prevent fiber collapse. Methods other than melt spinning may also be used.

[0050] Referring to Figure 1a, in the illustrated embodiment, a melt spinning method is used to make fibres 10 with an outside diameter 12 of 100 μm or less. The hollow area (or area of the lumen 14) of the fibre may be more than 10% or more than 30% or 40% of the cross-sectional area of the fibre. The hollow area is typically less than 60% or 50% of the cross-sectional area of the fiber. For example, a polymethyl pentene fibre may be made having an outside diameter 12 of between about 50 to 60 μm and an inside diameter 16 of 30 μm or more, resulting in a wall thickness 18 of 10 μm or less and a gas permeability of over 30,000 cc•mm/m^2•24hr•Bar or more.

[0051] In the embodiment illustrated in Figure 1, the textile PMP fibre 10 has about a 45 micron outside diameter 12 and about a 15 to 30 micron inside diameter 16. The fibre 10 was melt extruded using MX-001 or MX-002 PMP, produced by Mitsui Petrochemical of Japan and sold under the name TPX, as the raw polymer through a segmented spinneret as described above. This fiber 10 is used in the embodiments and examples described in this document, although other fibers 10 may also be used.

1.2 Fiber Aggregates (e.g. Tows)

[0052] Referring to Figure 1b, the hollow fibers 10 may be combined into fiber units 19 for handling. The fibre units 19 may be individual fibres 10, tows 20, for example, of 1 to 200 or 16 to 96 fibres 10 each, either twisted or untwisted (Figure 1b), threads, yarns, tubular, flat or cordage braids, or other units 19 for handling. Tows 20 are made by re-winding fibers from multiple take up spools in combination on to a second spool. Stronger inert fibres 22, such as yarns of PE or PP, may be included in a tow 20 or other unit 19. The fibres 10 may be curled for use in the units 19. Curled fibres 10 can be made by winding them onto a bobbin at varying tensions.

1.3 Sheet Structures

[0053] The fibers 10 and/or fiber units 19 can be provided in the form of sheets 26. In Figures 3a and 3b, the fibres 10 are woven as fibre units 19 into a basic two-dimensional structure or fabric sheet 26. In the embodiment illustrated, the units 19 run across the sheet, meaning perpendicular to the direction in which the sheet 26 advances out of a loom. Inert fibres 22 run along the length of the sheet 26 to provide support to the fibre units 19. Figure 3c illustrates steps involved in a weaving process. The fiber units 19 are carried on a shuttle through 2 groups of inert fibers 22 that are alternately raised or lowered after each pass of the shuttle. Other weaving or fabric making methods may also be used. Unit 19 type, unit 19 bundle size, spacing between units 19 and percent of fibre in each direction can all be tailored to meet the mechanical or biochemical requirements of each unique application.

[0054] In more detail, the fibre units 19 provide a support surface for the growth of a biofilm 30. The number of hollow fibre units 19, and the number of fibres 10 per unit 19, may be adjusted to provide a desired surface area for O_2 transfer compared to surface area of biofilm 30 or to the planar surface area of the fabric sheet 26. The planar surface area of the sheet 26 is simply the sheet length multiplied by its width, multiplied by two (since the sheet has two sides). The surface area of the biofilm 30 is the total area of the biofilm 30 exposed to the liquid in the reactor, which may be generally the same as the planar area of the sheet 26 for a substantially two dimensional sheet configuration.

[0055] The surface area for O_2 transfer is the total area of the hollow fibres 10 in the sheet exposed to the biofilm. This is approximately equal to the product of the effective diameter and length of the fibre 10, multiplied by the number of the fibres 10 in the sheet 26. The effective diameter for diffusion is a logarithmic average of the diameters of the fibre to account for the effect of the wall thickness. The inert fibres 22 crossing the hollow fibres 10 in the sheet 26, and contact between fibres 10, may interfere with oxygen transfer in some embodiments, for example a tightly woven fabric, but the interference is generally small and is ignored in surface area for oxygen transfer calculations.

[0056] Although the surface area of the biofilm 30 is generally the same as the planar area of the sheet, it may be slightly larger for very rough or open fabrics or fabrics having more dispersed fiber units 19. Varying fabric roughnesses may also be used to affect the thickness of the biofilm 30 or how readily the biofilm 30 can be reduced or controlled. High ratios of O_2 transfer surface area to biofilm area (SA O_2/SA biofilm) may be obtained, in the range of, for example, 6 to 10 or more. However, for treating feed water with a high concentration of COD, for example, 300 mg/L CODs or more, lower SA O_2/SA biofilm ratios, for example, between 1.6 and 10 are sufficient, and may be preferred to reduce module cost. An SA O_2/SA biofilm ratio in the range of

about 2 to 8, or about 4 to 6, can provide satisfactory results in many treatment applications.

[0057] The surface area of the biofilm 30 can also be larger than the planar area of the sheet 26 by providing a loose arrangement of fibres 10 and controlling the thickness of the biofilm 30 to a sufficiently thin layer so that the biofilm 30 on adjacent parallel fibres does not form a continuous layer. A sheet 26 with a rough or textured surface, the height of the surface undulations being in the range of the desired biofilm thickness, may also be desirable since it may facilitate biofilm control. Desired biofilm thickness may be 200 to 1,000 microns.

[0058] Provided that oxygen transfer through the module 40 does not limit reactions in the biofilm 30, the rate of COD reduction in the wastewater is roughly proportional to the concentration of COD in the wastewater. However, for oxygen transfer to not be a limiting factor, more oxygen is required to flow through the module 40 to support a biomass of the same surface area as wastewater COD concentrations increase. More oxygen can be provided by increasing the size or speed of operation of a blower. However, large head losses, for example 10 psi or more, may result due to resistance to oxygen flow through the fibre lumens 14. Head loss may be kept below 10 psi, or in the range of 6 to 9 psi, by choosing a fabric type and number of fibres that produces sufficient total lumen area for a given biofilm outer surface area.

[0059] Also, the inventors have observed that biofilms growing in wastewater with high concentrations of COD, for example 1000 mg/L CODs or more or 2000 mg/L CODs or more, are more resilient and tend to grow to undesirable thickness of a few mm or more, faster than biofilms growing in wastewater with lower COD concentrations. Thus, biofilms growing in high COD wastewater require more strenuous biofilm control methods which in turn make a stronger fabric desirable.

[0060] The various issues discussed above make it preferable for fabrics to be used in high COD wastewater that have more fibres, and

optionally more surface roughness, for the same overall planar area of a sheet or outer surface area of supported biofilm than for fabrics used to treat lower COD wastewater. This can be achieved by choice of method used to create the fabric and choice of thread or fabric unit count or tightness of the fabric. Multi-stage reactors may also be used. In a multi-stage reactor, an upstream reactor treats the feed at its highest COD concentration and is fitted with modules having dense fabrics with large numbers of fibres. A downstream reactor receives partially treated wastewater with a lower COD and is fitted with modules having a less dense fabric with fewer fibres for the same sheet or biofilm outer surface area. The less dense fabric is more economical since it has less fibres and may have a higher area of biofilm for a sheet of the same planar surface area.

[0061] The fabric sheets 26 may also be made by other methods such as braiding, stitching or knitting, such as warp knitting. Warp knitting is desirable, for example, when small units 19 or tows or even individual strands of fine fiber 10 are used. The fabric sheets 26 may be patterned, as in pattern knitting, if desired, to provide areas with fewer fibers or holes to enhance flow through the sheets 26.

[0062] In warp knitting, the fabric sheet 26, as shown in Figure 3d, contains interlaced loops of 'knitted stitches'. The column of stitches being formed on one needle make a fringe. The fringes in the length direction ('warp') of the fabric can be made by relatively inexpensive, commodity yarns, e.g. PET, PP, etc., as the inert fibres 22. The inert fibres 22 can withstand the stress and strain of processing and use. The fabric sheet 26 is generally strong and stiff in the warp (length) direction and elastic in the weft (cross) direction. The weft is a perpendicular yarn system, which is laid across the fringes and fixed by stitches (loops) of the warp fibres 22. The weft doesn't take part in the fabric (loop) formation, therefore the weft fibre units 19 can be processed very tenderly, being subjected to less stress and strain than the warp. Accordingly, preparing the sheet 26 with units 19 as the weft can minimize risk of damage to the fibres 10 during manufacturing the sheet 26.

The weft is usually a parallel layer or band of yarns being moved crosswise to the fringes (warp) during knitting. The fabric sheet width can be about to 2–3 m.

[0063] In the embodiment of Figures 4a and 4b, the sheets 26 are constructed of an open fabric made by weaving tows 20 through the shuttle of a loom and crossing the tows 20 with an inert fibre 22 only along the edges of the fabric 26. The fabric shown is approximately 1.3 m wide, that is it has active fibres 10 of about 1.3 m long, and has inert fibers 22 woven perpendicularly to the tows 20 in a strip of about 2 cm along the edges. As shown in Figure 4b, the fibers 10 in each tow 20 disperse beyond the strips so that the tows 20 remain unrestrained and partially open between the strips. The resulting roll of 1.3 m wide fabric is cut into sections of about 20-200 cm, or 30-60 cm, width to make individual sheets 26. In Figure 4b, the number of fibers 10 in each tow 20 is small for clarity but the tows 20 may each have, for example, between 1 and 200, for example 16, 48 or 96 fibers 10.

1.4 Modules

1.4.1 Loose Tow Module

[0064] In accordance with the present invention, multiple fiber units 19, including fibers 10, tows 20 or sheets 26, can be grouped together to form membrane modules 40. Figure 5 shows a module 40, which may be called a tow or loose tow module, with fibres 10 arranged and potted in tows 20 of fibres. The tows 20 are made of a loose collection of a plurality of fibres 10, for example between 1 and 200 or 16 to 96 fibres 10. The fibres 10 may be lightly twisted together or left untwisted. The fibres 10 may be curled, crimped or undulating to provide three dimensional structure to the each potted row. Curling may be achieved by re-winding the fibres 10 onto a bobbin while varying the tension on the fibres. The individual fibres 10 remain separable from each other in the tow 20. Such a tow 20, when coated with a thin biofilm, for example of less than 1 mm thickness, may provide a ratio of gas transfer area through the fibre walls to biofilm outer surface area ($SA_{oxygen}/SA_{biofilm}$) of less than 2.5, less than 1 or between 0.1 or 0.2 and 1. Inert fibres 22 may be

added to the tow to strengthen it if required. Each tow 20 is potted into a plug of resin 32 so that its ends 34 are open at one face of the resin 32. The plug of resin 32 is glued into a plastic header enclosure 35 having a port 36 which forms a header 44 connecting the port 36 to the open ends 34 of the fibers 10 through a cavity 37. There are two headers 44, one associated with each end of the fibres 10, although modules 40 with only an inlet header 44 may also be made. With two headers 44, air or other gases may be input into one header 44, flow through the fibres 10 and exhaust from the second header 44. Tows are potted in a resin 32, such as polyurethane, and the potted ends are cut to expose the fibre lumen. Alternately, a fugitive potting material may be used to block off fibre ends, as described in U.S. Patent No. 6,592,759, or other potting methods may be used. In Figure 5, the number of tows 20 and the number of fibers 10 per tow 20 are both small for clarity in the drawing and may be much larger in practice.

1.4.2 Sheet Module

[0065] A module 40 can also be constructed of a bundle or stack of sheets 26. The sheets 26 may have perpendicular inert fibers present across the entire width of sheet 26 as in Figure 3a or only across a portion of the width of the sheet 26, for example at the ends as in Figure 4. Raw material for the sheets 26 may be rolled onto a fabric roll. For example, where the sheets 26 are prepared by weaving, the material is rolled on to a take up roll at the end of a loom as material is produced. The fiber units 19 may extend across the roll while the inert fibers spiral around the roll. With the fibers oriented in this way, individual sheets 26 may be cut from the roll by rolling out a length of material from the roll and cutting it off with a hot knife or heat cutter. The heat cutter melts through the fiber units 19 and inert fibers and bonds them together to protect the fabric edge from disintegrating or fraying. Since the heat cutter melts a strip of fibers on either side of the cut line, for example a strip about 5 mm wide, the fibers remaining on the roll are similarly melted together to produce a stable edge. After a sheet 26 has been cut from the roll, the other two ends of the sheet, meaning the edges of the sheets 26 at right angles to the heat cut edges, are cut to open the lumens of the fiber

units 19. To minimize distortion or collapse of the ends of the fibers 10 under the cutting pressure, the area to be cut is first reinforced, for example by impregrating it with polyurethane to provide a reinforcing coating around the fibers 10 or fiber units. The cut across the fiber units 19 is then made with a sharp cutter, for example a razor edge cutter. The cutter is preferably kept very sharp, for example by changing blades regularly, to minimize distortions of the ends of the fibers 10. Other cutting machines or tools used in the garment and textile industries may also be used.

[0066] The end or ends of single or multiple sheets 26 can be potted into a header to provide one or more ports 36 in communication with the lumens of the fibers 10. To pot one or more sheets 26, sheets 26 are cut from a roll as described above. A plastic spacer strip is attached, for example with glue or adhesive transfer tape, on one or both sides of the sheet 26, at the end of the sheet 26 parallel to but offset from the razor cut line across the fiber units 19. For potting multiple sheets 26, the sheets 26 with spacer strips attached are laid on top of each other and attached together, for example by glue or adhesive transfer tape, between adjacent spacing strips or between the spacing strip of one sheet 26 and a second sheet 26. The strips space adjacent sheets 26 but also form a barrier between a potting material to be applied later and the cavity of the header containing the ends of the fibers 10. The ends of the sheet 26 or stack of sheets 26 is fitted into an elongated header cavity that may be made, for example, by injection molding. Spacing and sealing to the header walls is maintained with a self-adhesive closed cell neoprene gasket strip attached to each of the long header walls. Any openings in the header cavity left by the spacer strips may be covered with hot melt glue. Final sealing of the header is achieved by pouring a layer of potting material, for example a two-component polyurethane compound, over the spacer strips. The layer may be about 45 mm thick and extend between the insides of walls of the header. If there are multiple sheets, care is taken to force or ensure flow of the potting material, as completely and evenly as practicable, between the sheets 26. After the potting material hardens, a seal is formed between the outsides of the fibers 10 and the walls of the header

but the ends of the fibers 10 remain in communication with a cavity within the header.

[0067] Figures 6 to 9 show a module 40 in which a set of parallel sheets 26 are potted with gaps 42 between them in a header 44. Two headers 44 may be used as shown when a bleed of exhaust air is desired. One header 44 may also be used with exhaust bled through opposed open ends of the fibres 10 or with the other ends of the fibres 10 closed for dead end operation. The gap 42 may be between 2mm and 10 mm thick, or between 3 mm and 15 mm. The chosen gap 42 may depend on the water to be treated or the choice of method to control biofilm thickness. For example, a module 40 of tensioned sheets 26 may have a gap 42 of 6 mm when used with air scouring to control biofilm 30 thickness. Tension may be provided by mounting the headers 44 to a rigid structure, which may include parts of a tank, with one or both headers 44 movable relative to the structure. Alternately, the headers 44 may be attached to part of a frame held at an adjustable distance apart. The sheets of fabric 26 are potted and separated in the headers 44 by various potting materials 46 such as one or more of polyurethane, hot melt glue, adhesive strips, plastic spacing strips or epoxy. The spacing between adjacent sheets 26, or gaps 42, provides space for scouring air and substrate flow through the module 40. A large sheet of the fabric 26 may also be rolled or folded to produce a module 40 rather than using separate sheets. The length of the module 40 is a compromise between OTE and pressure drop and may range from 1 m to 5 m or between 1 m and 3 m.

[0068] Referring to Figure 8, to make the module 40 a sheet 26 of fibres 10 is laid onto strips 50 (one on each end) of adhesive located to cross the ends of the fibres 10. Additional strips 50 of adhesive and spacing strips 52 are placed over the sheet 26, followed by additional strips 50 of adhesive and an additional sheet of fabric 26. These steps are repeated as appropriate for the number of sheets 26 desired. The resulting assembly is then sealed into the header enclosures 35 of a pair of opposed headers 44 such that the lumens 14 of the fibres 10 are in communication with ports 36 in the headers

44 through cavities 37. The ends of the fibres 10 are cut before potting to open them, for example as described above. Additional glue or potting resin 41 may optionally be poured into the header enclosure 35 to further seal the fibers 10 to the header enclosure 35. Alternately, sheets 26 may be separately glued to spacing strips at their edges and inserted into a header cavity and additional glue or potting resin 41 placed around this assembly to seal it to the header enclosure 35. Further alternately, the first assembly method described above may be used.

[0069] Figure 9 shows a picture of a module 40 assembled as generally described above. The headers 44 are about 2 meters apart. Additional spacers 33 are used mid way between the headers to better preserve the sheet 26 separation. A thin steel rod 45 is attached to the edges of the fabric sheet 26 in the right half of the module to address the folding which can be seen in the left half of the module. The module 40 has a ratio of SA oxygen/SA biofilm of about 5.

[0070] Another embodiment of a module 40 can be seen in Figures 10a to 10c. The module 40 has a single sheet 26 with hollow fibre units 19 and inert fibres 22. The hollow fibre units 19 extend between headers 44 at either end of the sheet 26. The width 62 of the headers 44 is such that stacking multiple modules 40 adjacent each other with the headers 44 of adjacent modules 40 abutting each other provides the desired spacing between the adjacent sheets 26. The header enclosures 35 of this module 40 are clear allowing the cavity 37 to be seen. To pot the sheet 26, the header enclosure 35, which is a folded over plastic strip, is forced open and a sheet 26 is inserted. The header enclosure 35 springs closed on the sheet 26. Tubes which function as ports 36 are inserted into the ends of the header enclosures. Potting resin 31 is laid along the joint between the sheet 26 and the header enclosure 35, between the ports 36 and the header enclosure 35 and all other openings to seal the cavity 37.

[0071] Referring again to Figure 4, another module, which may be called a tow or tow sheet module, can be made of open sheets 26 of tows 20

cut along the woven edges to open the ends of the fibres 10 and potted with a 0 to 10 mm space between them into one or a pair of opposed headers. Depending on the potting method used, which may include potting methods described above, the fibres 10 may be cut open either before or after they are inserted into the potting resin. 1 to 100 or 8-20 sheets may be potted into a pair of headers to produce a module. Modules made in this way using the fibers of Figure 1 had $SA_{oxygen}/SA_{biofilm}$ ratios of between 1:2.5 (0.4) and 1/11 (0.1) with a biofilm thickness of 250 microns.

1.5 Cassettes/Reactors

[0072] In general, a plurality of modules can be grouped together to form a cassette, and one or more modules or one or more cassettes can be placed in a tank as part of a reactor. Referring to Figures 11 and 12, the modules 40 of a cassette 110 are mounted in a tank 112 of a pilot reactor for treating 1 cubic meter per day of industrial wastewater having a COD of over 1,000 mg/L, typically 7,000 mg/L. The feed is treated by either batch or continuous process to reduce its COD concentration to 300 mg/L as required for discharge into the municipal sewer that it outlets to. The tank 112 has a fill volume of 1.8 m^3. Fifteen modules 40 are provided in the tank 112, each module 114 containing six sheets 26 of 3.6 m^2 surface area of a woven fabric of PMP fibers units 19, woven as tows 20. The fibres 10 are 1.8 m long and extend between an inlet header 116 and outlet header 122 of the modules 40. Total number of PMP tows per sheet is 1968, and fibres per sheet are 94464, there being 48 fibers per tow and a two packing of 50 threads per inch in the sheet 26. Also, polyester yarn is woven perpendicular to the PMP fibre, and the total number of yarns per module is 1912. Air pressure drop in the fibre lumen is in the range of 5 to 10 psi. Total biofilm area per module is 17 m^2, and oxygen transfer area is about 5.1 times the biofilm area.

[0073] The modules in the embodiment illustrated are mounted in such a way that the tension of the sheets 26 extending between the headers 116, 122 can be adjusted. The cassette provides a rigid structure 150, which can include elements of the tank 112 or elements of a cassette sub-frame,

adjacent the modules 40, and one or both of the headers 116, 122 are movable relative to the rigid structure 150.

[0074] In the embodiment illustrated, the rigid structure 150 comprises a pair of side plates 152 that extend along the distal side surfaces of the outermost modules 40 of the stack of modules 40. As best seen in Figures 13 and 14, the modules 40 are attached to the side plate 152 by means of a mounting bracket 154 extending transversely between the side plates 152 at either end of the modules 40. The mounting brackets 154 are provided with grooves 156 shaped to receive T-shaped tongues 158 extending from surfaces of the headers 116, 122, opposite the sheets 26. The module 40 can be secured to the mounting brackets 154 by sliding the tongues 158 of the headers 116 and 122 into the grooves 156 of the brackets 154. The mounting brackets 154 can be secured to the side plate 152 by, for example, a bolt 160 passing through an aperture 162 engaging the plate 152 and a threaded hole 164 in an edge surface of the bracket 154.

[0075] The aperture 162 can be slot-shaped, so that the bracket 154 with the attached header 116, 122 can be shifted horizontally to increase or decrease the tension of the sheets 26. An eccentrically mounted cam member 166 can be provided between the head of the bolt 160 and the plate 152, with an outer diameter surface in engagement with an abutment surface 168 fixed to the plate 152. Rotating the cam member 166 can force the opposed brackets 154 further apart or allow them to draw closer together, thereby adjusting the tension of the sheets 26 in the modules 40.

[0076] The tension adjustment mechanism can be provided on only one end or on both ends of the modules 40, and can be modified to provide individual tension adjustment for each module 40 or for sub-groups of modules 40. Other mounting methods may also be used to allow modules 40 to be removed or tensioned.

[0077] In another embodiment of the invention, the elements or modules are stacked in a vertical configuration. Flow of scouring air from outside the modules or of water in the tank may be from top to bottom or

bottom to top. This minimizes the capital required for scouring air and the operating cost of air.

2.0 Operation/Applications

[0078] The fiber units 19 having one or more fibers 10 can be used as membranes to support biofilm in a reactor. In general, gas containing oxygen flows into at least one of the headers 44 of a module 40. The module 40 may be operated in a dead end mode, with no outlet other than through the fibres. Alternately, the module may be operated in a cross flow manner with gas entering through one header 44, flowing through the fibers 10, then exiting from the other header 44. The oxygen content and flow rate of the gas may be set to produce an oxygen transfer that provides aerobic conditions near the outer surface of the fibers 10, where the level of oxygen is highest. Aerobic reactions occur in this area, including conversion of organic compounds to carbon dioxide and water, and ammonia to nitrates. The biofilm may be maintained under anoxic conditions on its outer surface or near the substrate being treated and conversion of nitrogen to nitrates can take place. In this way, multiple and simultaneous reactions, including carbon based organics, ammonia and total nitrogen reduction, may be performed in the biofilm.

[0079] An example reactor 80 is shown in Figure 15. Figure 15 provides a near plug flow. The reactor 80 has a tank 82, a feed inlet 84 to the tank 82, an effluent outlet 86 from the tank 82, a flow path 88 between the feed inlet 84 and the effluent outlet 86, and a plurality of fiber units 19 in the form of modules 40 in the tank 82. Each module 40 can have one or more sheets 26 extending from one or more headers 44. The plurality of modules 40 can be provided as part of one or more cassettes 110.

[0080] The sheets 26 and modules 40 are sized to fit the tank 82 and fill a substantial part of its volume. The sheets 26 may be custom made to provide efficient use of the available space in the tank 82. The sheets 26 are preferably arranged in the tank 82 in a number of rows, one such row being shown in Figure 15. The sheets 26 may range from 0.25 to 2 mm in thickness and adjacent sheets 26 are placed in the tank 82 side by side at a distance of

2 to 15 mm to allow for biofilm growth and wastewater flow between adjacent sheets 26.

[0081] The tank 82 is longer than it is deep and may have a generally horizontal flow path 88 with minimal mixing. This is achieved by leaving some space near the ends (ie. near the inlet 84 and outlet 86) of the tank 82 for vertical movement of water and leaving minimal free space at the top, bottom and sides of the tank 82. A baffle 90 may also be placed upstream of the effluent outlet 86 to force the flow path 88 to go under it. A sludge outlet 92 is provided to remove excess sludge.

[0082] The flow path 88 is generally straight over a substantial portion of the tank 82 between the feed inlet 84 and effluent outlet 86. Each module 40 is held in the tank 82 by its headers 44 attached to a frame (not shown for clarity) which restrains each module 40 in positions in the reactor 80 whereby the sheets 26 of each module 40 are generally parallel to the flow path 88. Preferably, a plurality of sheets 26 are spaced in series along the flow path 88 so that the reactor 80 will more nearly have plug flow characteristics. Wastewater to be treated may be partially recycled from the effluent outlet 86 to the feed inlet 84. Such a recycle can increase the rate of gas transfer by increasing the velocity of wastewater along the flow path 88, but it is preferred if the recycle ratio is small so as to not provide more nearly mixed flow characteristics in the reactor 80.

[0083] Oxygen containing gas is provided to each module 40 through its inlet conduit 216 connected to an inlet manifold 94 located above the water to be treated. With the inlet manifold 94 located above the water, a leak in any module 40 will not admit water into the manifold nor any other module 40. Gas leaves each module 40 through its outlet conduit 218 which is connected to an exhaust manifold 95. Although it is not strictly necessary to collect the gases leaving each module 40, it does provide some advantages. For example, the gas in the exhaust manifold 95 may have become rich in volatile organic compounds which may create odour or health problems within a

building containing the reactor 80. These gases are preferably treated further or at least vented outside of the building.

[0084] Oxygen diffuses or permeates through the fibers 10. The amount of oxygen so diffused or permeated may be such that an aerobic biofilm is cultured adjacent the sheets 26, an anoxic biofilm is cultivated adjacent the aerobic biofilm and the wastewater to be treated is maintained in an anaerobic state. Such a biofilm provides for simultaneous nitrification and denitrification. A source of agitation 98 is operated from time to time to agitate the sheets 26 to release accumulated biofilm. A suitable source of agitation is a series of coarse bubble aerators which do not provide sufficient oxygen to the water to be treated to make it non-anaerobic.

[0085] Figure 16 shows a second reactor 80 having a tank 82, a feed inlet 84, an effluent outlet 86, a flow path 88 and a plurality of modules 40. Frames (not shown) hold each module 40 in a position whereby the sheets 26 of each module 40 are generally parallel to the flow path 88.

[0086] The sheets 26 are sized to fit the tank 82 and fill a substantial amount of its volume. The sheets 26 may be custom made to provide efficient use of the available space in the tank 182. The sheets 26 may range from 0.25 to 2 mm in thickness and are placed side by side at a distance of 2 to 15 mm to allow for biofilm growth and wastewater flow between adjacent sheets 26.

[0087] The tank 82 is deeper than it is long to encourage a straight and generally vertical flow path 88 over a substantial portion of the tank 82 with minimal mixing. This is done by leaving minimal space near the ends and sides of the tank 82 but a substantial amount of space near the top and bottom of the tank 82. Water to be treated may be partially recycled from the effluent outlet 86 to the feed inlet 84 but it is preferred that the recycle rate be small if a recycle is used.

[0088] Oxygen containing gas is provided to each module 40 through its inlet conduit 216 connected to a manifold 94. The manifold 94 may

alternately be located above the water to be treated so that a leak in any module 40 will not admit water into the manifold 94 nor any other module 40. Outlet conduits 218 are connected to an outlet manifold 95 which may alternately be located above the surface of the water to be treated.

[0089] Alternatively, gas flow through the module 40 is produced by applying a suction to the outlet conduits 218. The inlet conduits 216 are placed in fluid communication with the atmosphere. By this method, the rate of gas diffusion across the membrane is slightly reduced, but the exhaust from the blower may be connected to further apparatus for processing the exhaust gases.

[0090] Oxygen diffuses or permeates through the membranes 120 preferably such that an aerobic biofilm is cultured adjacent the sheets 26, an anoxic biofilm is cultivated adjacent the aerobic biofilm and the wastewater to be treated is maintained in an anaerobic state. A source of agitation 98 is operated from time to time to agitate the sheets 26 to release accumulated biofilm. A suitable source of agitation is a series of mechanical mixers.

[0091] Referring to Figure 17, a reactor 100 has a tank 112 with one or more membrane supported biofilm module cassettes 110 installed inside of it. The cassettes may have one or more modules 40, as described above. The module 40 may also be a tow module, a module of planar elements, or other types of modules using a membrane to support a biofilm. Each module 40 has a gas inlet header 116 fed with air, or another oxygen containing gas, through a blower 118. Gas passes from the inlet header 116 to the inside (or lumens 14) of one or more fibers 10. The walls of the fibers 10 serve as gas transfer membranes 120. A portion of the gas passes through the membranes 120 while another portion, and possibly some gasses taken up from the tank 112, flow to an outlet header 122 of the modules 40 and to an exhaust 124. The gases leaving the exhaust 124 may be post-treated or discharged to the atmosphere.

[0092] Feed water enters the reactor 100 through a feed valve 126 and feed pump 128. The feed is filled to a feed fill level 130 above the modules 40.

After a batch of feed has been treated, a drain valve 131 is opened to drain the tank 112 of treated water. The treated water may flow to a municipal sewer, to the environment, discharge directly to a receiving stream, or to another stage of a MSBBR (membrane supported biofilm batch reactor) or to another sort of reactor for further processing.

[0093] A biofilm 132 grows on the outside of the membranes 120. To control the thickness of the biofilm 132, one or more aerators 134 are provided below the modules 140 and connected to a scouring air blower 136 through an aeration valve 138. The scouring air blower 136 can be operated to provide bubbles when the tank 112 is full of water. The bubbles rise through the module 140 and physically remove some of the biofilm 132 from the membranes 120. The aerators 134 are also attached to a gas supply 140 through a gas supply valve 142. The gas supply 140 may contain a pressurized gas or a gas generator and pump or other device for supplying a gas when the tank 112 is empty. The reactor 100 also has a liquid pump 144 operable to fill the tank 112 with a liquid other than feed water. The liquid pump 144 may be connected to a reservoir holding the liquid or to a source of clean water passing through a modifier, such as a chemical injection device or heater. The tank 112 is generally open to the atmosphere and contains liquid at generally ambient pressure but has a lid 146 which may be closed from time to time to provide an enclosed space.

[0094] The main treatment process in the reactor 100 involves the batch application of feed to the biofilm 132. The tank 112 is filled with feed to the feed level 130 using the feed pump 128. The feed pump 128 is connected to the feed supply through an equalization reservoir 148 to permit batch operation from a non-batch feed. The feed remains in the tank 112 for a period of time, for example between 12 and 96 hours, while it is treated by the biofilm 32. During treatment, the lid 46 may remain open, but the water in the tank 112 is generally anoxic or anaerobic. However, oxygen, typically as a component of air, is supplied to the biofilm 132 through the membrane 120 by the blower 118 creating an aerobic region on the biofilm 132. From time to

time during the treatment period, a recirculaton valve 149 may be opened and feed pump 128 operated to mix the feed water in the tank 112.

[0095] After the biofilm 132 has digested the feed to the desired degree, the drain valve 131 is opened to drain the tank 112. The draining may occur in two steps. In the first step, the solids slurry present in the bottom of the tank is drained to remove settled solids which are then transferred to a sludge management system. In the second step, the clear decanted liquid is then drained to a second stage treatment or disinfection system, or discharged to a sewer, or discharged to a receiving stream.

[0096] The oxygen bearing gas supply may be continued throughout the filling operations to continue digestion of the material adsorbed on the biofilm, and to ensure that treatment starts immediately as soon as a portion of the biofilm is immersed in the wastewater. Similarly aeration may continue throughout the draining operation to continue treatment as long as a portion of the biofilm is immersed and to digest organics adsorbed in the biofilm for a short period of time even while not immersed, so as to maximize the time of treatment of each batch.

[0097] Referring now to Figure 18, a reactor 400 is shown having similar features as the reactor 100, but without the gas supply 140, gas supply valve 142, or liquid pump 144.

[0098] In a batch process, the concentration of the wastewater decreases towards the end of each processing period. Demand for oxygen supplied to the biofilm also decreases and so the gas supply to the modules may be reduced. Modules using fibres at least partially in the form of tows allow a very high surface area for oxygen transfer and biofilm growth. Tow modules are particularly useful in treating wastewater having a low COD, for example 1,000 mg/L or less, 500 mg/L or less or 300 mg/L or less, because they provide large surface areas. Pressure loss through the fine fibre lumens is not limiting with the amount of air supply required to deliver oxygen to a biofilm treating low COD wastewater. Although they may be useful for treating other wastewaters as well, tow modules can be used where the initial feed

has a low COD or as a second or third stage behind other treatment processes or apparatus that reduce the COD concentration of stronger feedwaters. With municipal wastewater or other feeds, for example feeds having a COD of 1,000 mg/L or more, a two stage apparatus may be used. In a first stage, membrane supported biofilm modules in the form of a fabric sheet are used as in Figure 9. The outlet from a reactor containing these modules is fed to a reactor containing tow modules with sheets as in Figure 4 which provides second stage treatment. The inventors have observed that rapid reduction in COD from a high COD wastewater limits the denitrification produced from a membrane supported biofilm reactor. With a two stage process, the first stage may be optimized for COD removal. The feed to the second stage has a reduced COD and the second stage may be optimized to support nitrifying microorganisms, for example of the species nitrobacter and nitrosomas, over carbon degrading microorganisms to provide improved ammonia oxidation in the second stage.

[0099] In general, when considering COD, soluble COD is used since soluble COD is most easily digested by a biofilm 30 and is easily measured. However, particularly for modules 40 with loose tows 20 over some or all of their area, some particles of insoluble COD are trapped in the biofilm. Over time, these particles are broken down into soluble COD and digested. Accordingly, total, or total biodegradable, COD also may be a relevant parameter in some embodiments.

[00100] For feeds having a CODs of 1000 mg/L or more, a module 40 may have an SA_{OXYGEN} /$SA_{BIOFILM}$ of 1 or more, for example between 1 and 10. Modules 40 having sheets 26 woven across the entire length of the fibers 10, in a dense weave with a high number of fibers for very high loadings, for example, are useful. For feeds having a CODs of 1000 mg/L or less, a module 40 may have an SA_{OXYGEN} /$SA_{BIOFILM}$ of between 0.2 and 2.5. Modules 40 having sheets woven across the entire length of the fibers but with a less dense weave, or sheets 26 with a central open tow 20 area, for example, are useful. For feeds having a CODs of 300 mg/L or less, a module

40 may have an SA_{OXYGEN} /$SA_{BIOFILM}$ of 1 or less, for example between 1 and 10. Modules 40 with sheets 26 have a central open tow 20 area, or modules 40 of loose tows 20, for example, are useful.

[00101] Figure 19a shows a bench scale reactor having a module 40 made by potting 100 tows 20, each of 96 fibres 10 as shown in Figure 1, into an opposed pair of headers 44. The module 40 was used to treat a feed water in a batch process. In the process, the module 40 was located in a tank 112 filled to 4 L of synthetic wastewater. The tank was drained and filled with fresh feed every 1 to 7 days. Air was applied to the module at 10 mL/min. A biofilm 30 of stable thickness grew on the module 40 for a period of over 6 months. The biofilm 30 was essentially endogenous, its rate of growth generally equal to its rate of decay, except that a small part of the biofilm 30 broke off and was discharged with some of the tank drains. A section of a tow 20 is shown in Figure 19b. Individual fibers 10 are covered in biofilm 30. In some places, the biofilm 30 around a small group of fibers 10 may merge together for a portion of the length of the fibers 10. The thickness of the biofilm 30 shown is about 250 microns.

[00102] Referring now to Figure 20, another reactor is shown as suitable, for example, for a septic tank, septic tank retrofit or shipboard treatment plant. The particular reactor shown is a septic tank retrofit using a standard septic tank 410 with an inlet 412 and an outlet 414 on opposite sides. The tank 410 has two stages including a primary chamber 416 and a secondary chamber 418. A dividing wall 420 has a submerged orifice 422 that allows flow between the chambers 416, 418. One or more modules 424 are placed in the secondary chamber 418. Air is supplied to the bottom headers of the modules 424 through inlet tubes 426. Exhaust air is vented from the upper headers of the modules 424 through exhaust tubes 428. Scouring air is periodically applied to a sparger 430 located under or near the bottom of the modules 424 through scouring air tube 432. The modules 424 each have 1 to 100 or 8-20 sheets as in Figure 4 potted into a pair of headers to produce a module 424. For example, a septic tank for a single household

may have one 8 to 10 sheet module 424 fed with a 1/4 hp air blower and creating a pressure drop of about 1 to 7 psi, or about 3 psi. With a typical household feed, a generally endogenous biofilm grows on the individual fibre 19 and tow 20 surfaces. Biological treatment in the biofilm results in a reduction in the suspended solids and chemical oxygen demand of the effluent, allowing the septic tile field to be reduced in size or eliminated.

[00103] In another embodiment of the invention, a number of bioreactors are installed in series to provide flow patterns approaching plug flow. This results in higher reaction rates and better utilization of oxygen.

[00104] In another embodiment of the invention, different oxygen levels are used in different stages of the bioreactor by oxygen spiking to meet different levels of oxygen demand and to achieve high bioreactor loadings. Different oxygen levels may also be used at different times in a single reactor or stage of a reactor. To increase the oxygen level, the pressure of the gas fed to the lumens of the fibers or the oxygen content of the feed gas can be increased. Similarly, to decrease the oxygen level, the feed gas pressure or oxygen content can be decreased. Higher oxygen levels may be used in upstream stages of multi-stage reactors or in highly loaded reactors. Oxygen levels may also be increased periodically or from time to time to correspond to periods of time when the loading on a reactor is temporarily increased, for example to respond to seasonal or daily variations in wastewater strength or quantity.

3.0 Biofilm Control

[00105] In a membrane supported biofilm reactor, it can be advantageous to control the thickness of the biofilm on the membranes. For example, in the reactor 100 (Figure 17), although the tank 112 is drained periodically, most of the biofilm 132 remains on the membranes 120, particularly where the feed has a high COD, for example over 300 mg/L. Excess thickness of the biofilm 132, for example 2 mm thick or more, provides minimal, if any, increase in digestion rate, over a thinner layer, for example of 1 mm thick or less. However, keeping the biofilm 132 thin allows the sheets

26 of the modules 40 to be placed closer together, providing more surface area per module volume. This increase in surface area generally more than offsets any minor increase in digestion that may, or may not, be achieved with a thicker biofilm 132.

[00106] Accordingly, means are provided to prevent the biofilm 32 from becoming unnecessarily thick. The following methods may be performed individually or in various combinations. The frequency of treatment varies with the growth rate of the biofilm 132. For example, a biofilm 132 may grow by 10 microns a day and the module 40 may be made to tolerate a biofilm of between 0.2 mm and 0.8 mm. Biofilm control procedures may then be required every 5 to 10 days. Alternately, the period between biofilm control procedures may be linked to the amount of COD that the biofilm has digested since the last control procedure, which is in turn related to the time and biofilm thickness increase since the last control procedure. For example, control procedures may be performed when the biofilm has digested about 20 to 200 grams of CODs per square meter of biofilm area since the last control procedure. When control or thickness reducing procedures are performed at this frequently, a stable biofilm layer is maintained over extended periods of time even though each control period does not have a drastic effect on biofilm thickness. Control procedures may be applied to the entire biofilm at once or to a portion of the biofilm at a time.

3.1 Mechanical Methods of Biofilm Control

[00107] Some methods for controlling the thickness of the biofilm 132 on the membranes 120 involve mechanically removing part of the biofilm 132. In one such method, still referring to Figure 17, one or more aerators 134 are provided below the modules 114 and connected to a blower 136 through an aeration valve 138. With the tank 112 full of liquid, blower 136 is operated to create bubbles from aerator 134 below the modules 40. The bubbles mechanically scour the biofilm 132 and also create a flow of water through the modules 40 that physically removes some of the biofilm 132. A high velocity of scouring air of 2-8 feet/second or an air application rate of 5 to 20, for

example about 10, cubic meters per hour per square meter of module footprint for intervals of 1 to 10 minutes may be used. This may be done, for example once every day to once every week. Also, air may be used to periodically mix the contents of the bioreactor.

[00108] Other mechanical methods include spraying the modules 40 with water while the tank 112 is empty and physically removing biofilm 132 such as with a comb, wire or brush. The removed biofilm 132 falls to the floor of the tank 112 and may be flushed out through drain 131 for further processing as for waste sludge. These mechanical methods may be performed less frequently than other methods and, when performed, may be performed after another method has weakened the biofilm 132.

[00109] Mechanical methods for controlling the biofilm are enhanced by providing the sheet 26 with a rough or textured surface, the height of the surface undulations being in the range of the desired biofilm thickness. Desired biofilm thickness may be 200 to 1,000 microns.

3.2 Chemical Methods

[00110] In another embodiment, ozone gas, introduced in the fibre lumen is used to oxidize a part of the biofilm to make it digestible. Oxygen is then provided to the lumens to permit the biofilm to digest the oxidized organics, thereby reducing the total amounts of solids generated and to control the biofilm thickness. The oxygen may be provided as a separate step or as part of the regular step of digesting wastewater. The reactor may be treated in this way one module or section at a time.

[00111] In another method, a control substance is applied to the tank side of the biofilm 132. For example, after the tank 112 is drained, clean water heated to, for example, 35-55 C, may be pumped into the tank 112 by the liquid pump 144. The heated water is kept in the tank 112 for a period of time (contact period), for example 3-5 hours, sufficient to kill a fraction of the biofilm 132 and dissolve some of the organics that form the biofilm matrix. The biofilm is also starved to some extent since feed has been removed. Oxygen may continue to be applied to the lumens or may be turned off. Air

scouring may also be provided during this period to enhance biofilm removal, although it may be more economical to carry out this operation without air scouring, particularly if the blower 136 and aerator 134 can then be eliminated from the reactor 100 entirely. The biofilm 132 is also starved to some extent. After the contact period, the water is drained through drain valve 131. In an industrial treatment system, the discharge water will have some COD but the duration of the contact period can be chosen such that the discharge is still suitable for discharge to a municipal sewer since most of the killed organisms will remain in the biofilm 32. During a later part of the contact period, the living inner part of the biofilm 32 will biodegrade the killed organisms. The effect of the heated water, or unheated water, may be enhanced with the addition of chemicals such as acids, for example with a pH between 1 and 6 or between 3 and 3, bases, for example with a pH between 8 and 13 or between 9 and 11, or enzymes. The chemicals and their concentration and contact time are chosen to partially dissolve or weaken some organics that are structural component of the biofilm but to kill only a fraction of the microorganisms while leaving the majority behind in an active biofilm for rapid restart of the reactor.

[00112] In another method, a gaseous control substance is applied to the tank side of the biofilm 132. The gas is applied from gas supply 140 while the tank 112 is drained at the end of a batch cycle. Lid 146 is closed so that the gas remains in the tank 112. The gas may be of various types, for example an acid such as chlorine. Alternately, ozone may be used. The primary purpose of the ozone is to break up the cell walls of the microorganisms in the biofilm 132 to make it more biodegradable. The amount of ozone applied would not be sufficient to oxidize more than about 5% of the biofilm directly and to kill only a fraction of the microorganisms present in the biofilm. However, refractory organic material is converted to organic material which is later reduced by biological oxidation when the tank is refilled. The ozone is generated in a gas phase (air or oxygen) and is easily dispersed in an empty tank 112. The ozone is kept in the tank 112 for a period of time allowing it to be absorbed by the biofilm 132. Redox conditions can be

controlled in the tank 112 while it is drained to promote sludge reduction. Alternating aerobic and anaerobic conditions can be established in the biofilm 132 by turning the feed to the inlet header 116 on and off while the tank 112 is filled with ozone to enhance the effects of the ozone. Killed and partially oxidized organisms remain in the biofilm 132 and are later digested in situ such that excess biomass need not be removed from the tank 112 for further treatment. Denitrification may also be improved because the carbon/nitrogen (C/N) ratio increases. Ozone may also be used in this method with membranes 120 that are sensitive to ozone since the membranes 120 are protected by the biofilm 32.

3.3 Biological Methods

[00113] In another method, worms or other animals or higher life forms are used in an isolated section of the reactor to digest excess biofilm to reduce bio-solids generation. The worms etc. are grown in a separate bioreactor. When desired, the worms etc. are applied to the biofilm by filling the tank with a liquid suspension or brine containing the worms etc.

[00114] Another method of biofilm control is endogenous respiration. By this method, the feed loading applied to the biofilm 132 is kept such that the rates of decay of the biofilm 132 equals its rate of growth. In practice, the rate of growth may exceed the rate of decay by a small amount in a batch process because some of the biofilm 132 may detach and leave the tank 12 when it is drained. However, endogenous respiration occurs practically only at low loading rates and so is more appropriate for feeds with low COD concentrations, for example 1000 mg/L CODs or less or 300 mg/L CODs or less.

[00115] Another method is periodic starvation. In this method, the feed is kept in the tank 112 for an extended period of time such that the COD concentration drops to below what it is at the end of a typical batch process. The biofilm 132 is not nourished and decays rapidly until the start of the next batch cycle. The biofilm can also be starve by removing the feed and filling

the tank with clean, for example tap or potable, water, or by loading the reactor at less than 0.1 kg CODs per kg MLSS per day.

[00116] In another method, the supply of gas to the inlet header 116 of the module 40 is turned on and off cyclically for a period of time. The varying supply of oxygen shocks the biofilm 132 and causes increased decay. Aerobic and anaerobic areas in the biofilm expand and contract while consuming, or being consumed by, the other. Alternately, gases such as ozone or chlorine, may be added to the inlet header 116 to enhance the shock.

[00117] With chemical or biological biofilm control, closer spacing between the sheets 26, for example 3-4 mm, may be used since hydraulic flow through the modules 40 is not required as with air scouring, agitation or other physical methods of biofilm removal. Chemical or biological methods are also useful where sheets 26 or fibers 10 or units 19 are not arranged so that a flow of scouring air will not reach all parts of the biolfim. Chemical or biological biofilm control methods may also be useful with open sheets 26 or modules with unsupported or loose fibers 10, fiber units 19 or tows 20 that would be damaged by air scouring, agitation or physical methods. Alternately, one or more chemical methods, one or more mechanical methods or one or more biological methods may be combined.

Examples:

Example 1: Chemical oxygen demand (COD) reduction in a membrane supported bioreactor

[00118] A bench scale bioreactor was made using a module generally as presented in Figures 6-9 except that only a single sheet of the fibres was used. The length of the sheet was 0.57 m and height 0.45 m, providing a total biofilm area of approximately 0.5 m^2 assuming a with both sides of sheet available for biofilm growth. The ratio of surface area for gas transfer to surface area of attached biofilm was between about 5 and 6. Inlet air flow was 25 ml/min at a pressure of 34.5 kPa. Reactor volume was 30L. Synthetic wastewater with a COD level of 1000 mg/l was introduced in a batch

manner periodically. The synthetic wastewater consisted of 1.0 g/L of soluble peptone and 0.03 g/L of sodium hydrogen phosphate dissolved in tap water. A series of batch reactions were conducted to determine the rate of reaction and oxygen transfer efficiency. Figure 21 presents the results of three batch periods: a three day period form day 2 to day 5, a three day period from day 6 to day 9 and a one day period from day 9 to day 10. It can be seen that 80-90% reduction of COD was obtained in each of the three-day batch periods. A COD reduction of about 40% was achieved in the one-day batch period suggesting that the rate of COD reduction is higher while the concentration of wastewater is higher and that the COD reduction rate levels off as the COD concentration in the batch decreases with time. Oxygen transfer efficiency during these series of tests ranged from 50 to 70%, as measured by the exit concentration of air.

Example 2: Bench Test with Synthetic Wastewater

[00119] A bench scale bioreactor was designed using a single sheet module as described for Example 1. Synthetic wastewater with a COD level of 1000 mg/l, as described in Example 1, was introduced and treated by the biofilm on the module. Rates of COD removal and oxygen transfer and the thickness of the biofilm were calculated or measured and recorded. For about the first 21 days, the reactor (which has a 30 L fill volume) drained and re-filled with feed after variable batch periods to keep the CODs in the tank generally between 500 and 1000 mg/L. At day 8 and day 16, in addition to emptying the tank and re-filling it with new feed, the module was powerwashed with a water sprayer to remove biofilm. From about day 21 to day 30, the biofilm was subjected to starvation (i.e. the tank was filled with tap, i.e. clean or drinkable, water while oxygen supply continued to the module) and air scouring treatments. On about day 30, the tank was emptied and re-filled with feed. From then on, the tank was emptied and re-filled with wastewater daily but no biofilm control steps were taken, to allow the biofilm to grow in thickness and observe the effect and rate of such growth. The results of the test are presented in Figure 21. It can be observed that the

COD removal rate varied between about 19 to 38 grams per square metre per day without being proportional to the biofilm thickness. The oxygen transfer varied between about 10 to 15% grams per square metre per day, also over a relatively wide range of biofilm thickness, namely, from about 0.5mm to over 2.3mm, at which thickness the measurement device reached its maximum thickness.

Example 3: Pilot Study with Industrial Wastewater

[00120] A small pilot study was conducted using four modules generally as shown in Figures 6 to 9. Each module has 6 sheets of fibers and a total planar surface area, or area of biofilm, of about 3.6m^2, and a ratio of surface area for gas transfer to surface area of attached biofilm of between about 5 and 6. The modules were installed in a 300 litre tank. The reactor was initially operated with peptone (about 2000 mg/l) and then peptone added to wastewater in a declining ration to accelerate the initial growth of biofilm on the sheets but then acclimatize the biofilm to the wastewater. After acclimatizing the biofilm, batch operations were conducted, filling the tank with industrial wastewater. The wastewater was drawn from multiple sources in ratios chosen to create an feed COD of about 3000 mg/l. "Pure" oxygen was supplied to the modules at a feed pressure of about 5 psi. As shown in Figure 23, bulk CODs concentration dropped to less than 1000 mg/l in about 2 to 3 days. It was also noted that COD removal rates declined with bulk CODs concentration in the wastewater, and with time, during each batch.

[00121] COD removal rates were calculated at different periods of time during the batches corresponding to different concentrations of CODs in the tank. Batches having initial CODs of 5000 mg/l and 7000 mg/l were also tested to observe the effect of higher initial COD concentrations on COD removal rate. The results are presented in Figure 24. As indicated in Figure 24, removal rate was generally higher at higher loadings except that, in the reactor tested, very high loadings did not always produce very high removal rates suggesting that one or more of air feed pressure, surface area for air

transfer to biofilm surface area or total module area were less than optimum for very high loadings.

[00122] The same reactor was used for a series of trials conducted under continuous operation. In the trials, HRT and inlet CODs were varied. The feed gas was "pure" oxygen at a feed pressure of 5 psi. For each trial, the average inlet CODs, outlet CODs and removal rate, organized by HRT of the trial, are presented in Figure 25. COD removal rates generally decreased as HRT increased or as inlet CODs decreased.

[00123] The effectiveness of biofilm control procedures were also verified in the reactor during the batch trials mentioned above. Gentle aeration of about 1 scfm/module for 15 seconds every hour was applied, primarily for mixing, and more aggressive air scouring of about 4 scfm/module for 2-3 minutes every 2-3 days was applied primarily to remove biofilm. The biofilm thickness was successfully maintained in a range from about 0.2 mm to less than 0.8 mm regardless of the average bulk CODs in the reactor, which varied from about 300 mg/L to about 5,500 mg/L.

Example 4: Pilot Study with Municipal Wastewater

[00124] Another pilot study was conducted using two modules as described in Example 3, each having a surface area of about 3.6m^2, installed in an 85 litre tank. Air was supplied to the modules at a feed pressure of about 5 psi. Peptone was added initially to the sewage to accelerate the initial growth of biofilm on the sheets as described for example 3. Batch operations were conducted, filling the tank with municipal wastewater, screened through a 3mm screen, having an initial CODs of averaging about 100 to 200 mg/l, but occasionally up to 700 mg/L. At the ends of the batches, CODs concentration had generally dropped to less than 30 mg/l and COD removal rate had also generally dropped to less than 1 g/m2/d. The levels of CODs and CODt with respect to time within a sample period in a batch are presented in Figure 26.

[00125] A study was also conducted with a continuous process, with different trials performed over a total period of about 60 days. In the trials,

HRT varied from 24 hours to 3 hours and inlet CODs from 100 mg/l to 200 mg/l. Average removal rates tended to be lower with lower loading rates.

[00126] Nitrification and denitrification kinetics were also measured in the continuous process study. The results of 4 trials are presented in the following table.

Table 1: Nitrification and Denitrification in Continuous Operation

HRT (hr)	Inlet CODs (mg/L)	Inlet NH3-N (mg/L)	Outlet CODs (mg/L)	Outlet NH3-N (mg/L)	Outlet NO3-N (mg/L)
11.5	165	18.2	29	3.5	3.4
7.8	117	19.6	25	5.4	4.4
4.4	105	17.7	35.9	5.6	4.3
3.1	84	18.7	37.6	11.6	1.3

[00127] Biofilm control was also tested in the municipal wastewater study. Biofilm thickness averaging 0.2 mm was observed with air scouring, but thicker biofilm appeared to collect between some individual sheets indicating that these areas were not receiving full scouring air.

Example 5: Bench Scale Study with a Tow Module with Wastewater

[00128] A module similar to the one shown in Figure 5, having 100 PMP fibre tows, each tow having 96 fibres of dense walled PMP, was tested. The total surface area of the fibres in the module was 0.54 m^2. In the module, each tow was individually potted into an upper and lower header. The module was fed with a supply of air at a rate of 10 ml/min to the bottom header and exhausted out of the top header. The module was suspended, with the top header held in a clamp at the water surface and the bottom header weighed down, in a container filled to a volume of 4 L. The module was operated in a batch mode using a synthetic wastewater of 1000 mg/L CODs and also wastewater from a septic tank. At the start of each batch processing period, the container was filled with wastewater. Air was supplied to the module to support a biofilm growing on the fibres for processing periods ranging from

between about 1 to 7 days while wastewater was neither added to nor withdrawn from the tank. Shorter batch periods were generally used with wastewater having lower concentrations of COD. At the end of the processing period, the tank was drained. New wastewater was added to start the next processing period. At various times, the module was removed to non-destructively measure the thickness of the biofilm on them and measurements of the COD in the wastewater were taken.

[00129] The thickness measurements from the tests using synthetic wastewater are recorded in Figure 27 which shows the thickness of the biofilm on the fibres over the period of 180 days of operation. There was initially no biofilm but after about 20 or 40 days a biofilm had developed having a thickness that generally ranged between about 100 and 300 µm. For most of the test run, no additional methods were used to control the biofilm thickness and yet the biofilm thickness remained generally stable and acceptable. Small portions of biofilm were observed to be shed from the module during at least some of the tank draining operations, and biofilm control was otherwise provided by endogenous growth of the biofilm. However, for a period of approximately 15 days, the module was operated in a starvation mode. In this mode, the tank was filled with tap water and air feed was continued. The biofilm was reduced in thickness from about 250 µm to about 100 µm during the starvation period indicating that the starvation period was effective at reducing the thickness of the biofilm.

[00130] Figures 28 and 29 show the removal rate of COD in tests using the synthetic wastewater. Figure 28 shows removal rate as a function of time and Figure 29 shows removal rate as a function of COD concentration. Referring first to Figure 28, each vertical line within the figure indicates the start of a new batch processing period. Accordingly, at the times indicated by the vertical lines, new wastewater having a COD of 1,000 mg/L was added to the tank. As the batch progresses, the wastewater is treated and accordingly its COD concentration reduces. As shown in Figure 28, the COD removal rate tended to drop with time in each batch processing period suggesting that the

removal rate is related to the COD concentration in the wastewater. Further, the removal rate in the batch between day 154 and day 159 approached zero indicating that further processing time would have marginal value. In Figure 29, the COD removal rate is plotted directly against the average COD concentration in the wastewater. As indicated in Figure 29, the relationship between COD removal rate and COD concentration in the wastewater is nearly linear with the removal rate being generally proportional to the COD concentration.

[00131] For the tests using septic tank wastewater, the wastewater was taken from the second chamber of a septic tank. For one trial, the characteristics of the wastewater were as follows:

Total Chemical Oxygen Demand (COD_t): 377 mg/L

Soluble COD (COD_s): 199 mg/L

Ammonia Nitrogen (AN): 55.1 mg/L

Total Suspended Solids (TSS): 70 mg/L

The module was operated in a batch mode with batch processing periods of approximately 24 hours to simulate actual reaction conditions in a septic tank. Air was supplied during these periods at the rate given above to provide oxygen to the biofilm. After one processing period of 22 hours and 35 minutes in duration, a sample of the treated wastewater was analyzed and results were as follows:

COD_t: 140 mg/L

COD_s: 73 mg/L

AN: 24.7 mg/L

TSS: 1 mg/L

A significant improvement in effluent quality was achieved. In particular, a huge reduction in TSS was achieved. By visual observation, a large portion of the TSS removed was in the form of colloidal matter.

[00132] Figure 30 records the results from another trial using septic tank wastewater. The reactor was operated for a two-day batch period with concentration of CODt, CODs TSS and ammonia nitrogen measured at the beginning, middle and end of the batch period. For comparison purposes, another sample of wastewater taken from the same septic tank on the same day was placed in a 500 mL graduated cylinder and monitored as a control. After two days of operation, reduction of Total COD (CODt) in the reactor approached 75 mg/L, with a removal in excess of 70%. TSS dropped from 34 mg/L to almost no appreciable TSS after two days of treatment. Ammonia was also reduced during this period. During the same period, the control had a less than 40% reduction in COD and an increase in TSS. The batch process and reactor effectively treated the septic tank wastewater by removing COD but also removing suspended solids, in part because of the quiescent nature of the process.

Example 6 – Chemical Biofilm Control:

[00133] A biofilm control study was done using the single sheet reactor described in Example 1 with a very thick biofilm on it. At the start of the test, the tank was drained and 30 L of sodium hydroxide solution in deionized water at a pH of 9.43 and a temperature of 40 C was added to the reactor. After a first 4 hours of soak, air scouring at 2 scfm was started and was continued for more than 18 hours while sodium hydroxide solution remained in the tank. Air supply to the lumens remained on. The biofilm thickness was reduced slightly (4.6 mm to 4.3 mm) over the first four hour period. After the 18 hours of soaking and air scouring, the thickness of the biofim was reduced further to 3.2 mm.

[00134] In another biofilm control study, 6 single sheet modules as shown in Figure 10a and 10b were used. Each sheet was about 27 cm long by 20 cm wide and had an available surface area of about 0.11 square meters. The sheets were woven with the hollow fibers running lengthwise and open at both ends. The ratio of air transfer area to biofilm area was about 6 to 1. The modules were placed in a 20 L (working volume) reactor operated

in batch mode at room temperature with batch periods of about 3 days. The reactor was fed with synthetic sewage at concentrations from 2000 to 8000 mg/L CODs. Air was fed to the lumens of the modules at about 2 psi with a flow rate of about 20 mL/min to an inlet header of each sheet. At intervals of from 3 to 7 days, between batches, the modules were soaked for 4 hours in a solution of NaOH in hot water with a pH of 10 at 50 C. Air supply to the lumens remained on. After the 4 hours, the reactor was re-filled with feed. No air scouring was provided during the soak periods or during the batch periods. Figure 31 shows the biofilm thickness over time which was maintained between 0.2 and 0.8 mm and averaged about 550 microns over a 140 day period. Calculated results from the batches during that period indicate that during the interval between cleanings the biofilm removed from 66 to 120 grams of CODs per square meter.

[00135] Many modifications and variations of the present invention are possible within the teachings of the invention and the invention may be practiced other than as described above. The scope of the invention is defined by the following claims.

Claims:

We claim:

1. An apparatus for supporting a biofilm in a liquid comprising:

a) a plurality of gas permeable hollow fibers, each hollow fiber having a lumen, an outer surface and an open end; and,

b) a header, the header having a cavity and a port open to the cavity,

wherein the hollow fibers extend from the header, with the outer surfaces of the open ends of the hollow fibers sealed to the header and the lumens of the hollow fibers communicating with the port through the cavity.

2. The apparatus of claim 1 wherein the hollow fibers have an outside diameter of 100 microns or less.

3. The apparatus of claim 1 or 2 wherein the hollow fibers have a hollow area of 10% or more, more preferably 30% or more.

4. The apparatus of any preceding claim wherein the hollow area is 50% or less.

5. The apparatus of any preceding claim wherein the hollow fibers are non-porous or dense walled.

6. The apparatus of any preceding claim wherein the hollow fibers comprise polymethyl pentene.

7. The apparatus of any preceding claim wherein the hollow fibers have a second end and are between 0.25 metres and 3.0 metres long.

8. The apparatus of any preceding claim wherein the hollow fibers have a second end and are between 1.0 metres and 2.0 metres long.

9. The apparatus of any preceding claim wherein the hollow fibers are arranged into groups.

10. The apparatus of claim 9 wherein the groups comprise between 24 and 96 hollow fibers.

11. The apparatus of claim 9 or 10 wherein the groups further comprise second fibers that are stronger than the hollow fibers.

12. The apparatus of any of claims 9 to 11 wherein the group is a tow of fibers.

13. The apparatus of any of claims 9 to 11 wherein the group is a thread, yarn or twisted fibers.

14. The apparatus of any preceding claims wherein the hollow fibers are curled, crimped or undulating along their length.

15. The apparatus of any preceding claim wherein the hollow fibers extend along their length generally in a first direction.

16. The apparatus of claim 15 further comprising third fibers extending along their length generally in a second direction, the second perpendicular to the first direction.

17. The apparatus of claim 16 wherein the third fibers and hollow fibers are intertwined.

18. The apparatus of claim 17 wherein the hollow fibers and third fibers form a fabric.

19. The apparatus of claim 18 wherein the fabric is generally continuous across the length of the hollow fibers.

20. The apparatus of claim 18 wherein the fabric extends over a portion of the length of the hollow fibers near their open ends and does not extend over a central portion of the length of the fibers.

21. The apparatus of any of claims 16 to 20 wherein the hollow fibers and third fibers are woven, knitted, stitched or warp knitted together over at least a portion of the length of the hollow fibers.

22. The apparatus of any preceding claim wherein the hollow fibers have second open ends.

23. The apparatus of claim 22 wherein the second open ends of the hollow fibers are potted in a second header.

24. The apparatus of claim 23 wherein the second open ends communicate with a second port of the second header through a second cavity of the second header.

25. The apparatus of claim 23 or 24 wherein the header and the second header are spaced apart from each other and the hollow fibers are arranged into one or more flat sheets or generally parallel planar structures extending between the headers.

26. The apparatus of claim 25 wherein the flat sheets or planar structures are generally parallel to each other.

27. One or more of the apparatus of claims 25 or 26 wherein adjacent planar structures have a spacing between them of between 2 mm and 20 mm or, more preferably, of between 3 mm and 15 mm.

28. The apparatus of any of claims 23 to 27 wherein the first header and second header are held apart at a distance that applies a tensile force to the hollow fibers.

29. The apparatus of any of claims 25 to 28 further comprising spacers between the flat sheets or planar elements outside of the header.

30. The apparatus of any of claims 25 to 29 wherein the flat sheets or planar structures further comprise a rigid member extending between the headers.

31. The apparatus of any preceding claim having a surface area for oxygen transfer to surface area of supported biofilm ratio of about 1.6 or more.

32. The apparatus of any preceding claim having a surface area for oxygen transfer to surface area of supported biofilm ratio of about 2 or more.

33. The apparatus of any preceding claim having a surface area for oxygen transfer to surface area of supported biofilm ratio of about 5 or more.

34. The apparatus of any preceding claim having a surface area for oxygen transfer to surface area of supported biofilm ratio of about 1 or less.

35. The apparatus of any of claims 18 to 21 wherein the roughness of the fabric is between 0.1 and 2 mm.

36. A reactor comprising:

a) a tank for holding a liquid to be treated, the tank having an inlet and an outlet;

b) an apparatus according to any preceding claim; and,

c) a gas delivery system for providing a gas to the port.

37. The reactor of claim 36 further comprising an agitator or aerator adapted to agitate the liquid around the apparatus.

38. The reactor of claim 36 or 37 further comprising a chemical injection system for injecting chemicals into either the lumens of the hollow fibers or a part of the reactor in communication with the outer surfaces of the hollow fibers.

39. The reactor of any of claims 36 to 38 having a heater to heat either the gas provided to the port or the liquid held in the tank.

40. A multi-stage reactor having two or more reactors according to any of claims 36 to 39, the outlet of a first reactor connected to the inlet of a second reactor.

41. The multi-stage reactor of claim 40 wherein the first and second reactors are plug flow reactors, batch reactors or continuously stirred reactors.

42. The multi-stage reactor of claim 37 or 38 wherein the apparatus of the second reactor has a lower surface area for oxygen transfer to surface area of supported biofilm ratio than the apparatus of the first reactor.

43. The multi-stage reactor of any of claim 40 to 42 wherein the apparatus of the first reactor has a surface area for oxygen transfer to surface area of supported biofilm ratio between of 5 or more and the apparatus of the second reactor has a surface area for oxygen transfer to surface area of supported biofilm ratio of 5 or less.

44. The reactor or multi-stage reactor of any of claims 36 to 43 wherein the reactor(s) have a plurality of the apparatus arranged in parallel between the inlet and outlet.

45. The multi-stage reactor of any of claims 40 to 42 wherein the fibers of the apparatus of the first reactor are formed into a sheet along their entire length while the fibers of the apparatus of the second reactor are unsupported by perpendicular fibers over a portion of their length.

46. A process for treating a liquid comprising the steps of:

a) contacting an apparatus according to any of claims 1 to 35, or another apparatus having a port in communication with one or more inner surfaces of a gas permeable biofilm support medium with the liquid; and,

b) providing a gas to the port of the apparatus, the gas permeating to outer surface(s) of the medium to support a biofilm growing on the outer surface(s).

47. The process of claim 46 wherein the liquid comprises wastewater.

48. The process of claim 46 or 47 wherein the gas comprises oxygen.

49. The process of any of claims 46 to 48 wherein the gas comprises hydrogen.

50. The process of claim 47 or 48 wherein the biofilm is maintained in an aerobic state adjacent the outer surface(s) and in an anoxic or anaerobic state adjacent the liquid.

51. The process of any of claims 46 to 50 wherein the liquid is contacted with the apparatus in a batch or continuous process.

52. The process of any of claims 46 to 51 wherein the liquid is generally continuously or intermittently stirred.

53. The process of any of claims 46 to 51 wherein the liquid moves past the outer surface(s) in a generally plug flow.

54. The process of any of claims 46 to 53 wherein the biofilm is maintained in a state of generally endogenous growth.

55. The process of claim 54 performed in a septic tank or shipboard system or to treat a wastewater taken generally directly from one or more houses or businesses or parts of a ship.

56. The process of any of claims 46 to 55 wherein the biofilm is maintained at a thickness between 0.05 mm and 2 mm, more preferably between 0.1 mm and 1 mm.

57. The process of any of claims 46 to 53 or 56 further comprising the steps of maintaining a least a portion of the biofilm so that its thickness alternately increases and decreases, the biofilm increasing in thickness over first periods of time and, between the first periods of time, reducing the thickness of the biofilm.

58. The process of claim 57 wherein the thickness of only a portion of the biofilm is reduced at a time.

59. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by air scouring or agitating at least a portion of the liquid.

60. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by contacting at least a portion of the biofilm with a second liquid containing worms or other animals which digest the biofilm.

61. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by applying ozone to at least a portion of the biofilm from the lemen

side of the fibers or from the outside of the biofilm to oxidize the portion of the biofilm and then maintaining the biofilm to digest the oxidized portion.

62. The process of claim 61 wherein the thickness of the biofilm is reduced by introducing ozone gas into the port followed by supplying oxygen to the port.

63. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by supplying air to the port while the liquid is removed from contact with the biofilm or provided at a loading of less than 0.1 kg CODs per kg MLSS per day to digest the biofilm aerobically.

64. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by applying a control agent to at least a portion of the outer surface of the biofilm.

65. The process of claim 64 wherein the control agent is clean water.

66. The process of claim 64 wherein the control agent is heated clean water, preferably heated to between 40 and 60 C.

67. The process of claim 64 wherein the control agent is ozone gas.

68. The process of claim 64 wherein the control agent is an alkali solution with a pH between 8 and 13, more preferably between 9 and 11.

69. The process of claim 64 wherein the control agent is an acid with a pH between 1 and 6, more preferably between 3 and 4.

70. The process of claim 64 wherein the control agent is a second liquid and the second liquid is agitated or aerated while in contact with the biofilm.

71. The process of any of claims 64 to 70 wherein the biofilm is digested aerobically after the control agent is applied.

72. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by draining the liquid away from contact with the biofilm.

73. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by stopping or reducing the supply of oxygen to the port from time to time or periodically to create alternating aerobic and anoxic or anaerobic conditions in a portion of the biolfilm.

74. The process of claim 57 or 58 wherein the thickness of the biofilm is reduced by physically removing a portion of the biofilm.

75. The process of claim 74 wherein the biofilm is physically removed by spraying it with a third liquid or scraping it with a brush or scraper.

76. The process of any of claims 57 to 75 wherein the liquid is removed from a portion of the biofilm while the thickness of that portion of the biofilm is being reduced.

77. The process of any of claims 46 to 76 wherein the amount of oxygen supplied to the port is increased during a period of time when the CODs of the liquid is increased.

78. The process of any of claims 46 to 49 wherein the liquid is periodically removed from the biofilm and replaced with a fresh batch of liquid and the supply of the gas is continued while the liquid is being removed, while the biofilm is not in contact with the liquid or while a fresh batch of liquid is being replaced in contact with the biofilm.

79. The process of any of claims 46 to 78 wherein the liquid, after being treated, has less than 10 mg/L of suspended solids and less than 50 mg/L of CODs.

80. The process of any of claims 46 to 79 operated in a two stage process wherein the first stage of the process reduces the CODs of the liquid to less than 300 mg/L, more preferably to between 200 and 300 mg/L.

81. The process of any of claims 46 to 80 wherein the liquid, before treatment, has a CODs of 1000 mg/L or more and the apparatus has a surface area for gas transfer to surface area of attached biofilm of 1 or more, more preferably between 1 and 10.

82. The process of any of claims 46 to 80 wherein the liquid, before treatment, has a CODs of 1000 mg/L or less and the apparatus has a surface area for gas transfer to surface area of attached biofilm of between 0.2 and 2.5.

83. The process of any of claims 46 to 80 wherein the liquid, before treatment, has a CODs of 300 mg/L or less and the apparatus has a surface area for gas transfer to surface area of attached biofilm of 1 or less, more preferably between 0.1 and 1.

84. A method for cutting the ends of fibers in the apparatus of any preceding claim comprising the steps of gluing a potting resin around the open or looped ends of a plurality of the fibers and then cutting through the resulting block of hardened resin and fibers.

85. A method of producing an apparatus according to any of claims 18 to 27 comprising the steps of and adhering spacers to the planar member(s) parallel to but displaced from the open ends of the hollow fibers, a first edge of the spacers being nearer the ends of the hollow fibers and a second edge

of the spacers being farther from the ends of the hollow fibers, inserting the planar member(s) into a header cavity, and applying a potting resin over the second edge of the spacers extending from the planar member(s) to walls of the header cavity.

86. The process of any of claims 57-76 wherein the thickness of the biofilm is reduced at least every 10 days or after the biofilm has digested between 20 and 200 grams of CODs per square metre of biofilm area since the last reduction.

87. The process of any of claims 46 to 83 operated as a batch process having steps of draining the liquid from a tank containing the apparatus, the draining step further comprising a step of draining a first part of the liquid containing settled solids to a first treatment system and draining a second part of the liquid to a second treatment system.

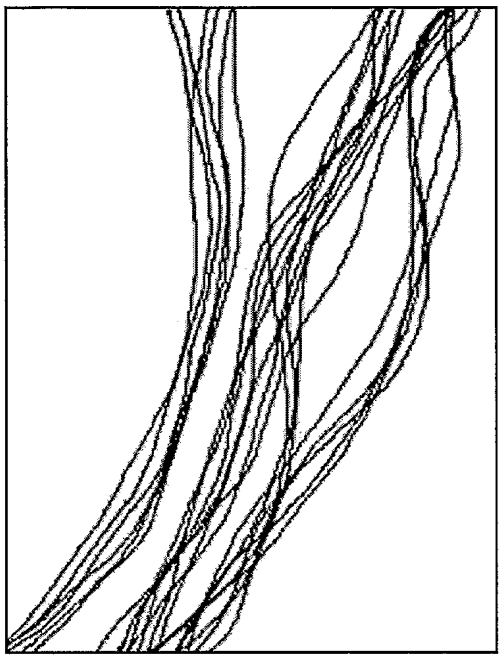


FIG. 1

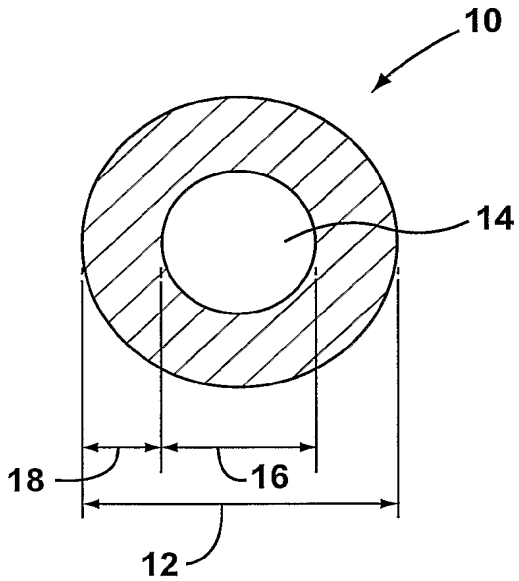


FIG. 1a

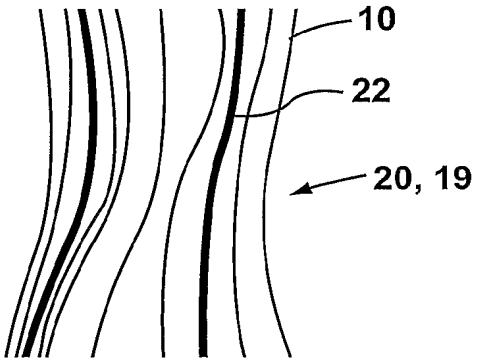


FIG. 1b

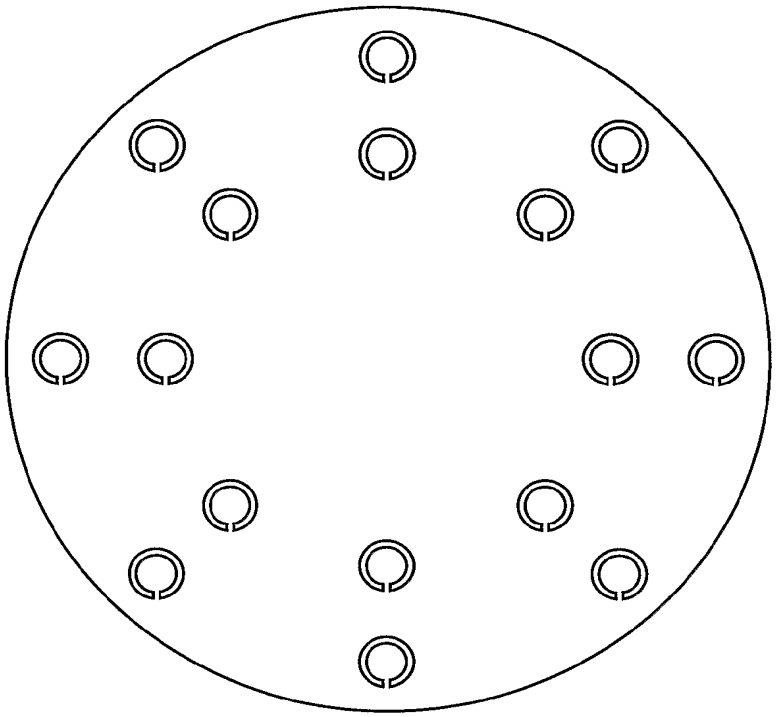


FIG. 2 E



FIG. 2a

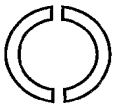


FIG. 2b



FIG. 2c



FIG. 2d

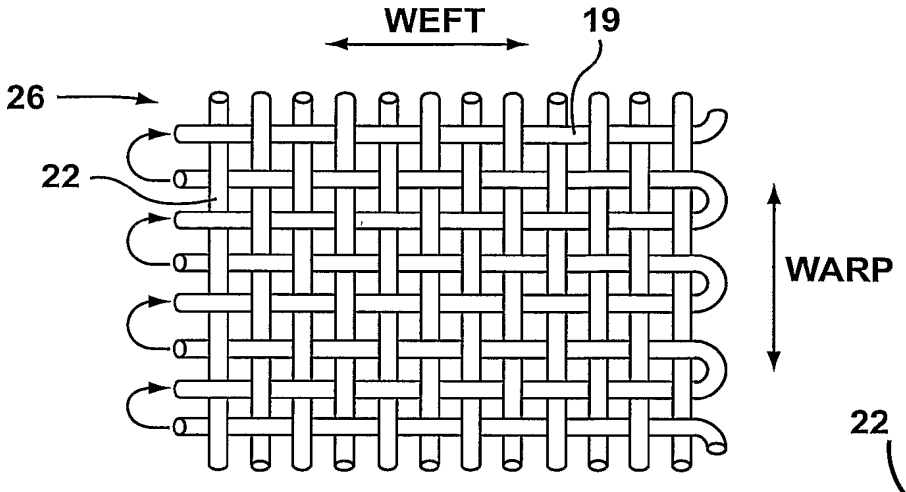


FIG. 3a

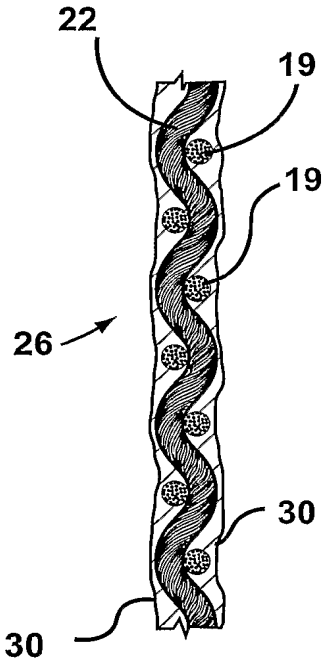


FIG. 3b

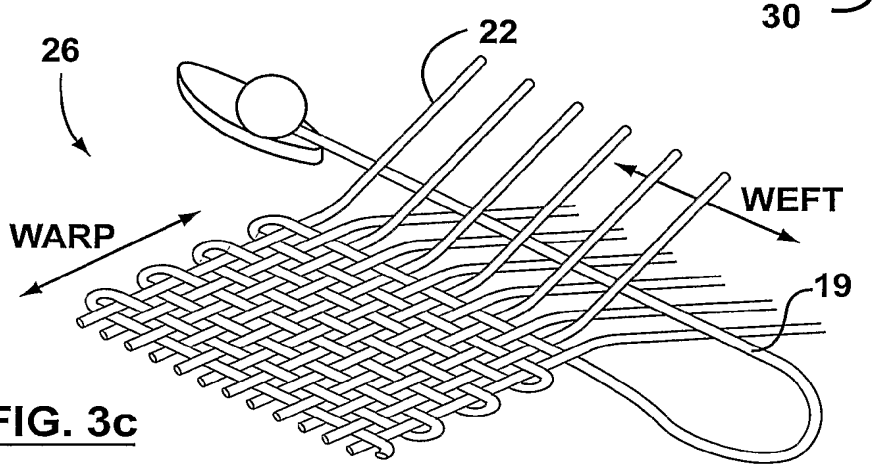


FIG. 3c

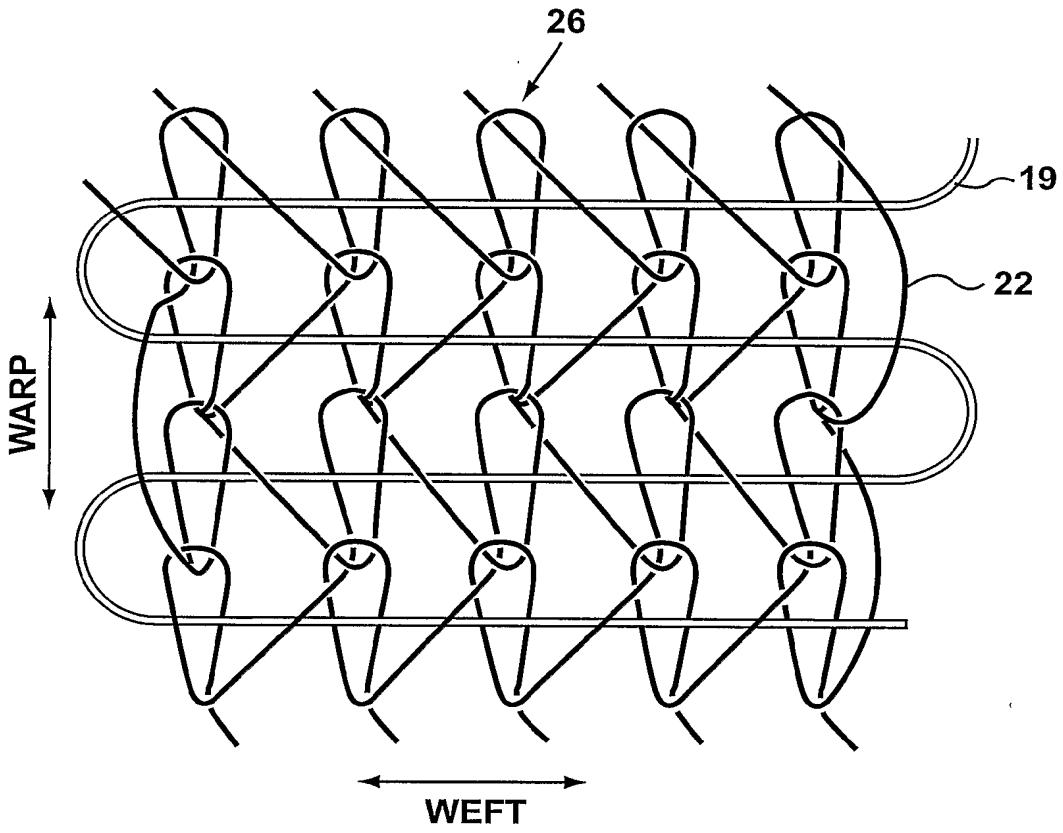


FIG. 3d

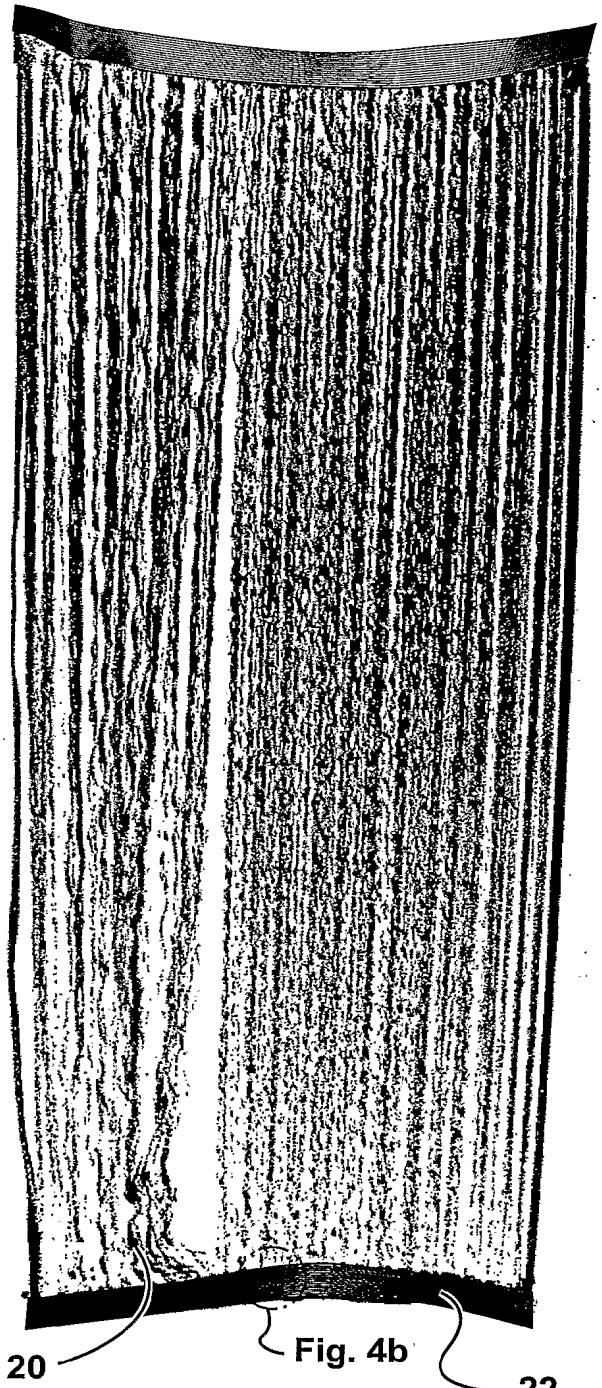


FIG. 4a

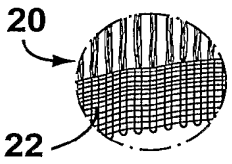


FIG. 4b

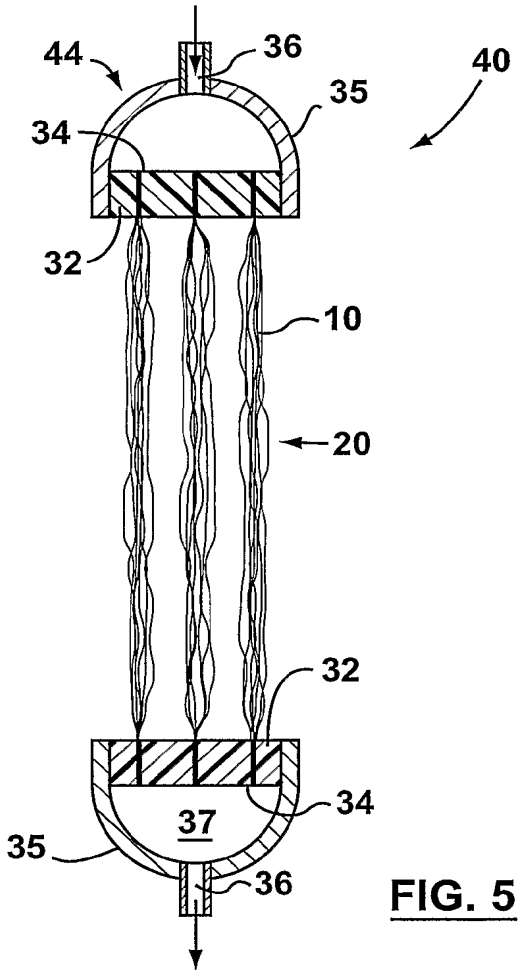


FIG. 5

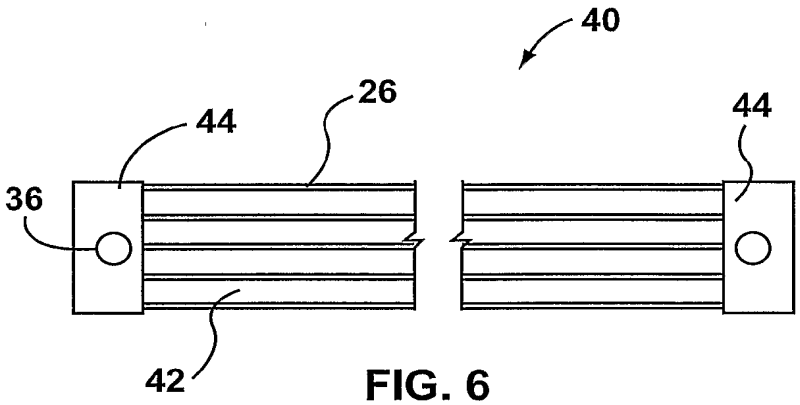


FIG. 6

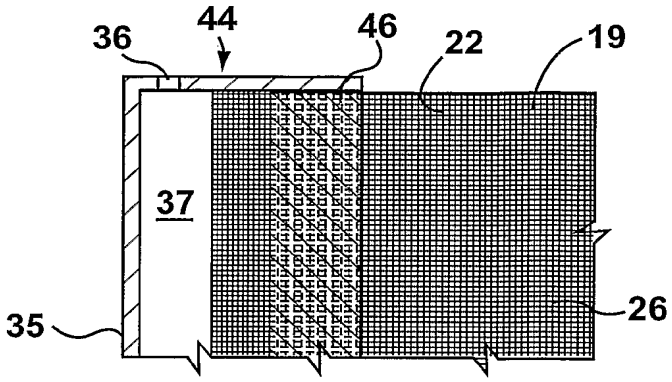


FIG. 7

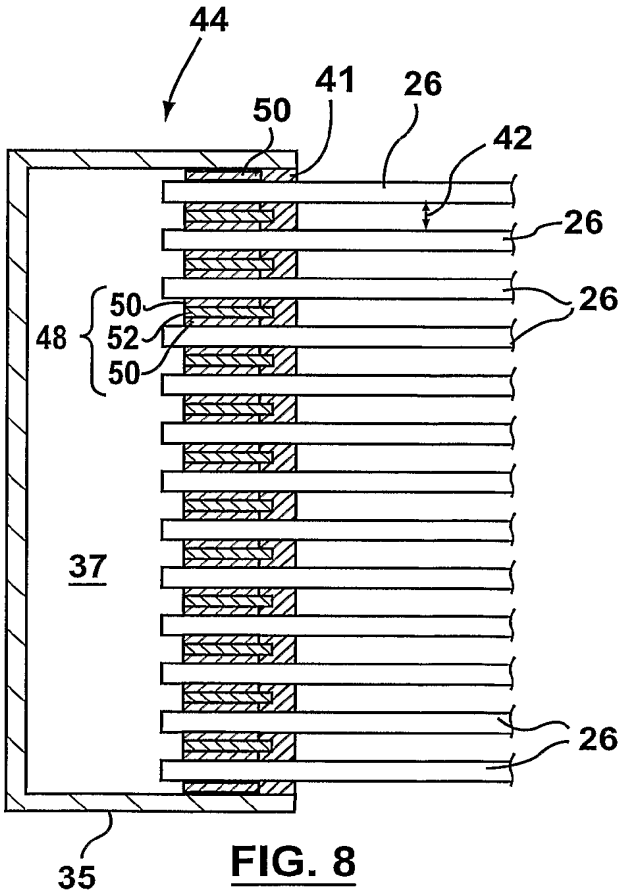


FIG. 8

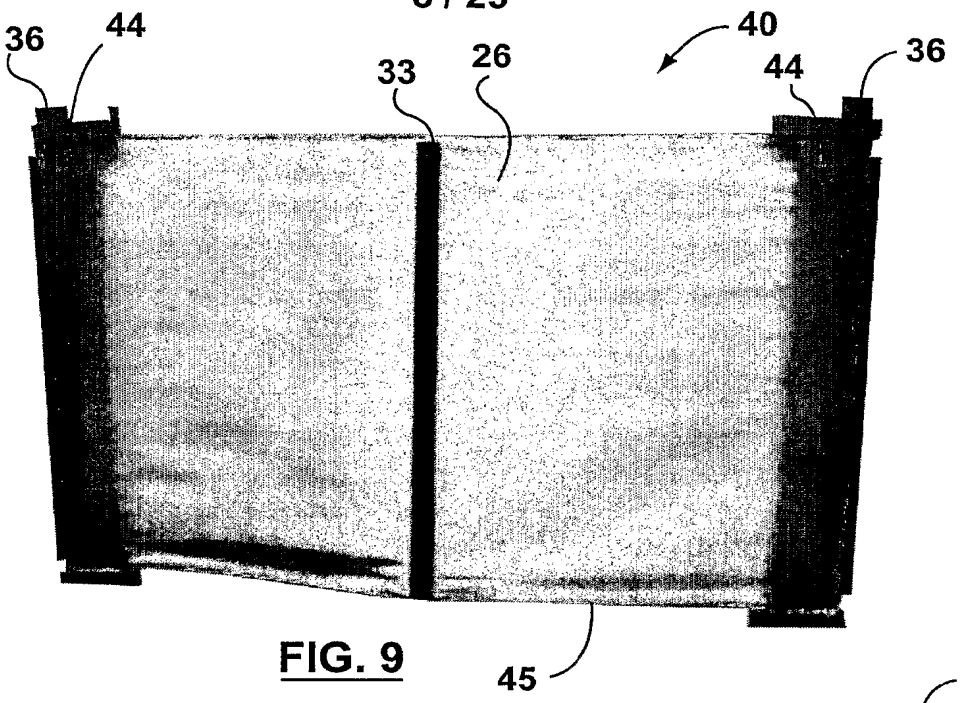


FIG. 9

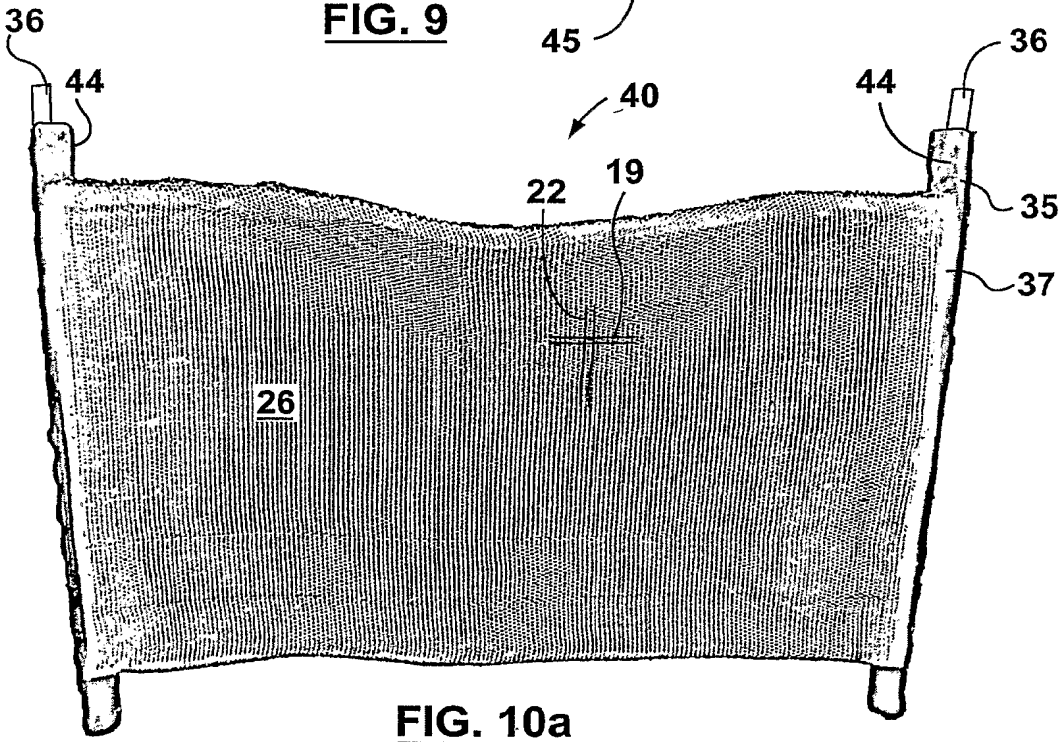


FIG. 10a

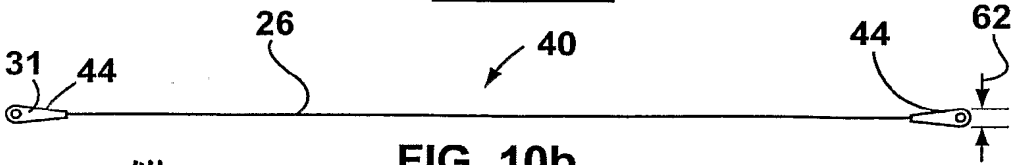


FIG. 10b

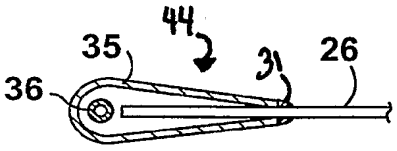


FIG. 10c

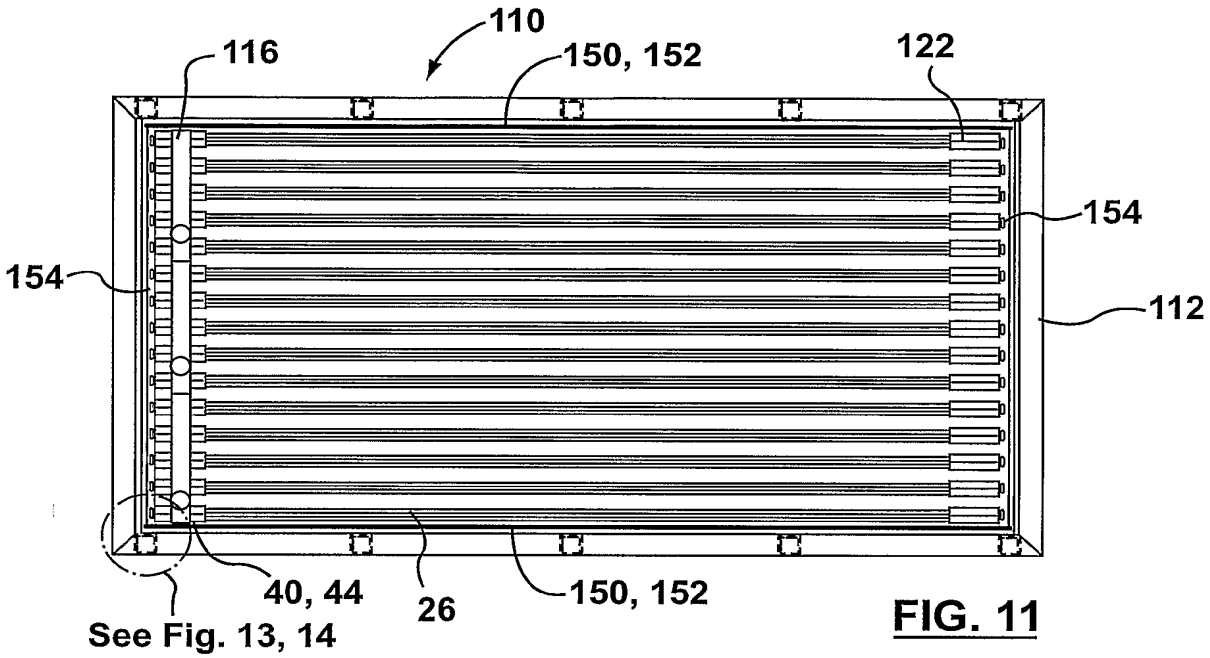

110
116
150, 152
122
154
154
112
40, 44
26
150, 152
See Fig. 13, 14

FIG. 11

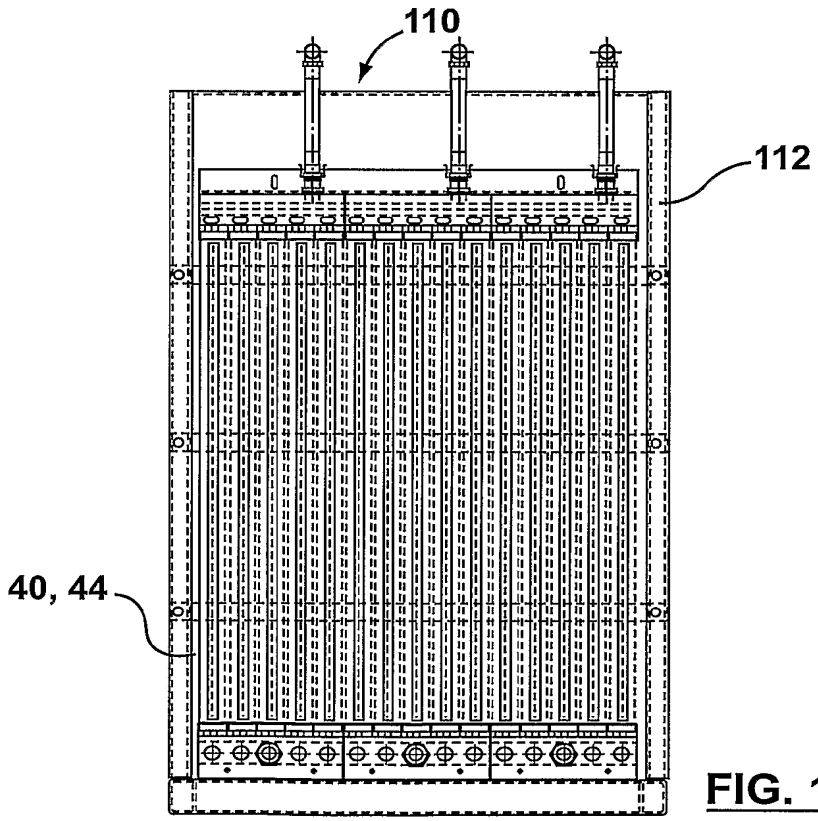

110
112
40, 44

FIG. 12

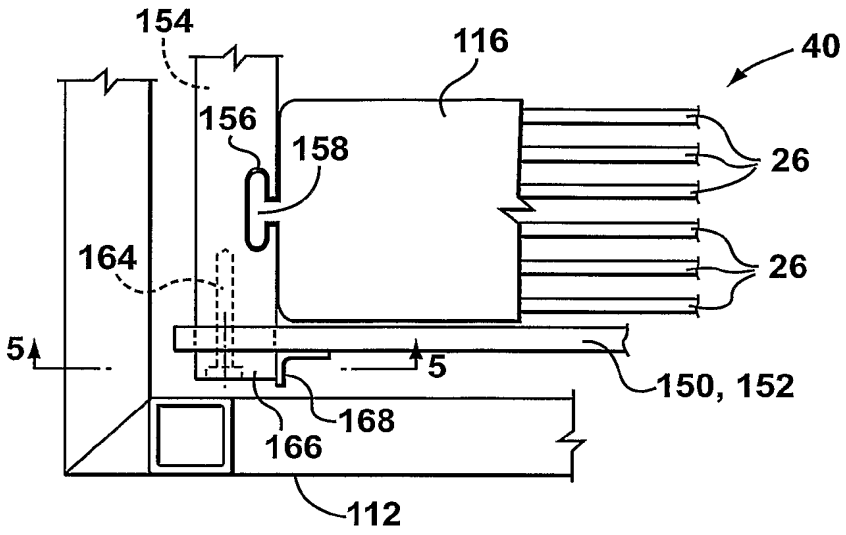

154
116
40
156
158
26
164
26
5
5
150, 152
168
166
112

FIG. 13

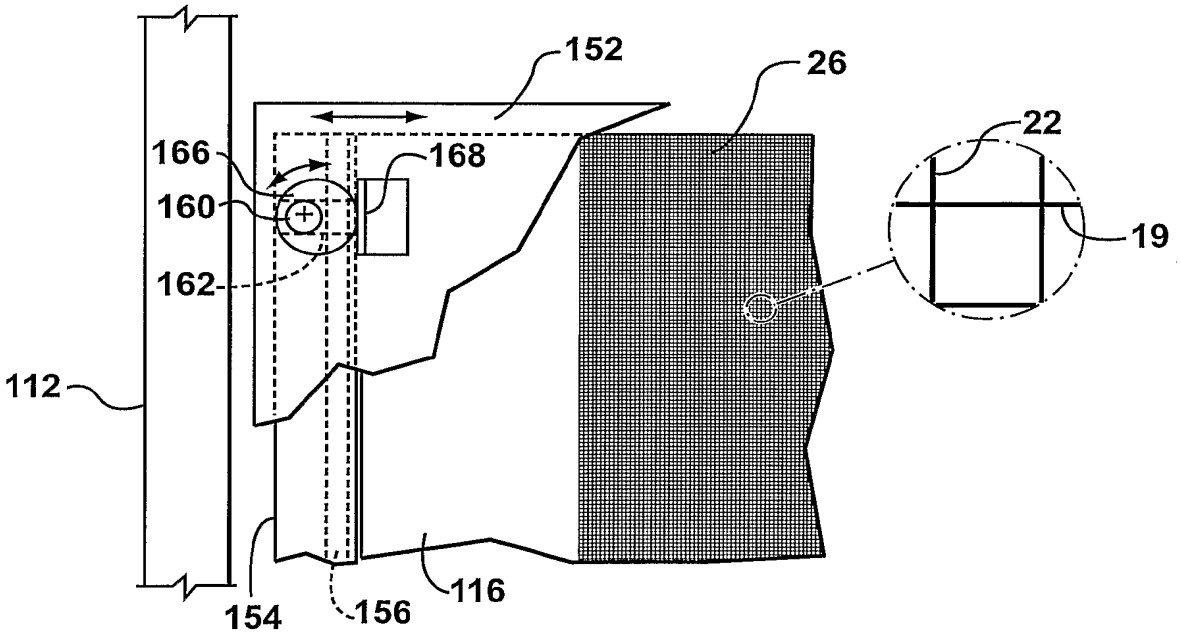

152
26
22
166
168
160
19
162
112
154
156
116

FIG. 14

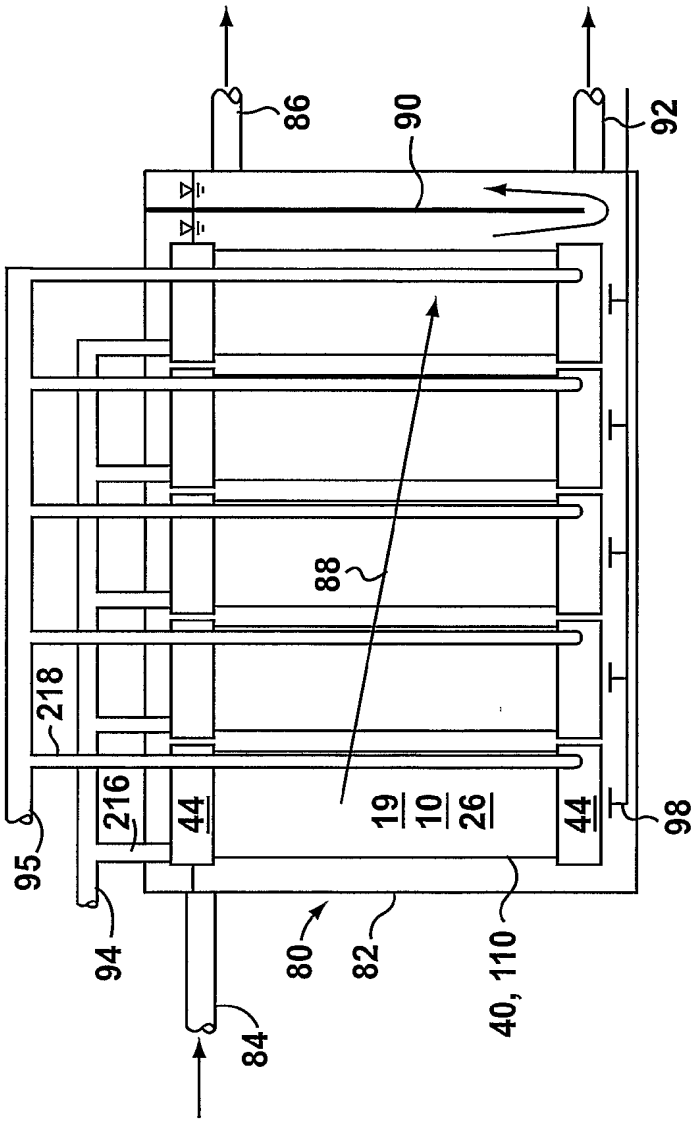


FIG. 15

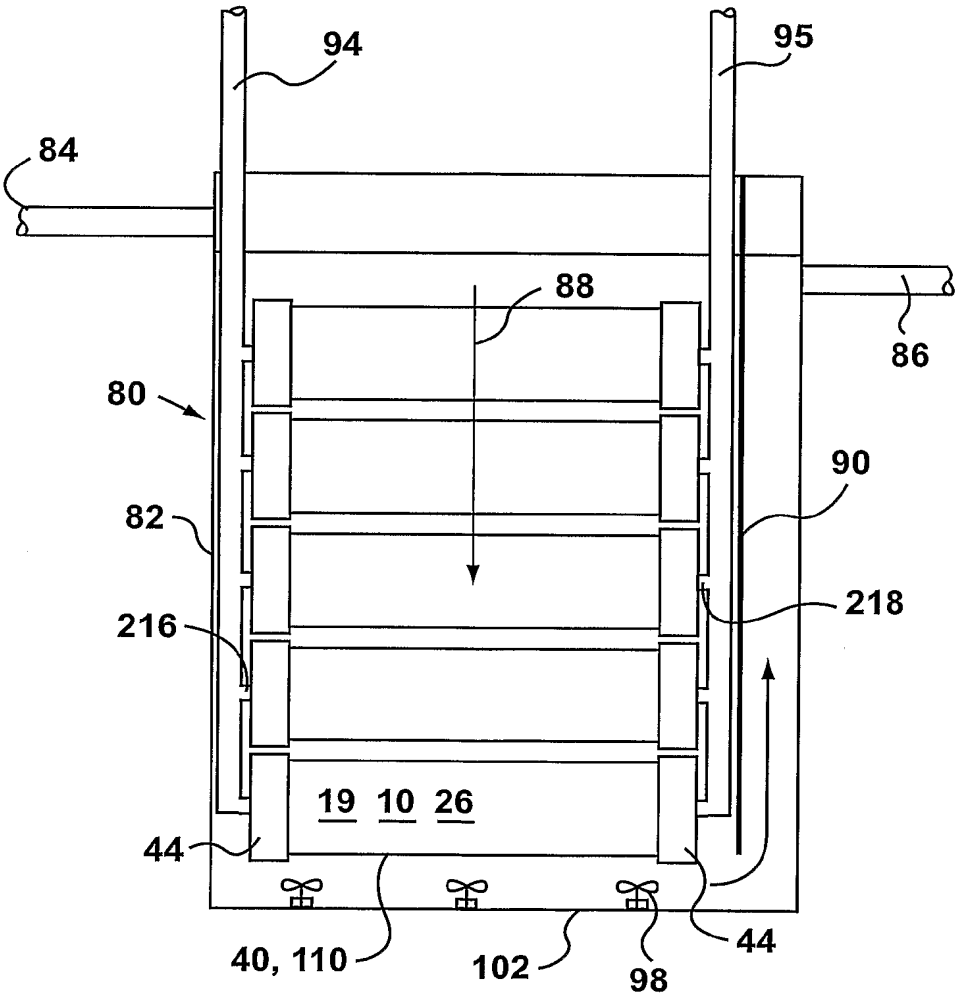


FIG. 16

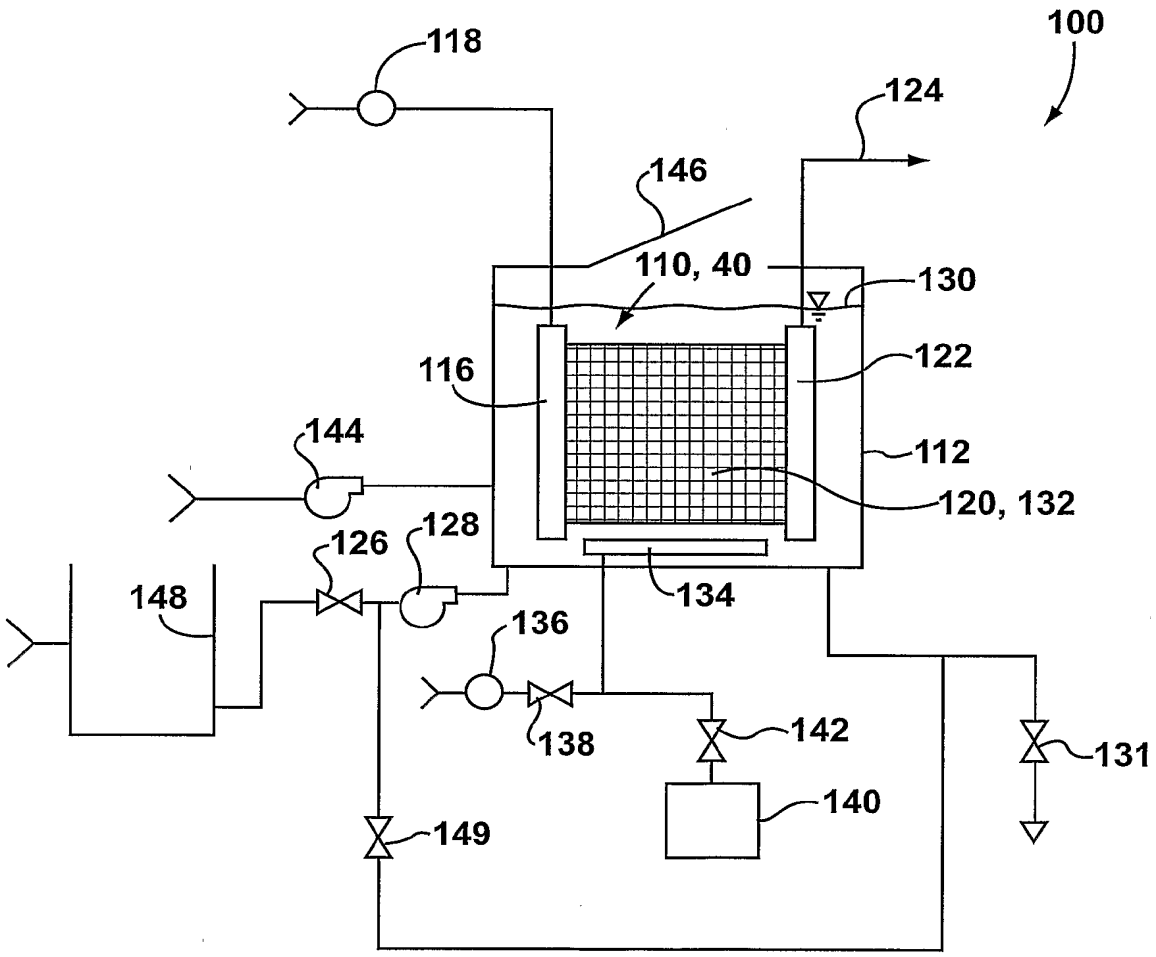

100
118
124
146
110, 40
130
116
122
144
112
120, 132
126
128
148
134
136
138
142
131
140
149

FIG. 17

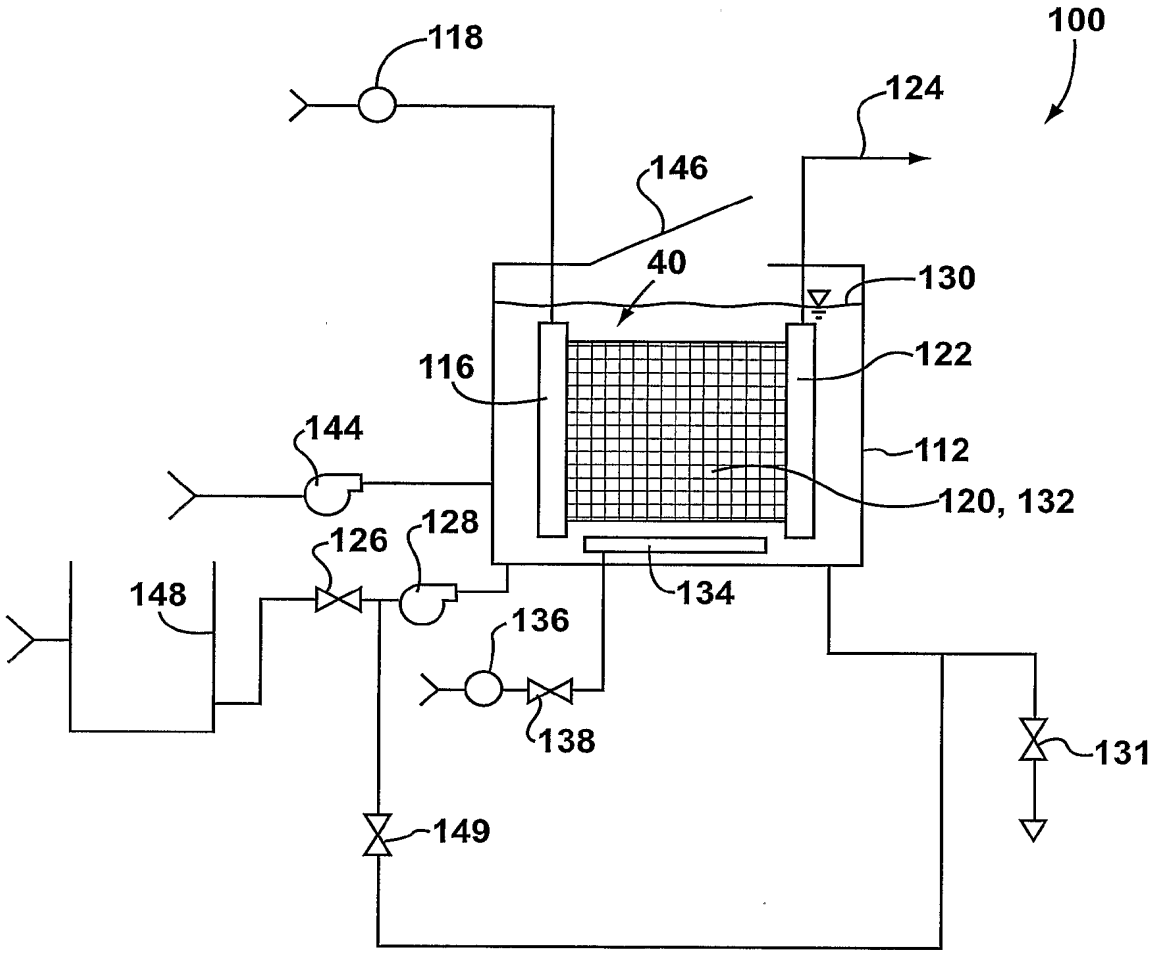

100
118
124
146
40
130
116
122
144
112
120, 132
126
128
148
134
136
138
131
149

FIG. 18

FIG. 19b

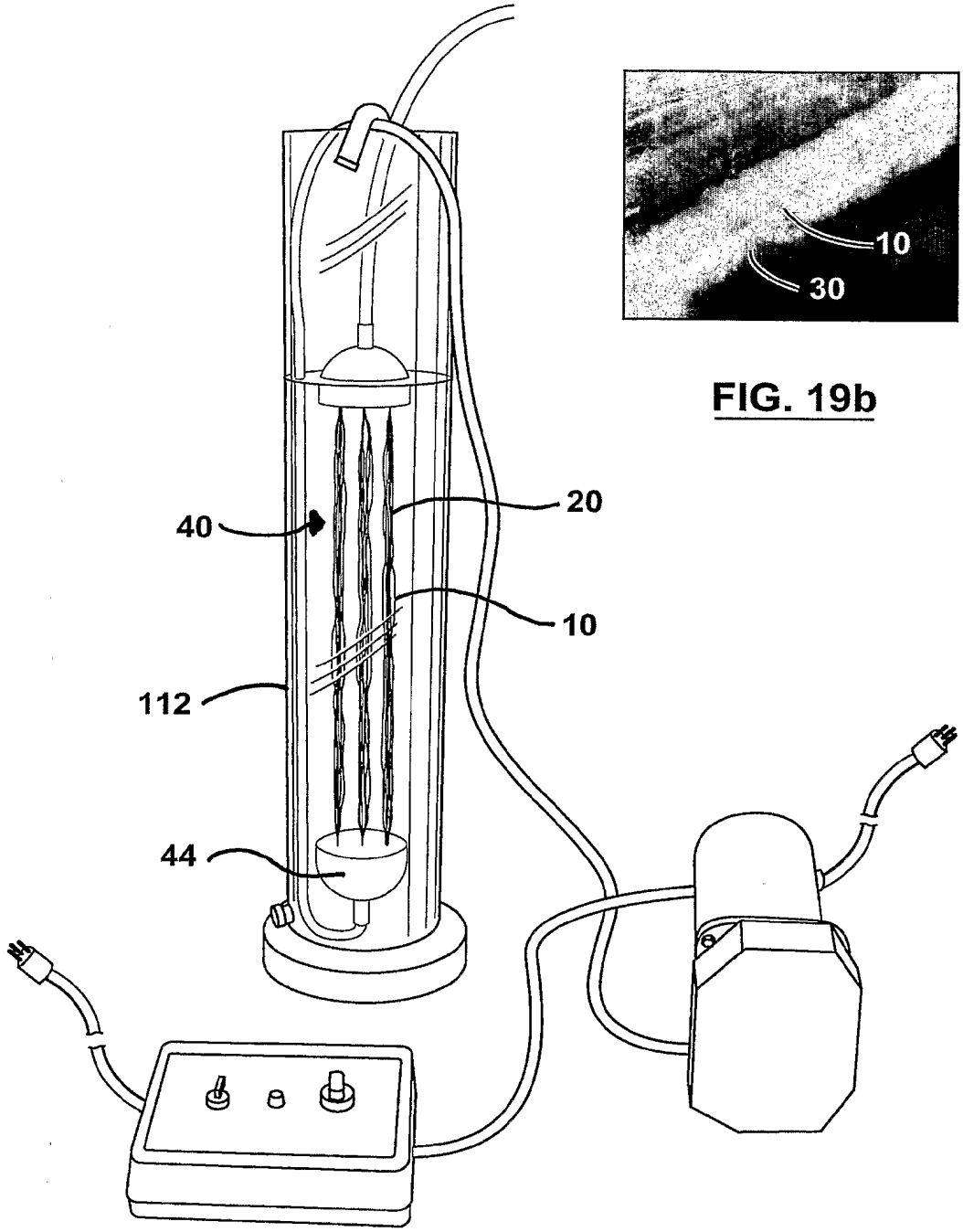


FIG. 19a

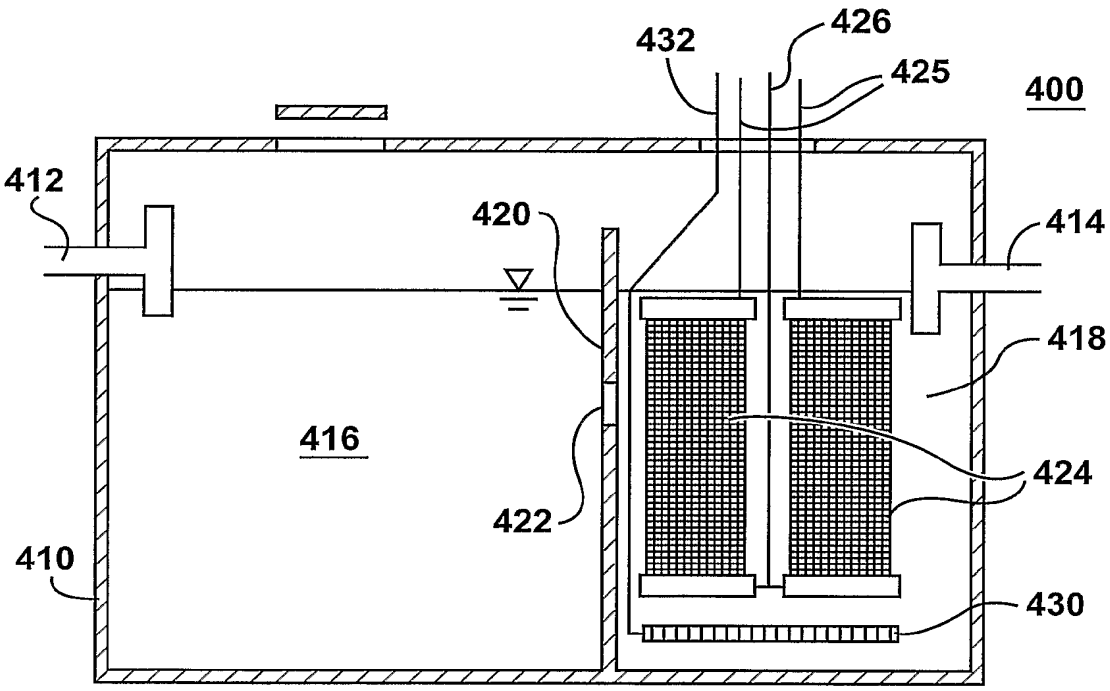


FIG. 20

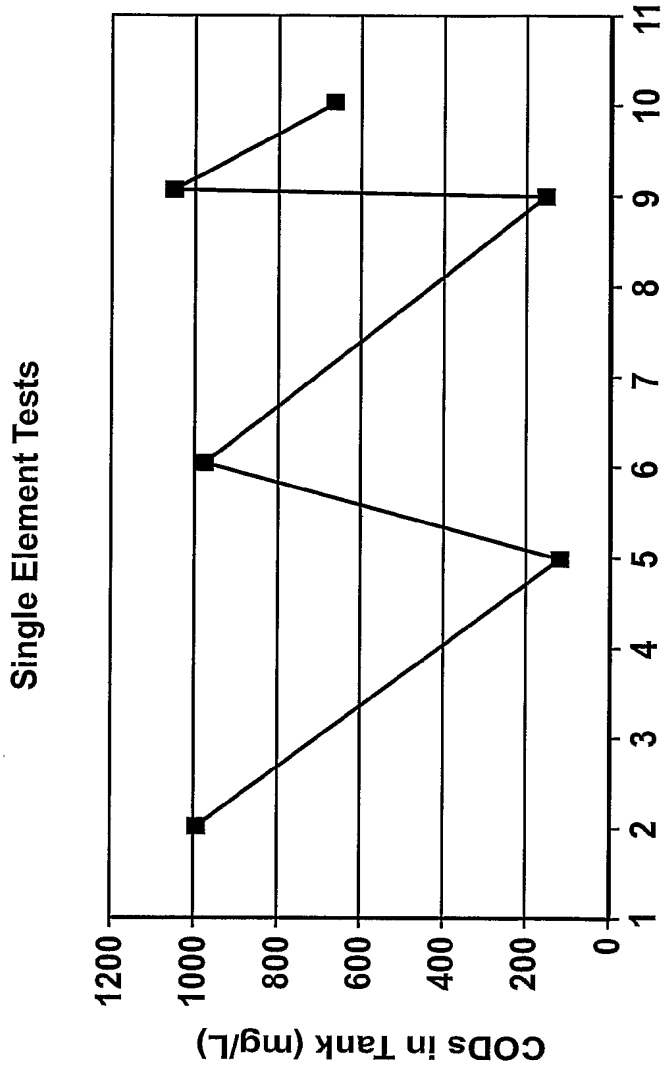

Single Element Tests
CODs in Tank (mg/L)
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
10
11
Time (Days)

FIG. 21

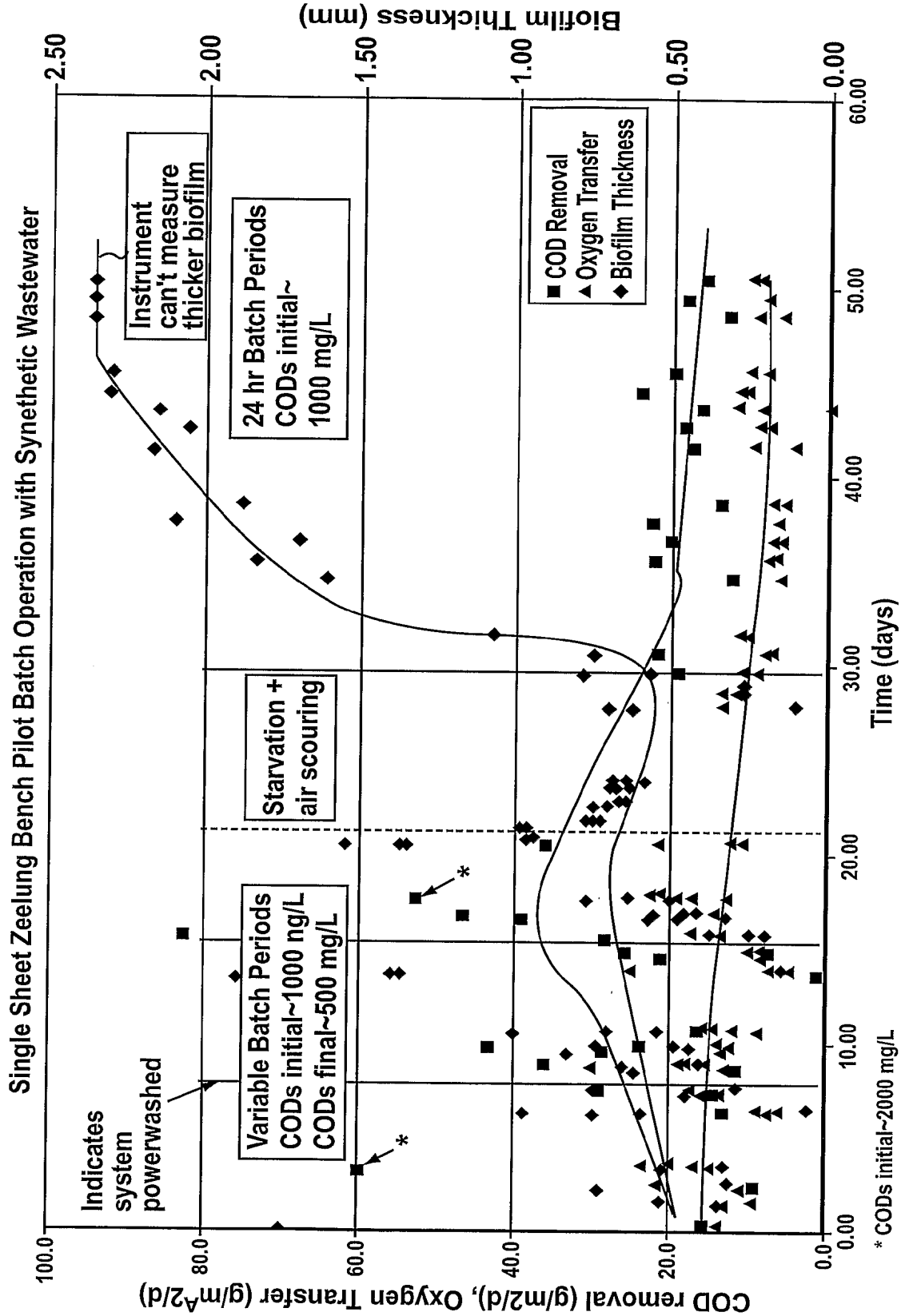

Single Sheet Zeelung Bench Pilot Batch Operation with Synethetic Wastewater
COD removal (g/m2/d), Oxygen Transfer (g/m^A2/d)
Biofilm Thickness (mm)
Time (days)
Instrument can't measure thicker biofilm
24 hr Batch Periods CODs initial~ 1000 mg/L
Starvation + air scouring
Variable Batch Periods CODs initial~1000 ng/L CODs final~500 mg/L
Indicates system powerwashed
COD Removal
Oxygen Transfer
Biofilm Thickness
* CODs initial~2000 mg/L

FIG. 22

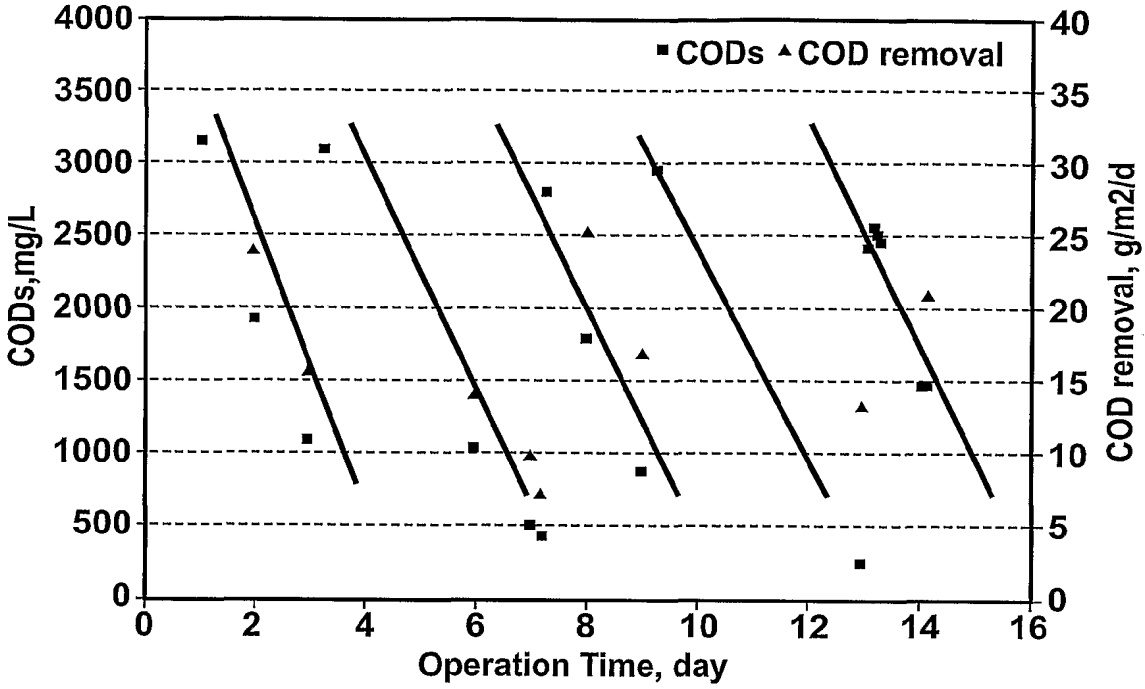

CODs
COD removal
CODs,mg/L
COD removal, g/m2/d
Operation Time, day
4000
3500
3000
2500
2000
1500
1000
500
0
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16

FIG. 23

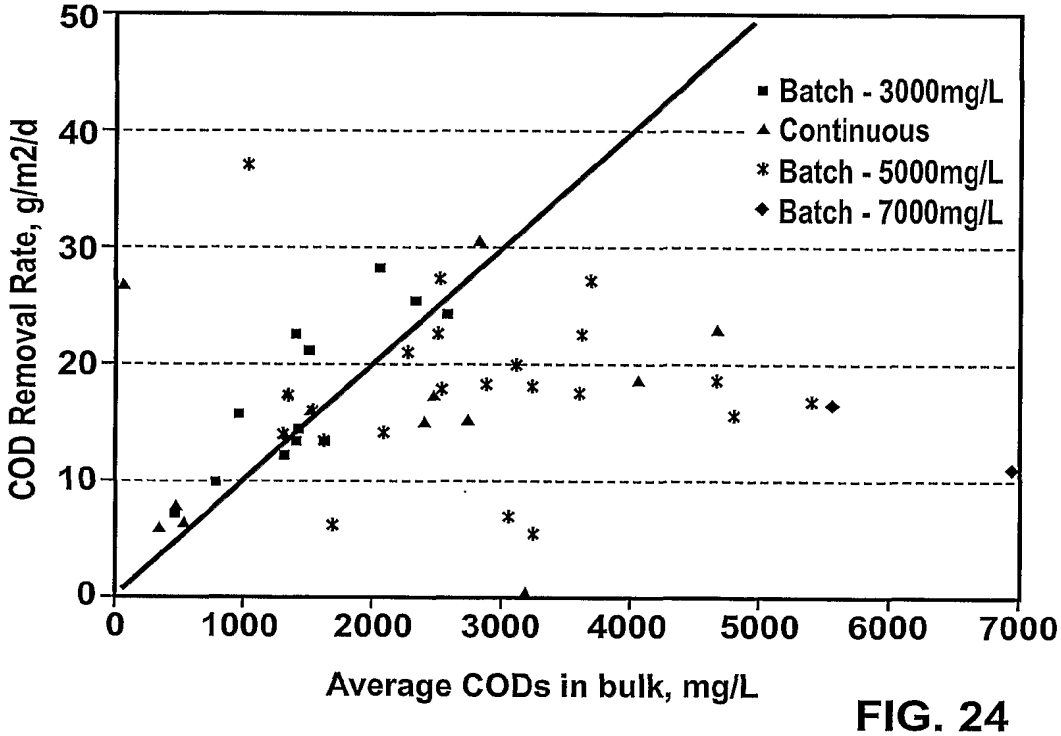

Batch - 3000mg/L
Continuous
Batch - 5000mg/L
Batch - 7000mg/L
COD Removal Rate, g/m2/d
Average CODs in bulk, mg/L
50
40
30
20
10
0
0
1000
2000
3000
4000
5000
6000
7000

FIG. 24

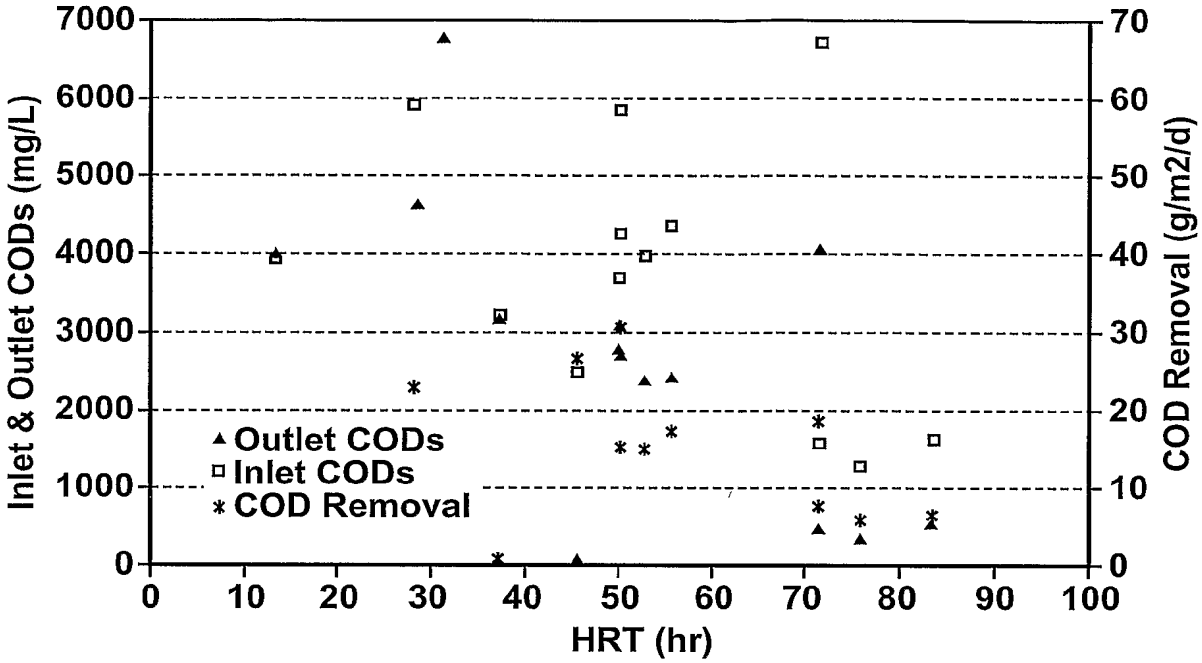

Inlet & Outlet CODs (mg/L)
COD Removal (g/m2/d)
HRT (hr)
Outlet CODs
Inlet CODs
COD Removal
0
1000
2000
3000
4000
5000
6000
7000
0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70
80
90
100

FIG. 25

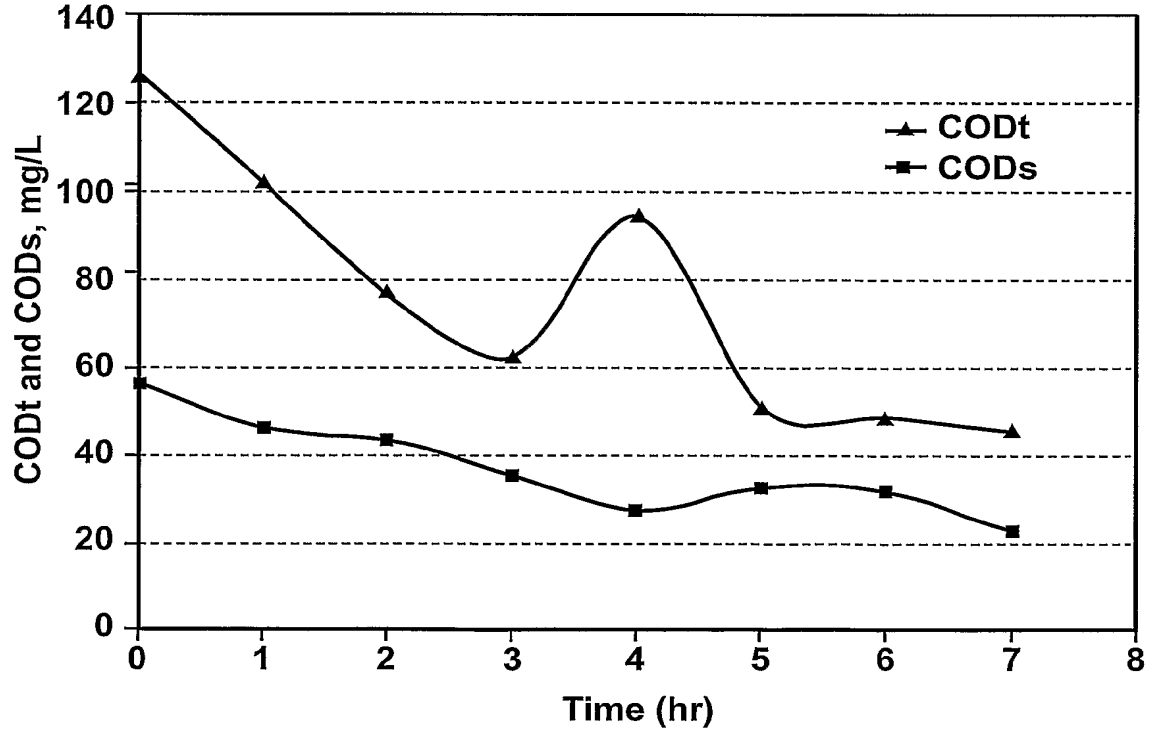

CODt and CODs, mg/L
Time (hr)
CODt
CODs
0
20
40
60
80
100
120
140
0
1
2
3
4
5
6
7
8

FIG. 26

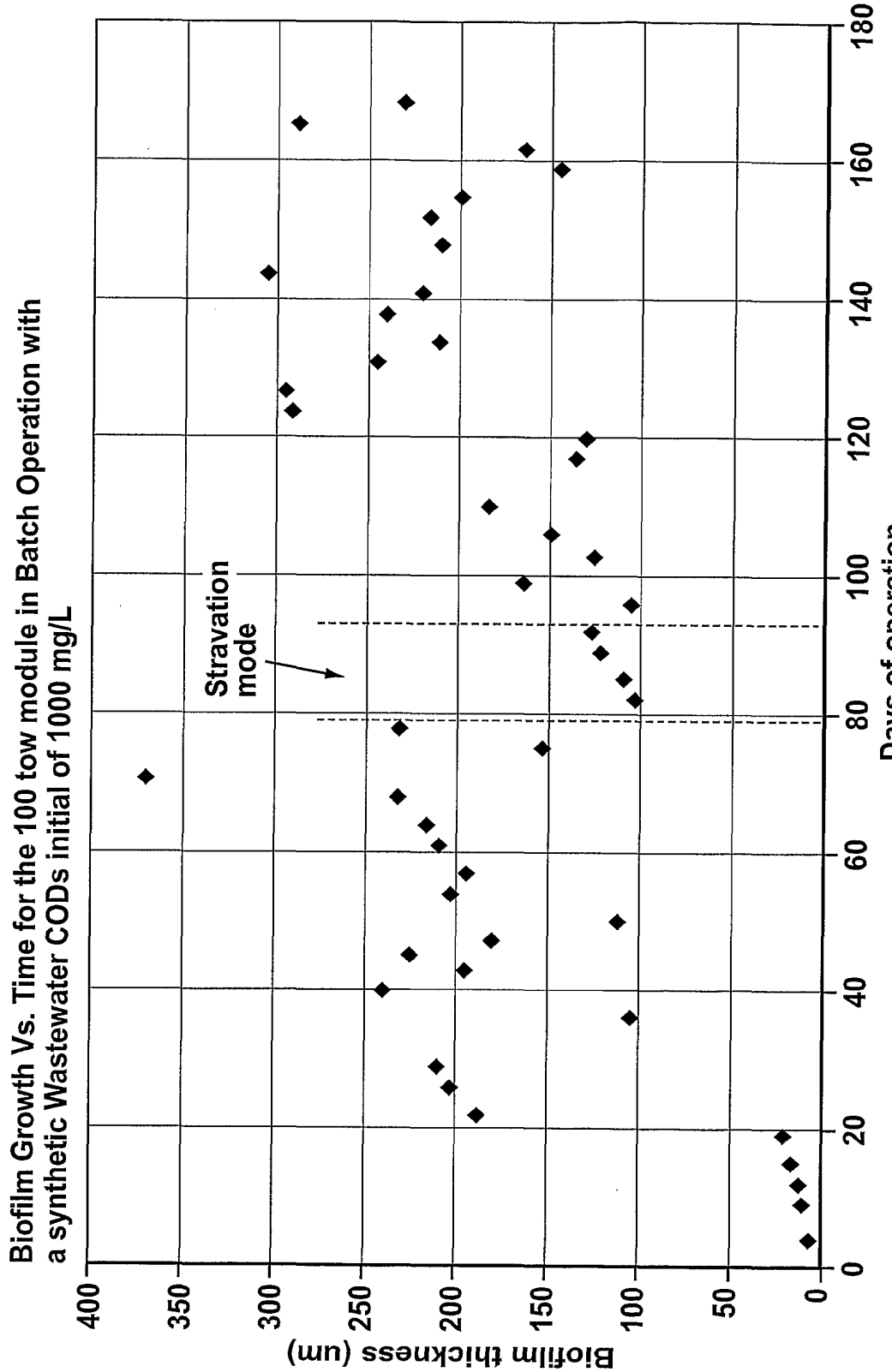

Biofilm Growth Vs. Time for the 100 tow module in Batch Operation with a synthetic Wastewater CODs initial of 1000 mg/L
Stravation mode
Biofilm thickness (um)
Days of operation
0
50
100
150
200
250
300
350
400
0
20
40
60
80
100
120
140
160
180

FIG. 27

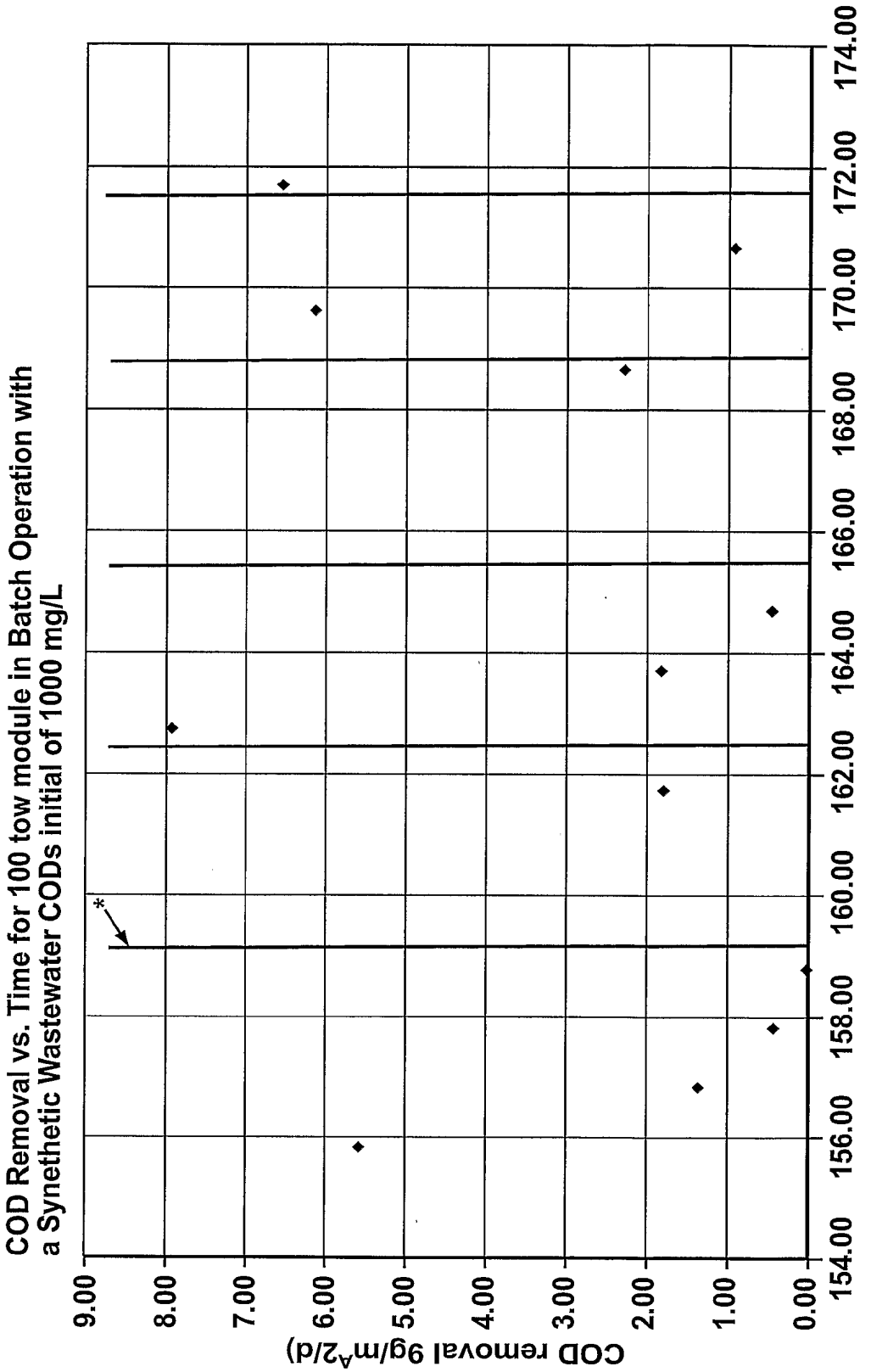

COD Removal vs. Time for 100 tow module in Batch Operation with a Synethetic Wastewater CODs initial of 1000 mg/L
COD removal 9g/m^2/d)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
154.00
156.00
158.00
160.00
162.00
164.00
166.00
168.00
170.00
172.00
174.00
Time (days)
*
* New batch run started

FIG. 28

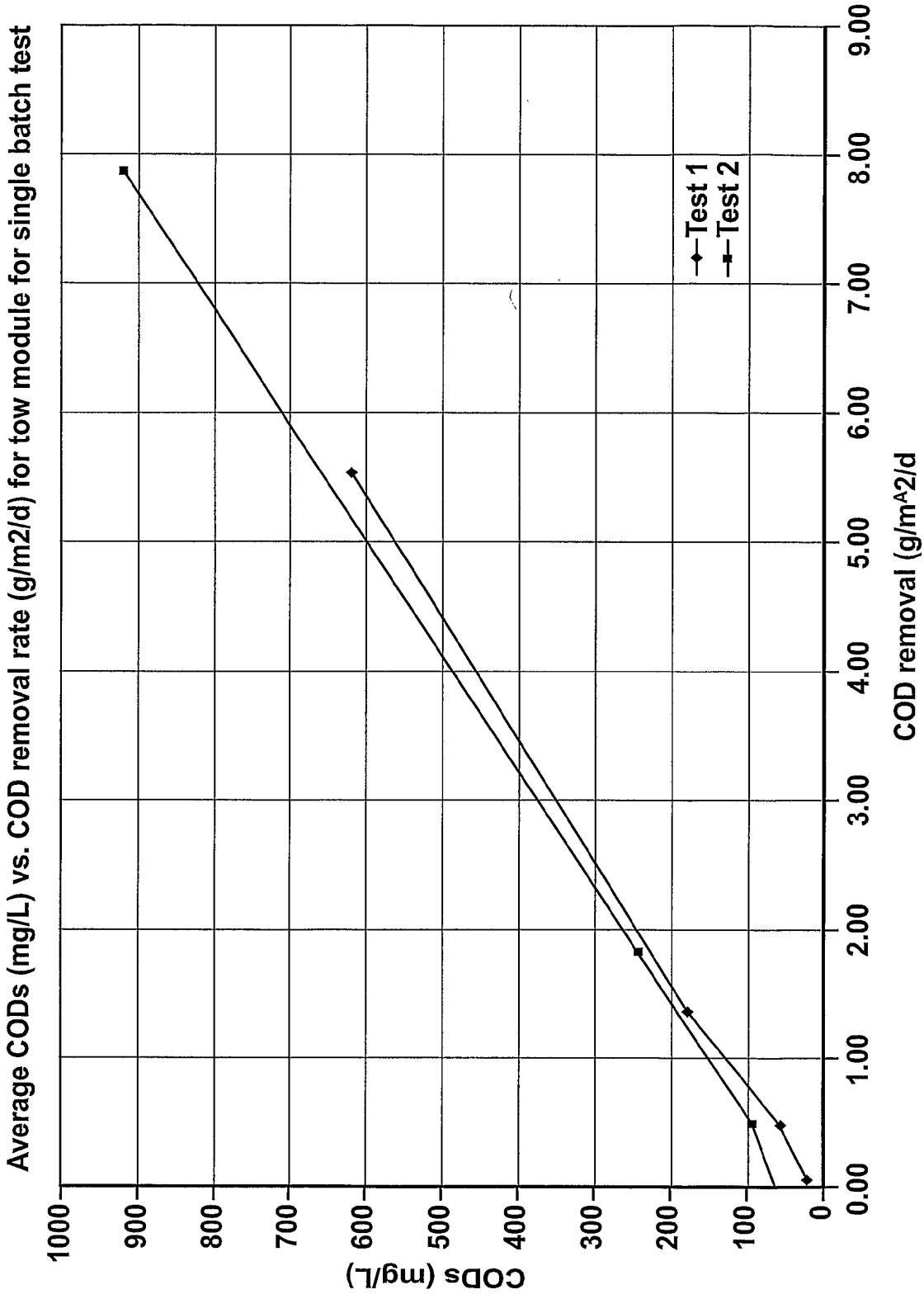

Average CODs (mg/L) vs. COD removal rate (g/m2/d) for tow module for single batch test
CODs (mg/L)
1000
900
800
700
600
500
400
300
200
100
0
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
COD removal (g/mA2/d
Test 1
Test 2

FIG. 29

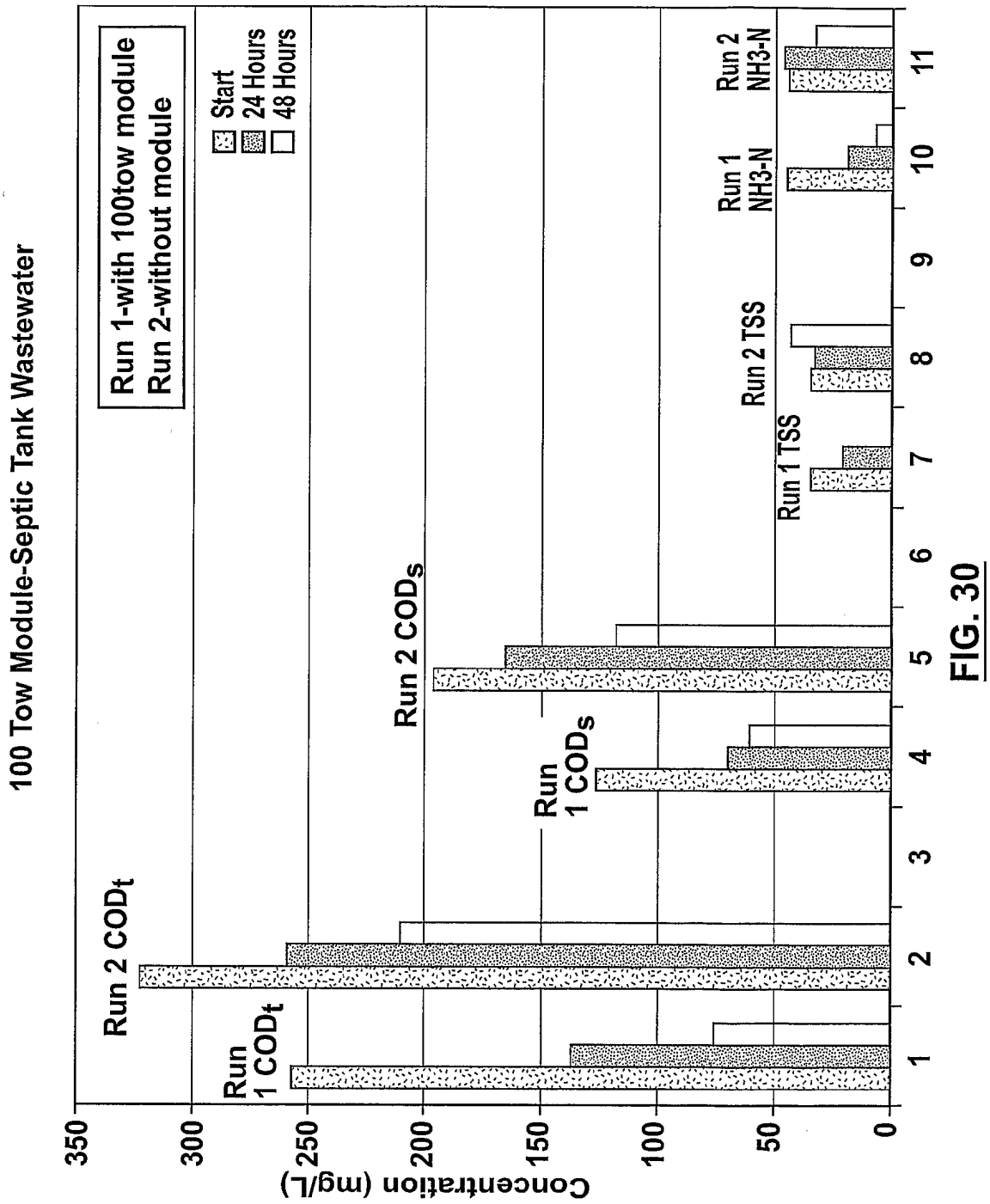

100 Tow Module-Septic Tank Wastewater
Run 1-with 100tow module
Run 2-without module
Start
24 Hours
48 Hours
Run 2 CODt
Run 1 CODt
Run 2 CODs
Run 1 CODs
Run 2 TSS
Run 1 TSS
Run 1 NH3-N
Run 2 NH3-N
Concentration (mg/L)
0
50
100
150
200
250
300
350
1
2
3
4
5
6
7
8
9
10
11

FIG. 30

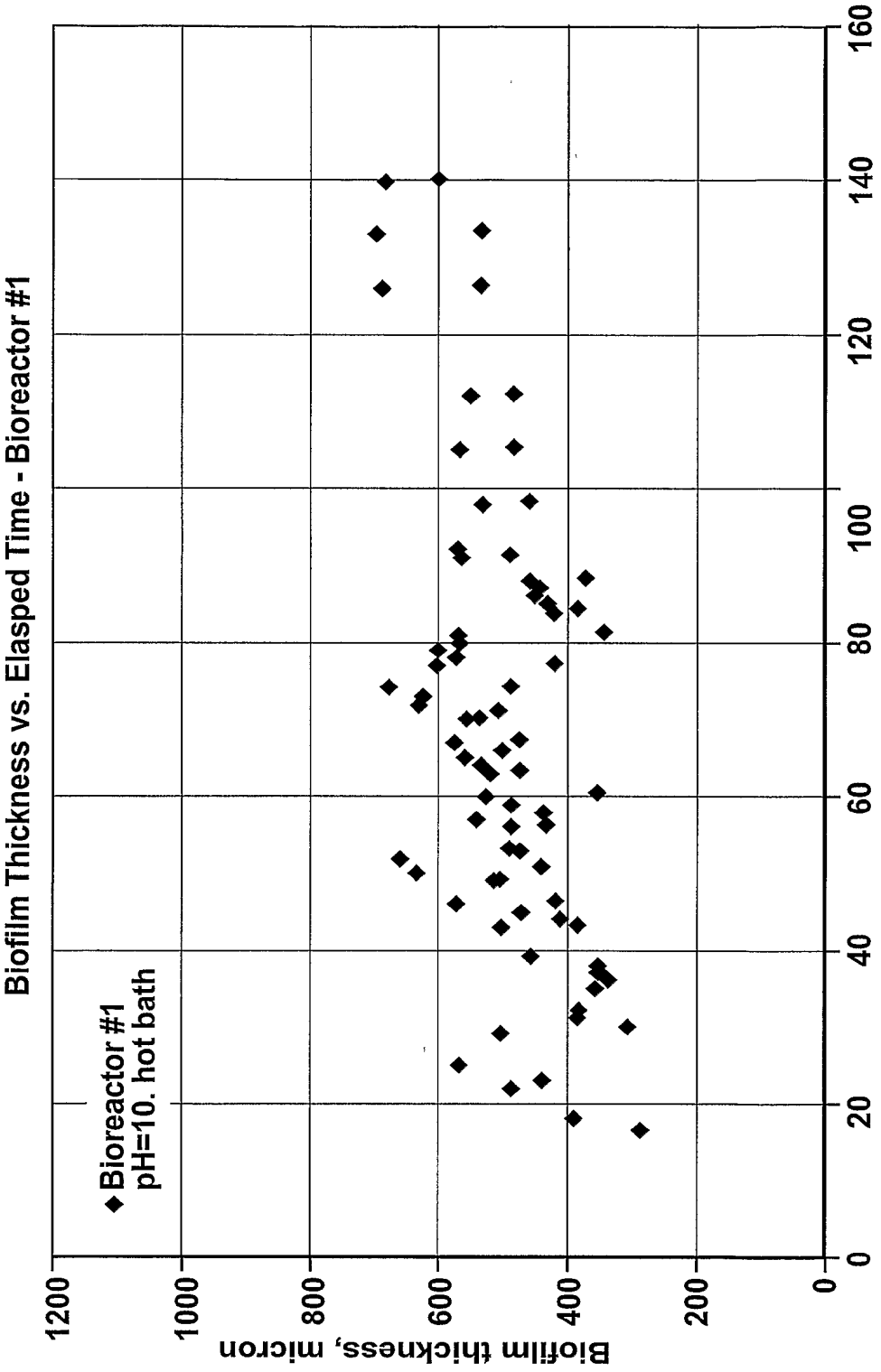

Biofilm Thickness vs. Elasped Time - Bioreactor #1
◆ Bioreactor #1
pH=10. hot bath
Biofilm thickness, micron
0
200
400
600
800
1000
1200
0
20
40
60
80
100
120
140
160
Elasped Time (days)

FIG. 31

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA2004/000206

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C02F3/10 C02F3/20 C02F3/12 C02F3/30 B01D63/02
B01D63/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C02F B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAZUAKI YAMAGIWA ET AL: "SIMULTANEOUS ORGANIC CARBON REMOVAL AND NITRIFICATION BY BIOFILM FORMED ON OXYGEN ENRICHMENT MEMBRANE" JOURNAL OF CHEMICAL ENGINEERING OF JAPAN, SOCIETY OF CHEMICAL ENGINEERS. TOKYO, JP, vol. 27, no. 5, 1 October 1994 (1994-10-01), pages 638-643, XP000478070 ISSN: 0021-9592	1-5,9, 11, 15-18, 21-26, 36-38, 46-48, 50,51, 54-56
Y	the whole document	84-87
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 May 2004	19/05/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Marti, P

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 126 050 A (IRVINE ROBERT L ET AL) 30 June 1992 (1992-06-30) column 6, line 22 -column 7, line 6 column 11, line 3 - line 59; figures 1-5	1,5, 15-21, 36-41, 46-52, 54, 56-59,74
X	BRINDLE K ET AL: "Nitrification and oxygen utilisation in a membrane aeration bioreactor" JOURNAL OF MEMBRANE SCIENCE, ELSEVIER SCIENTIFIC PUBL.COMPANY. AMSTERDAM, NL, vol. 144, no. 1-2, 10 June 1998 (1998-06-10), pages 197-209, XP004123666 ISSN: 0376-7388 page 197 -page 198 page 200 -page 206	1,7-10, 22-24, 36,39, 46-48, 50-54, 56-59, 73,79
X	US 4 181 604 A (NUMAZAWA RYOZO ET AL) 1 January 1980 (1980-01-01) cited in the application column 2, line 64 -column 4, line 53; figures 1,2; examples 1-3	1-4,36, 46-48, 50-52, 57,59
X	EP 0 049 954 A (MITSUBISHI RAYON CO) 21 April 1982 (1982-04-21) cited in the application the whole document	1-4
Y	US 2002/020666 A1 (BEHMANN HENRY ET AL) 21 February 2002 (2002-02-21) the whole document	85-87
Y	US 2001/037967 A1 (DEANE MARSHAL ET AL) 8 November 2001 (2001-11-08) cited in the application paragraph '0023! - paragraph '0042!	84
A	CASEY E ET AL: "Review of membrane aerated biofilm reactors" RESOURCES CONSERVATION AND RECYCLING, ELSEVIER SCIENCE PUBLISHER, AMSTERDAM, NL, vol. 27, no. 1-2, July 1999 (1999-07), pages 203-215, XP004169804 ISSN: 0921-3449 page 209, paragraph 3 -page 210, paragraph 1 page 211, paragraph 3 -page 212, paragraph 1	1-87

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA2004/000206

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 209 855 B1 (GLASSFORD CRAIG L) 3 April 2001 (2001-04-03) column 3, line 18 - line 54 column 5, line 16 - line 64	1,15-21

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA2004/000206

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5126050	A	30-06-1992	NONE		
US 4181604	A	01-01-1980	JP	1118394 C	15-10-1982
			JP	53128142 A	08-11-1978
			JP	57007558 B	10-02-1982
			JP	54066552 A	29-05-1979
			CA	1096976 A1	03-03-1981
			DE	2815866 A1	26-10-1978
EP 0049954	A	21-04-1982	JP	1459656 C	28-09-1988
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			CA	2300719 A1	15-09-2001
			US	2003150798 A1	14-08-2003
			US	2004079692 A1	29-04-2004
			AU	3716101 A	17-09-2001
			WO	0166474 A2	13-09-2001
US 2001037967	A1	08-11-2001	CA	2308234 A1	05-11-2001
			US	2003173706 A1	18-09-2003
			AU	5809901 A	20-11-2001
			WO	0185315 A1	15-11-2001
			CA	2377814 A1	15-11-2001
			EP	1214140 A1	19-06-2002
			EP	1249268 A2	16-10-2002
			HU	0201794 A2	28-09-2002
			JP	2003532521 T	05-11-2003
US 6209855	B1	03-04-2001	WO	0067886 A1	16-11-2000
			EP	1173271 A1	23-01-2002

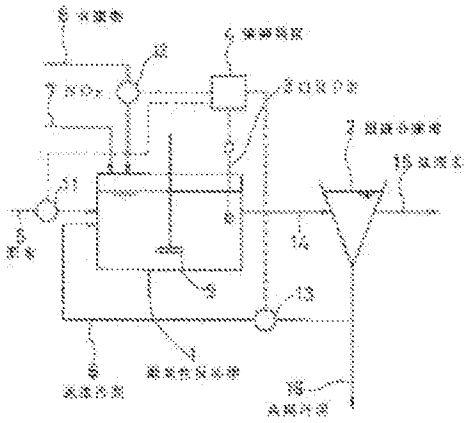
TREATMENT OF SELENIUM-CONTAINING WATER

Publication number: JP8323391 (A)
Publication date: 1996-12-10
Inventor(s): FUKASE TETSURO; NISHIKAWA SHOZO
Applicant(s): KURITA WATER IND LTD
Classification:
- international: ***C02F3/28; C02F3/34;*** **C02F3/28; C02F3/34;** (IPC1-7): C02F3/34; C02F3/28
- European:
Application number: JP19950135437 19950601
Priority number(s): JP19950135437 19950601

Also published as:
JP3358388 (B2)

Abstract of **JP 8323391 (A)**

PURPOSE: To provide a treating method of a selenium-containing water capable of stably and efficiency removing a selenium compd. to detoxicate the water with a simple device and operation. CONSTITUTION: The selenium-containing water 5 is introduced to an anaerobic treating tank 1 to bring it into contact with a biological sludge in an anaerobic state, and an oxidation-reduction potential(ORP) of contrasting silver/silver chloride electrodes is detected with an ORP meter 3, and a feed water introduce rate, an org. matter feed rate or returning sludge rate are controlled to reduce the selenium by controlling pumps 11, 12 or 13 so that the ORP value may be <=-50mV.



Data supplied from the ***esp@cenet*** database ☐Worldwide

BIOLOGICAL TREATMENT OF HEAVY METAL IN DESULFURIZATION WASTE WATER

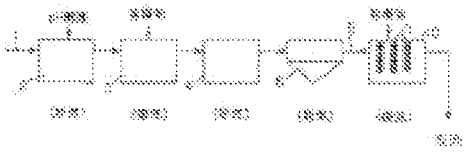
Publication number: JP2001062486 (A)
Publication date: 2001-03-13

Inventor(s): NOGUCHI YOSHIKAZU; NAKUI HIROYUKI; KAKEGAWA HIROYUKI; MAEDA KOICHIRO
Applicant(s): ELECTRIC POWER DEV CO; DENPATSU KANKYO RYOKKA CT KK

Classification:
- international: ***B01D53/50; B01D53/77; C02F3/10; C02F3/12; C02F3/28; C02F3/30; C02F3/34;*** **B01D53/50; B01D53/77; C02F3/10; C02F3/12; C02F3/28; C02F3/30; C02F3/34;** (IPC1-7): C02F3/30; B01D53/50; C02F3/10
- European:
Application number: JP19990238004 19990825
Priority number(s): JP19990238004 19990825

Abstract of **JP 2001062486 (A)**

PROBLEM TO BE SOLVED: To provide a treating method capable of reducing the content of selenium in desulfurization waste water to <=0.1 mg/l, which is the regulation value, and easily recovering activated sludge containing the whole microbial cells and the flocculated heavy metal. SOLUTION: In a nitrification and denitrification method by combining a nitrification vessel 2 for oxidizing ammonia or the like into nitric acid or the like by activated sludge treating the desulfurization waste water under an aerobic condition with a denitrification vessel 3 for reducing nitric acid into gaseous nitrogen by activated sludge treating under an anaerobic condition, a treating vessel 10, in which a carbon fiber assembly 11 cylindrically surrounded by a mesh net is hung, is provided in a waste water discharging line.; The heavy metals containing selenium is separated by keeping the treating vessel 10 under the anaerobic condition to aggregate and concentrate the activated sludge containing selenium reducing bacteria or the like on the carbon fiber assembly 11 by self-adsorption and supplying the organic material from the outside to reduce and adsorb the heavy metals containing selenium in the activated sludge aggregated and concentrated on the carbon fiber assembly 11.



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European Patent Office

SUPPLEMENTARY EUROPEAN SEARCH REPORT

Application Number
EP 06 76 1180

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X Y	WO 87/00161 A (EPOC LTD [GB]) 15 January 1987 (1987-01-15) * page 3, line 24 - page 4, line 1 * * page 4, line 29 - page 5, line 7 * * page 5, line 32 - page 6, line 10 * * page 8, line 27 - page 9, line 12 * * page 10, lines 6-8,19-32 * * page 11, lines 6-11,25-29 * * page 13, line 6 - page 14, line 1 * * page 15, lines 25-32 * * page 17, lines 2-21 * * example 5 * * figure 1 * * claims 1,4-6,8,13,14 * -----	1-3,6 4,5	INV. C02F3/30 B01D53/22
X	JP 2001 062486 A (ELECTRIC POWER DEV CO; DENPATSU KANKYO RYOKKA CT KK) 13 March 2001 (2001-03-13) * abstract * -----	6	
Y	JP 08 323391 A (KURITA WATER IND LTD) 10 December 1996 (1996-12-10) * the whole document * -----	4	TECHNICAL FIELDS SEARCHED (IPC) C02F B01D
Y	EP 0 769 479 A (KEMA NV [NL]) 23 April 1997 (1997-04-23) * page 1, lines 20-55 * * page 3, lines 17-20 * * claims 1,3,4,11 * -----	5	
A	EP 0 773 192 A (MITSUBISHI HEAVY IND LTD [JP]) 14 May 1997 (1997-05-14) * the whole document * -----	1-6	
A	US 5 271 831 A (OREMLAND RONALD S [US]) 21 December 1993 (1993-12-21) * the whole document * -----	1-6	

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

Place of search	Date of completion of the search	Examiner
The Hague	9 July 2008	Rozanska, Agnieszka

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

4

EPO FORM 1503 03.82 (P04C04)

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing claims for which payment was due.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

☒ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-6

   Biological removal of nitrate and selenium from waste stream.

   ---

2. claims: 7-9

   Process for operating a media bed reactor by considering the ORP potential in determining a nutrient addition amount, location or rate.

   ---

3. claim: 10

   Process for operating a fixed media bioreactor by bumping the reactor periodically.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 06 76 1180

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

09-07-2008

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8700161 A	15-01-1987	AU 590786 B2	16-11-1989
		AU 6139786 A	30-01-1987
		CA 1274925 A1	02-10-1990
		EP 0229145 A1	22-07-1987
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JP 8323391 A	10-12-1996	JP 3358388 B2	16-12-2002
EP 0769479 A	23-04-1997	JP 9187793 A	22-07-1997
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US 5271831 A	21-12-1993	NONE	

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

Biological Treatment of FGD Wastewaters

NCDENR
Raleigh, NC
January 27, 2005

applied biosciences

Presentation Outline

- Applied Biosciences Background
- Biological Water Treatment
- The ABMet® Process
- FGD Wastewater Treatment

Applied Biosciences

- Founded 1996
- Salt Lake City, Utah
- Services
  - Treatability/ Technology Evaluation
  - R & D / Microbiology Laboratory
  - Consulting
  - Pilot Testing
  - Full Scale implementation
  - In situ and ex situ treatment
- Zenon

Biological Treatment Basics

- Inorganics cannot be used as food.
- Treatment requires nutrient addition.
- Removal is based on biological reduction to insoluble elements or compounds.
- Contaminants precipitate from solution.
- Metals collect inside the system.
- Nitrogen and cyanide compounds degrade.

A successful biological solution must be:

1. **EFFECTIVE.**
   - low discharge targets
2. **RELIABLE.**
3. **AFFORDABLE.**
   - low capital costs
   - low O&M
4. **ENVIRONMENTALLY SOUND.**
   - must not trade one liability for another

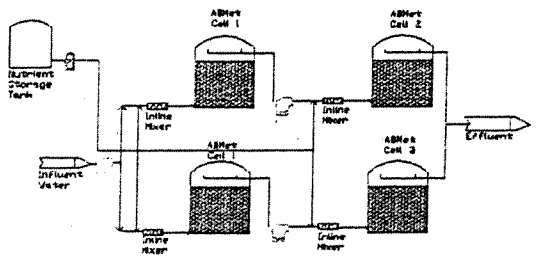
The ABMet® Treatment Process:

- Reduces arsenate and arsenite to arsenic sulfide precipitate
- Biologically reduces selenate and selenite to insoluble elemental selenium.
- Reduces nitrate and nitrite to nitrogen gas.
- Also removes other metals (Cr, Ni, Cd, Cu, Zn, Hg, Co)
- Removal efficiencies >98% achievable

Culture Selection

- Microbial Culture
  - Selected for site conditions
  - Environmental microbes
  - No clinical pathogens

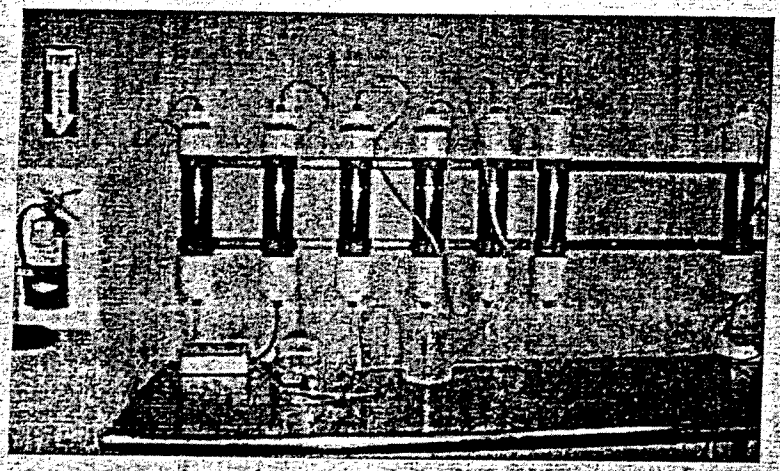
How it Works



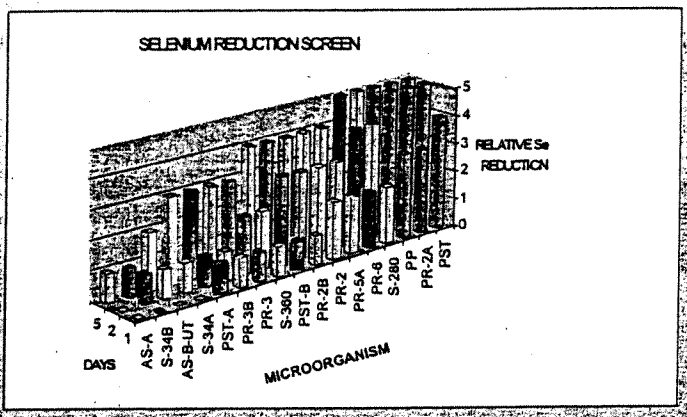
ABMet® Implementation

- Treatability testing
- On-site pilot testing
- System Design
- Full Scale implementation

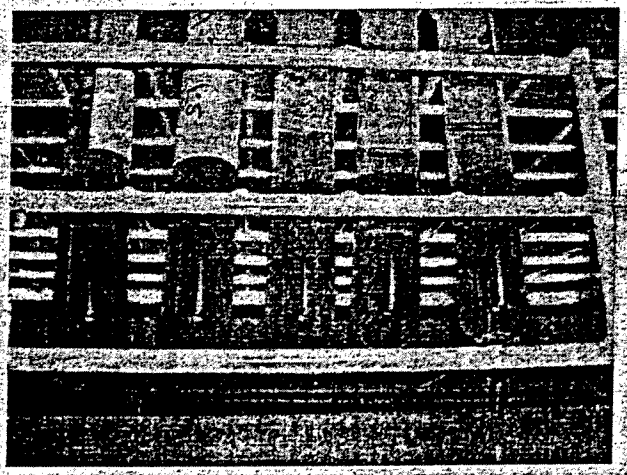
Treatability Testing



Optimized Cultures



Microbial Screening

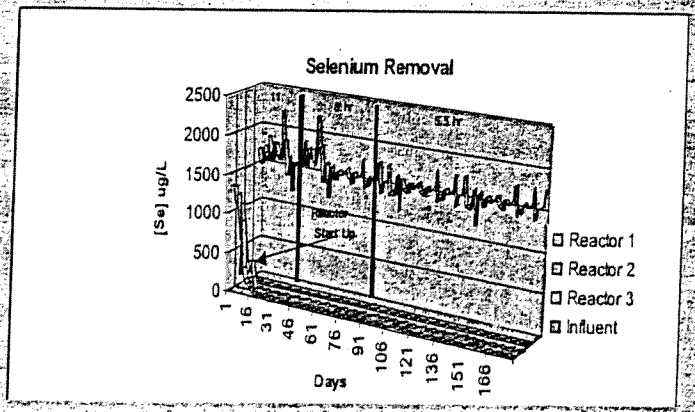


Pilot Testing

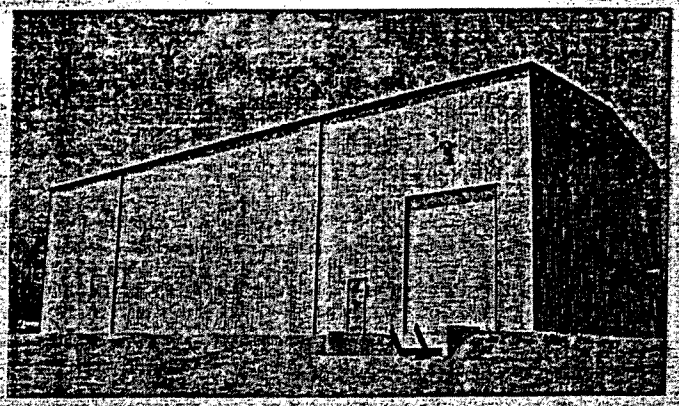


US EPA/DOE Pilot Test

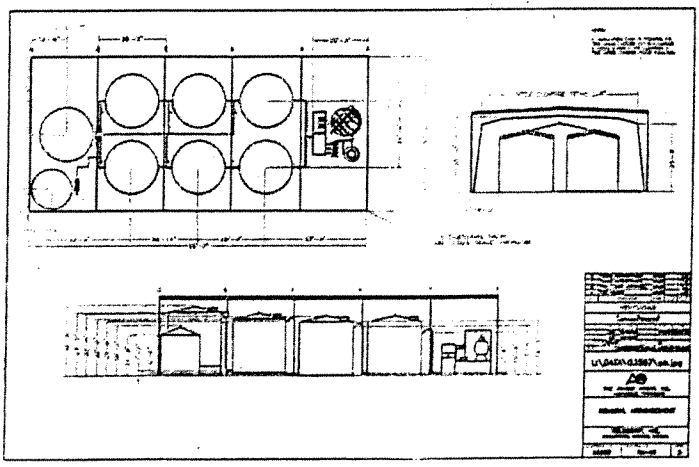
MWTP, 2000

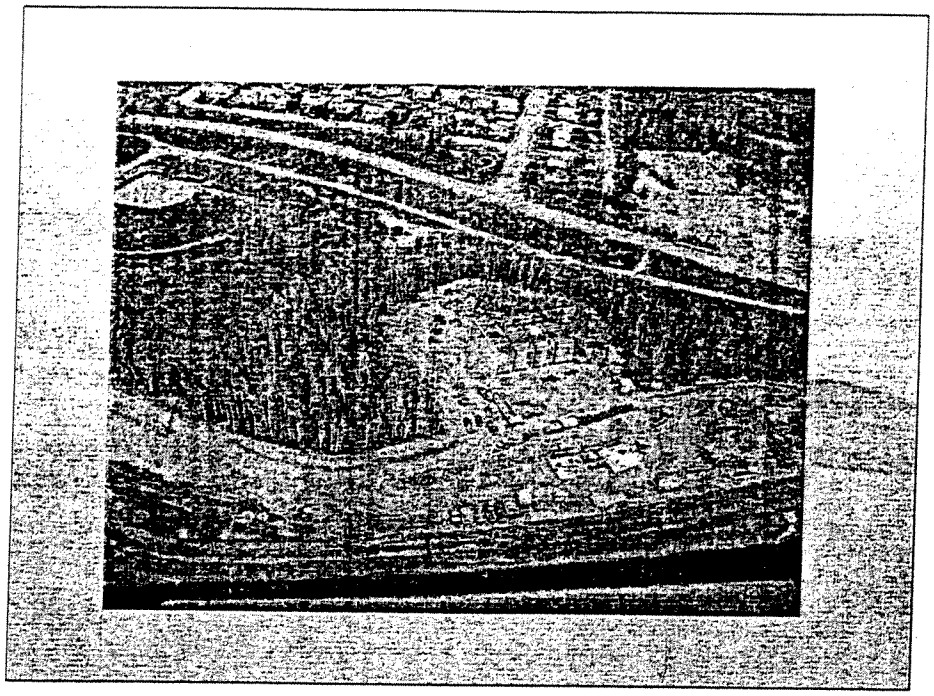


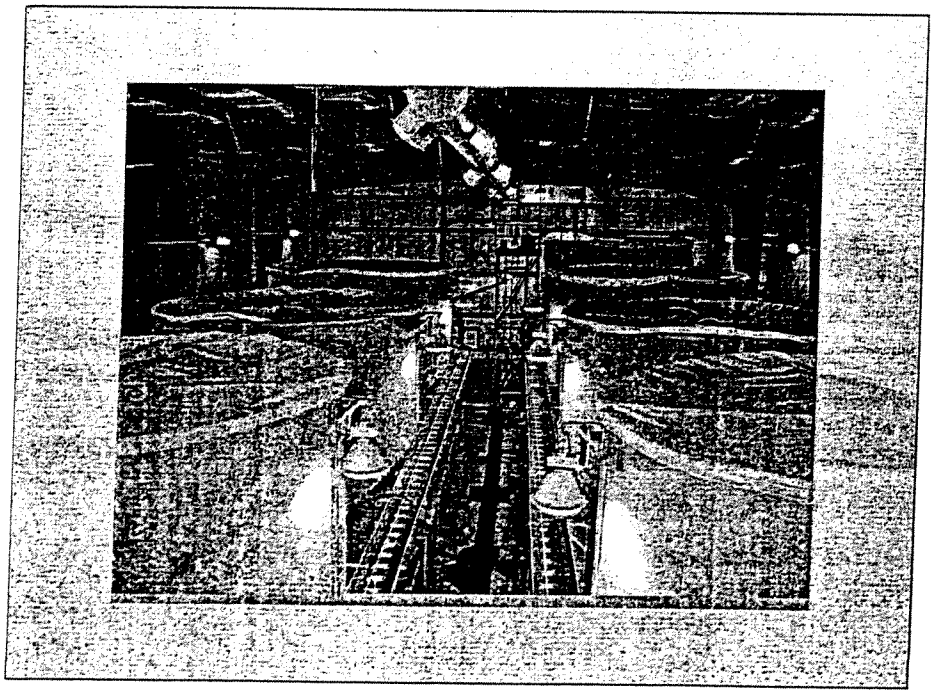
ABMet® Facility, Goldcorp Ontario

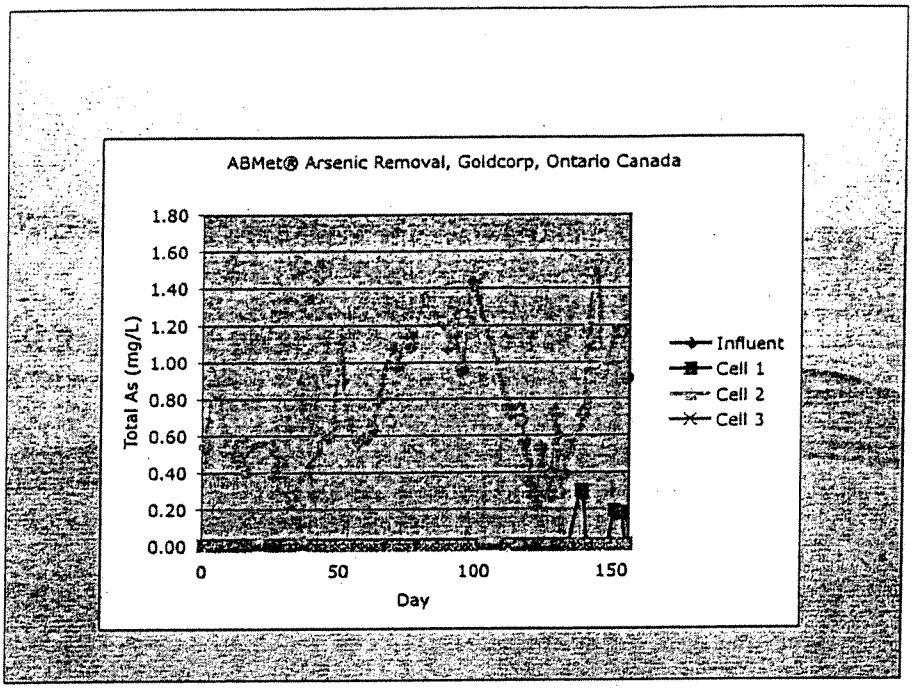


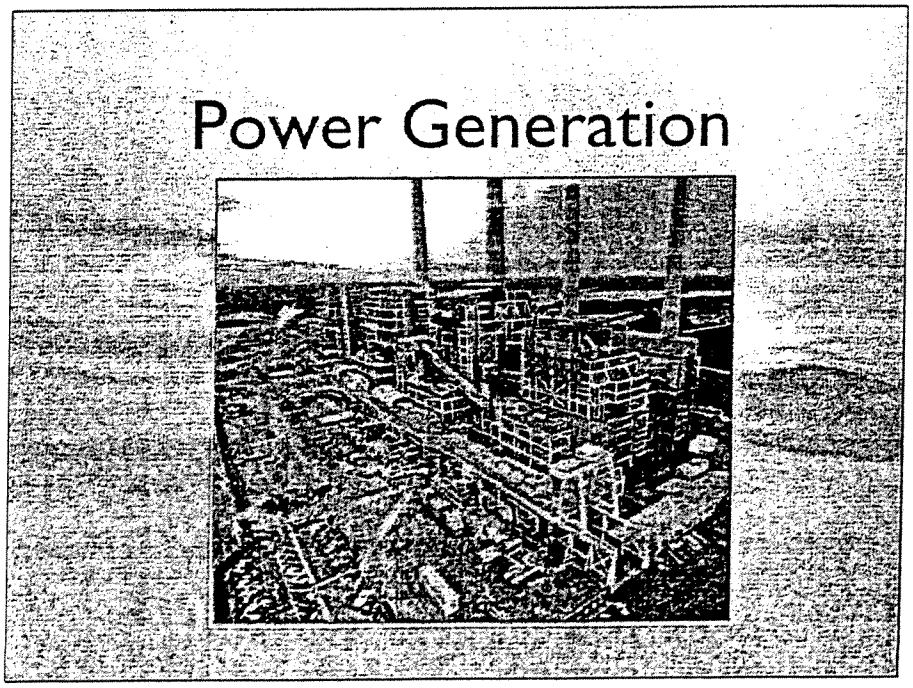
General Arrangement, Goldcorp








ABMet® Arsenic Removal, Goldcorp, Ontario Canada
Total As (mg/L)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
50
100
150
Day
Influent
Cell 1
Cell 2
Cell 3

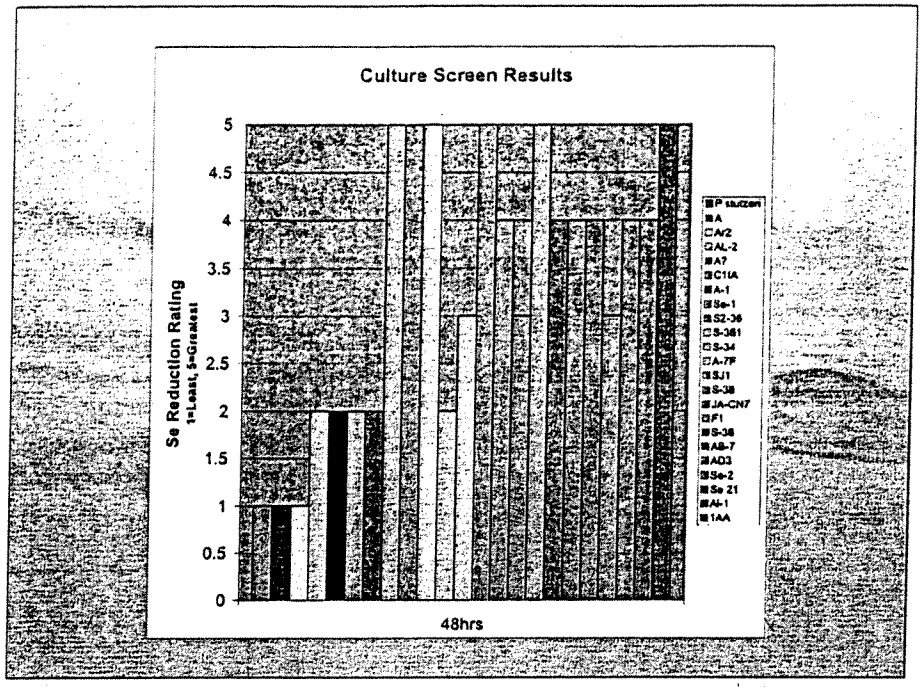

Power Generation

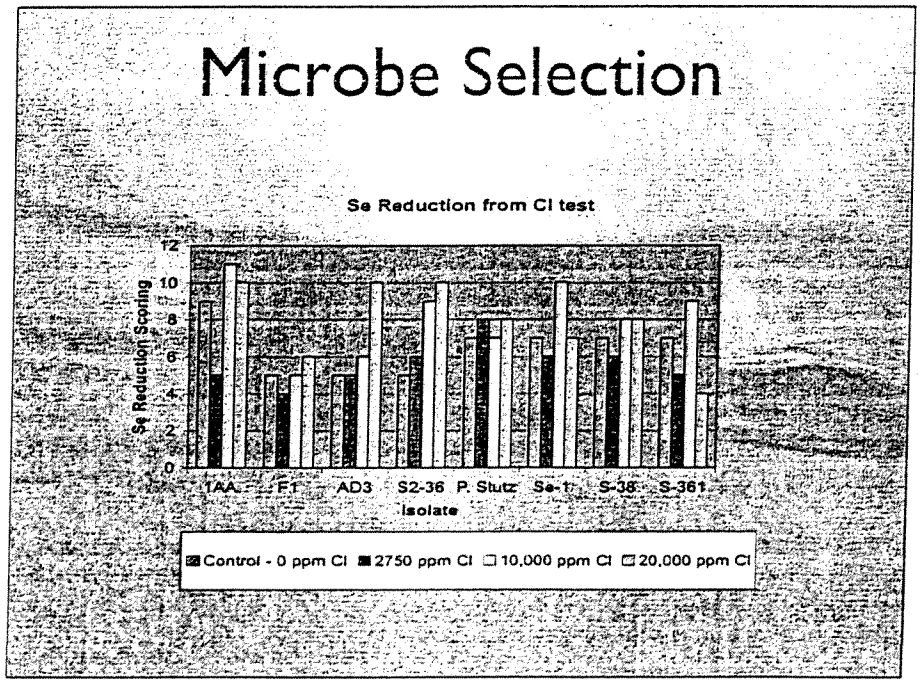
FGD Design Considerations

- Influent conditions
  - –TDS / Chloride
  - –Se concentration
  - –Temperature
  - –DBA
  - –Toxicity
  - –pH

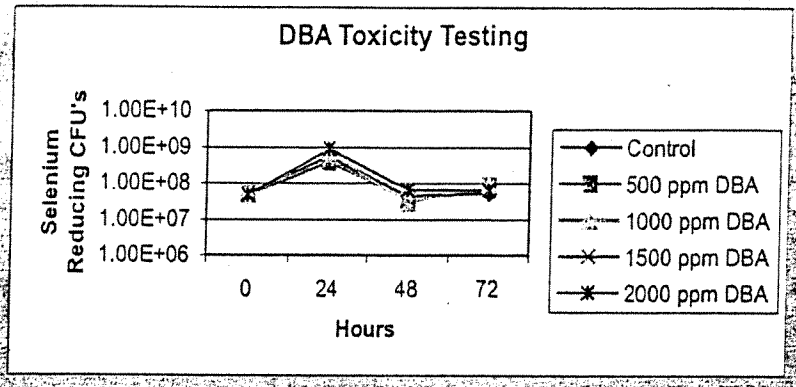
FGD Wastewaters

Parameter, mg/L	Marshall Station Week 1	Marshall Station Week 2	Marshall Station Week 3	Marshall Station Week 4	Michigan South Central Power Agency	Cincinnati B & W Millcreek Unit #3	Louisville G&E	Conemaugh Station
TDS	10,600	11,100	10,400	10,600	13,400	5,620	8,510	35,000
TSS	14	48	46	55	45	3880	47	200
Chloride	4250	3880	4500	4750	2750	1500	700	15,000
Sulfate	1780	1280	1200	1210	4150	2180	2580	2000
Arsenic	0.016	0.022	0.023	0.024	<0.005	0.142	<0.005	0.023
Cadmium	<0.001	0.002	0.002	0.006	0.008	0.067	0.033	0.0035
Chromium	0.007	0.199	0.187	0.002	0.007	0.689	0.014	0.056
Mercury	0.0003	0.0373	0.0225	0.0006	0.0015	0.0664	0.001	0.002
Nickel	0.226	0.217	0.255	1.35	0.93	0.402	0.183	0.0065
Selenium	0.885	2.67	2.75	1.2	0.405	0.792	0.37	1
Silver	<0.001	0.004	0.004	<0.001	<0.001	0.008	<0.001	0.7

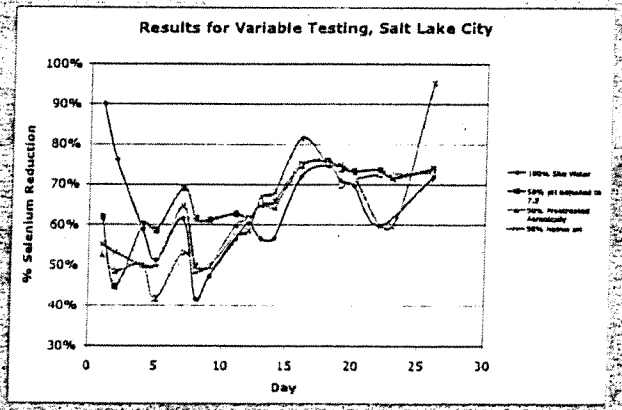

Culture Screen Results
Se Reduction Rating
1=Least, 5=Greatest
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
48hrs
P stutzeri
A
CA2
AL-2
A7
C11A
A-1
Se-1
SZ-36
S-361
S-34
A-7P
SJ1
S-38
JA-CN7
F1
S-36
AB-7
AD3
Se-2
Se 21
AI-1
1AA


Microbe Selection
Se Reduction from Cl test
Se Reduction Scoring
12
10
8
6
4
2
0
1AA
F1
AD3
S2-36
P. Stutz
Se-1
S-38
S-361
Isolate
Control - 0 ppm Cl
2750 ppm Cl
10,000 ppm Cl
20,000 ppm Cl

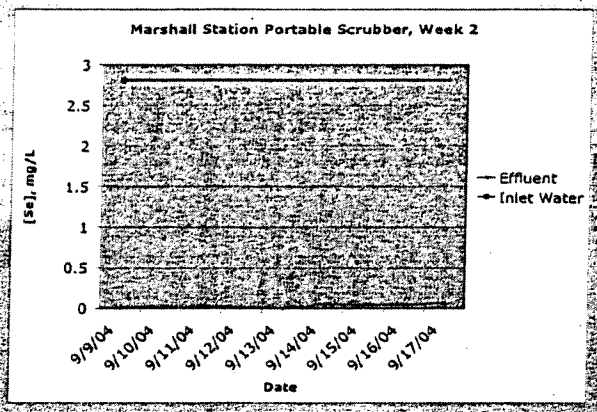
Toxicity Testing



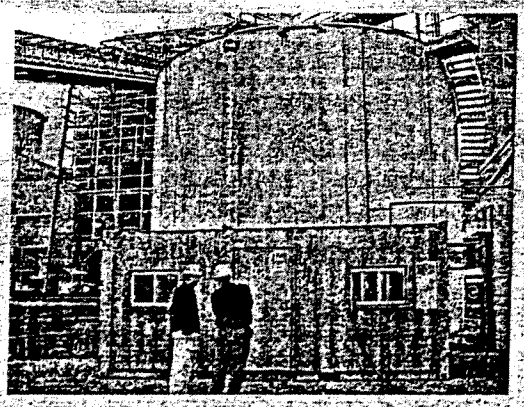
FGD Variable Testing



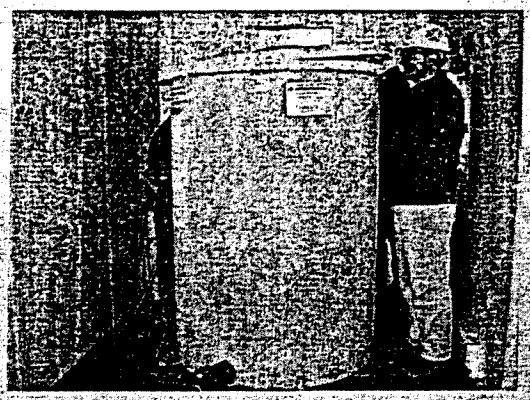
Bench Scale Reactor Testing



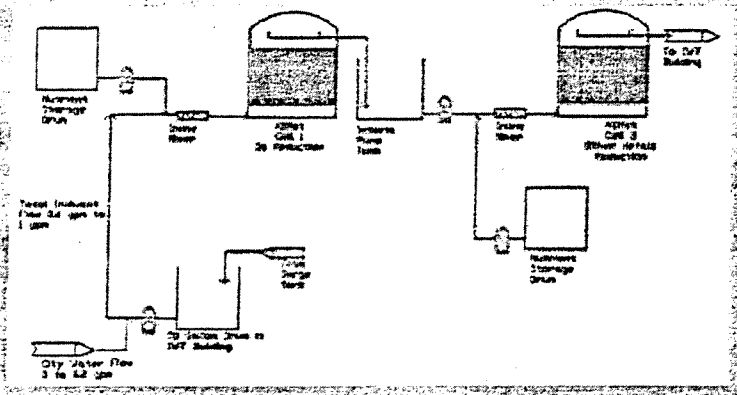
Pilot Testing, Conemaugh Station, PA



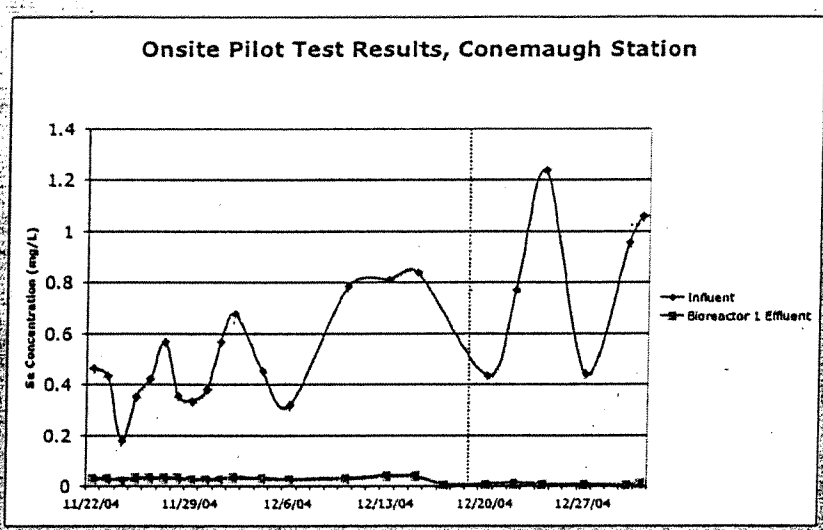
Pilot Testing, Conemaugh Station, PA



Pilot Testing, Conemaugh Station, PA



Field Results



Field Data, Conemaugh Station

	Influent	Bioreactor 1 Effluent		
Date	Se (mg/L)	Se (mg/L)	% Reduction	Method
11/22/04	0.464	0.029	93.75%	ICP-MS
11/23/04	0.433	0.031	92.84%	ICP-MS
11/24/04	0.181	0.023	87.29%	ICP-MS
11/25/04	0.354	0.033	90.68%	ICP-MS
11/26/04	0.423	0.035	91.73%	ICP-MS
11/27/04	0.567	0.033	94.18%	ICP-MS
11/28/04	0.358	0.035	90.17%	ICP-MS
11/29/04	0.335	0.026	92.24%	ICP-MS
11/30/04	0.38	0.027	92.89%	ICP-MS
12/1/04	0.57	0.028	95.44%	ICP-MS
12/2/04	0.679	0.038	94.40%	ICP-MS
12/4/04	0.454	0.031	93.17%	ICP-MS
12/8/04	0.32	0.027	91.56%	ICP-MS
12/10/04	0.787	0.029	96.32%	ICP-MS
12/13/04	0.811	0.042	94.82%	ICP-MS
12/15/04	0.84	0.043	94.88%	ICP-MS
12/17/04	0.58	0.0027	99.53%	HGAF
12/20/04	0.434	0.00788	98.23%	HGAF
12/22/04	0.772	0.012	98.45%	HGAF
12/24/04	1.24	0.0081	99.35%	HGAF
12/27/04	0.441	0.0081	98.16%	HGAF
12/30/04	0.957	0.0029	99.69%	HGAF
12/31/04	1.060	0.00957	99.09%	HGAF

Metal Removal, Conemaugh Station

Sample ID	Collection	Cr (µg/L)	Co (µg/L)	Cu (µg/L)	Zn (µg/L)	As (µg/L)	Ba (µg/L)
PARS INF	12/17/04	16.1	3.3	288	129	14.1	153
PARS1 EFF	12/17/04	<3.5	<2.5	2.2	34	<7.5	93
PARS2 EFF	12/17/04	8.1	21.8	14.1	44	15.8	388
PARS INF	12/22/04	19.3	185	278	578	9	475
PARS1 EFF	12/22/04	8.4	6.2	10.4	34	<7.5	508
PARS2 EFF	12/22/04	8.5	24.8	20.4	48.8	13.1	634
PARS INF	12/27/04	56.8	12.3	761	212	23.2	282
PARS1 EFF	12/27/04	6.5	<2.5	11.7	12.3	<7.5	58.1
PARS2 EFF	12/27/04	7.5	5.7	20.3	53.6	<7.5	261

Sample ID	Collection	Mo (µg/L)	Ag (µg/L)	Cd (µg/L)	Hg (µg/L)	Tl (µg/L)	Pb (µg/L)
PARS INF	12/17/04	3.8	1.17	1.76	3.28	2.46	73.4
PARS1 EFF	12/17/04	<1.0	<0.75	<0.40	0.0618	<0.20	<0.75
PARS2 EFF	12/17/04	1.9	<0.75	<0.40	0.134	0.48	<0.75
PARS INF	12/22/04	81.8	7.61	10.4	2.1	16.3	23.4
PARS1 EFF	12/22/04	<1.0	<0.75	<0.40	0.184	<0.20	<0.75
PARS2 EFF	12/22/04	1.3	6.54	<0.40	0.113	0.69	0.97
PARS INF	12/27/04	8	7.22	3.51	16.7	5.64	166
PARS1 EFF	12/27/04	<1.0	0.79	<0.40	0.054	<0.20	1.14
PARS2 EFF	12/27/04	<1.0	0.79	<0.40	0.0703	<0.20	0.92

Pilot Status

- Continued operations through February
- Operate at higher selenium level
- Test effluent for BOD and residual nutrients
- Backwash and characterize solids

Full Scale Considerations

- Pretreatment
- Inlet Temperature
- Post-treatment
  - Nutrients
  - BOD
  - Re-aeration
- Solids handling
  - Recycling vs. Disposal

Conclusions

- ABMet® Process is effective in FGD Wastewaters
- Low effluent values are achievable
- Effective at various inlet variables
  - Selenium concentration
  - DBA
  - Chloride
  - Temperature
  - Chloride
- Selenium removal at >98%

ABMet® Biological Selenium Removal from FGD Wastewater

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IWC-05-72

Keywords: FGD wastewater, biological reduction, bacteria, selenium, arsenic, copper, mercury, zinc

Summary: The biological precipitation and removal of selenium and metals from flue gas desulfurization (FGD) blowdown utilizing a fixed-film biotreatment process has been successfully pilot tested and demonstrated to remove dissolved selenium at a > 99% efficiency. Selenium levels less than 0.01 mg/L were achieved both in the lab and in field tests.

The process has been tested in more than eight FGD wastewaters, and implemented at full scale in other industries. The wastewaters tested contained up to 500 mg/L Dibasic Acid, (DBA), as well as elevated levels of chloride, (>15,000 mg/L), and TDS, (>29,000 mg/L). The biofilm maintained its integrity and efficiency in temperature tests up to 105 °F and at pH levels as low as 5.35. Other metals significantly removed from the FGD wastewater during the process included arsenic, copper, nickel, mercury and zinc. Minimal sludge volumes were generated as a waste product as compared to chemical flocculation and precipitation.

A 1,400 gal/min facility using the process is currently under design for full scale operation starting in 2007. This paper presents a process overview and pilot scale performance data, and a full scale, conceptual design approach.

INTRODUCTION

Coal-fired power plants produce more than half the electricity used in the United States. The process of combusting coal to generate power converts naturally occurring sulfur contained in the coal to gaseous sulfur dioxide (SO_2), a criteria pollutant and a precursor to acid rain.

The 1990 Clean Air Act and its subsequent amendments mandated the reduction of power plant SO_2 emissions. The Best Demonstrated Technology for reducing SO_2 emissions is wet scrubber flue gas desulfurization (FGD) systems. These systems are designed to introduce an alkaline sorbent, consisting of lime or limestone in a spray form, into the exhaust system of a coal-fired boiler. The alkali reacts with the SO_2 gas and is collected in a liquid form as calcium sulfite or calcium sulfate slurry. The slurry is discharged to a treatment system where the calcium sulfate may be removed by gravity settling or centrifugation. The pre-treated FGD wastewater, (blowdown) can contain elevated levels of mercury, arsenic and selenium as well as other metals and metalloids found in various coal sources. Directly following gypsum removal, the blowdown can be delivered to bioreactor trains for biological contaminant reduction.

Many diverse species of bacteria have been proven capable of reducing selenate and selenite in an aqueous reductive environment (Frankenberger et al. 1994), by linking the oxidation of organic substrates to the dissimilatory reduction of the selenium oxyanions (De Souza, 2001). The oxidized selenium compounds serve as electron receptors during the electron transport process. This effectively reduces the oxidized selenium compounds to nanospheres of granulated elemental selenium which accumulate in and around the bacterial cells. The unique physical properties of these granules cannot currently be produced by purely chemical means and exhibit a higher level of stability than other chemically induced formations (Oremland, 2004).

PROCESS OVERVIEW

The biological treatment technology presented utilizes biofilms of facultative anaerobic bacteria in an up-flow bioreactor, through which the wastewater is passed. Proprietary, site-specific, naturally occurring bacterial cultures are produced in bulk and used to inoculate the bioreactors. Microbial reduction occurs within the confines of the bioreactor. The elemental selenium and other contaminant precipitates are retained within the matrix of the bioreactor until the employment of a flushing cycle to release and collect the solids for recycling or disposal.

Implementation of the bio-treatment is a multi-step process. Initially, site water is qualitatively screened with microbial stock cultures to select specific bacteria that demonstrate the ability to metabolize the target contaminants. Site water is also analyzed for indigenous microbial populations that have the ability to effectively reduce metal inorganic compounds. The selected bacteria are then scaled up to inoculate bench scale bioreactors for treatability testing within a laboratory environment.

Bench scale reactors are filled with an inert, proprietary substrate on which the bacteria form a high density, fixed biofilm and are therefore retained within the system. The bioreactors are quantitatively analyzed for selenium reduction while being monitored. Site-specific variables such as water quality, composition, temperature, as well as nutritional requirements are evaluated.

At the conclusion of bench scale testing, a pilot test is set up on site. The selected bacterial consortia is again scaled up and used to inoculate the pilot bioreactors. Fluctuations in the composition of site water influent, temperature and nutritional supplementation are evaluated with respect to contaminant reduction and hydraulic retention time. A maintenance schedule for solids removal is developed.

The parameters established during pilot testing are incorporated into recommendations for a full-scale design. Implementation logistics and timelines, as well as, full-scale design criteria are addressed at this time.

MATERIALS AND METHODS

Site waters were obtained from several operating FGD facilities. The FGD wastewaters tested contained up to 500 mg/L Dibasic acid, (DBA), as well as elevated levels of chloride, (>15,000 mg/L), and TDS, (>29,000 mg/L). Inlet Selenium concentrations ranged from (0.3 – 4.0 mg/L).

Culture Screening

In a series of tests, individual stock cultures were screened for selenium reduction and chloride tolerance. The testing procedures involved adding a stock selenium reducing culture to a test tube filled with site water and nutrient amended with selenium. The isolates were allowed to incubate for 48 hours and selenium reduction was assessed by visually evaluating the red precipitate in the tube. Isolates determined to have the greatest selenium reducing capability in site water were selected for further screening in chloride amended site water.

Selenium reducing isolates were selected from the initial selenium reduction screening and grown in three chloride variables (low = no chloride added to site water, medium = chloride added to 10 g/L total chloride, high = chloride added to 20 g/L total chloride). Isolates were added to test tubes with site water, nutrient, selenium, and the respective amount of chloride. Selenium reduction was assessed after 48 hours. Selenium reduction was assessed by visually rating the red precipitate in the tube. Isolates that performed well at all three chloride concentrations were selected for further testing.

Temperature Testing

The cultures previously screened for selenium reduction, chloride tolerance, and growth potential were further tested for temperature tolerance. The testing entailed inoculating activated carbon with the mixed culture and evaluating the growth of the culture at several temperatures. The temperatures tested in the laboratory included room temperature, 85 °F, 90 °F, 95 °F, 100 °F, 105°F, and 113 °F. The testing procedures involved taking a sample of inoculated carbon and conducting microbial plate counts at time zero, then adding the carbon sample to a flask with nutrient, and allowing the carbon to incubate in a temperature controlled water bath for 48 hours. After 48 hours in the water bath, microbial plate counts were conducted on the carbon matrix. Comparing the T48 hour counts to the initial counts assessed growth.

Bench Scale Bioreactor Testing

Bench scale bioreactors were set up to test metals reduction in scaled ABMet® reactors. The bioreactors have a capacity of 3 liters and operated

at a 12-hour retention time. The bioreactors were filled with an inert, proprietary support material and inoculated with the mixed culture selected from the culture screening. The bench scale bioreactor testing consisted of five runs using three different FGD scrubber waters. The reactors were sampled daily, and samples were submitted for selenium and metals analysis at an independent laboratory.

On Site Pilot Testing

The pilot test consisted of a train of two reactors set up in series, the first being inoculated with site-specific selenium reducing consortia and the second with sulfate reducing bacteria. See Figure 1.

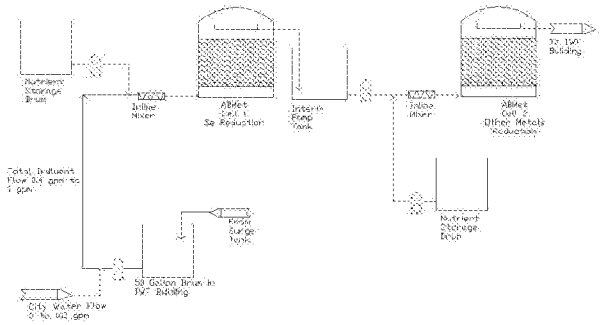


Figure 1.
FGD Wastewater Treatment Pilot System Process Flow Schematic

The bioreactors were fed a proprietary molasses based nutrient using an automated delivery system. The influent and effluent of each bioreactor were sampled three times per week and analyzed for total selenium. Once per week, the influent and final effluent were sampled and analyzed for other total metals arsenic, copper, nickel, mercury and zinc.

RESULTS

The culture screening assessed selenium reduction capability in site water spiked with 25 mg/L selenium. All culture screenings used water from an operating FGD system. Twenty-three isolates were tested and selenium reduction was rated between 1 and 5, with 5 being the highest. Results indicated that selenium reduction occurred in site water. Eight isolates showing the greatest selenium reduction capability were chosen for further testing. See Figure 2.

The eight isolates from Test 1 were screened further in site water spiked with varying levels of chloride. Results indicated that selenium reduction occurred

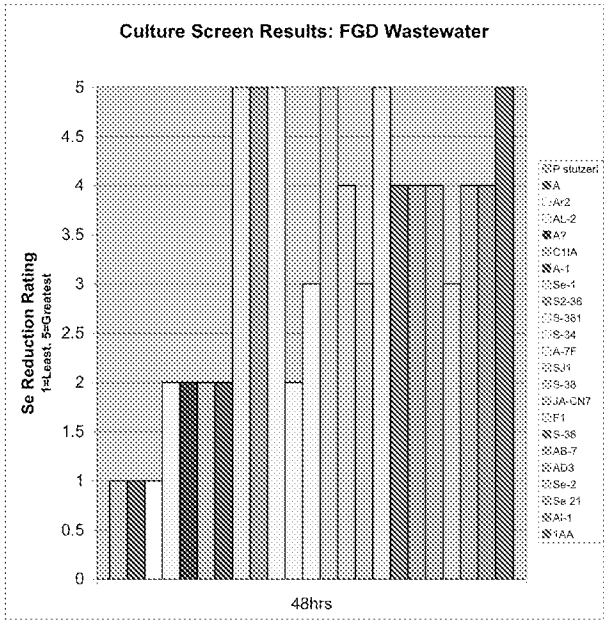


Figure 2.
Culture Screening – Selenium Reduction Test in FGD Site Water.

at all chloride concentrations and certain isolates performed well even at high concentrations. It is also noted that some isolates performed better at higher chloride concentrations than lower chloride concentrations. See Figure 3.

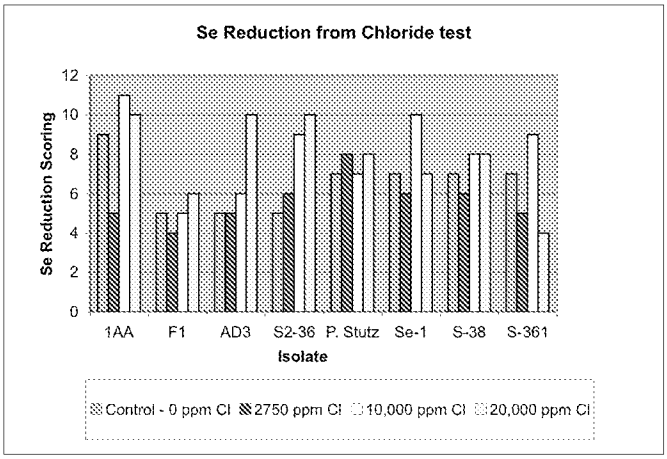


Figure 3.
Selenium Reduction in Varying Chloride Concentrations

Bench scale laboratory analysis included toxicity evaluations for DBA concentrations, low pH values and extreme temperature testing. Results of the tests concluded DBA concentrations up to 2,000 mg/L, native pH of 5.35 and inlet water temperatures

of up to 105°F did not inhibit selenium reduction or the viability of the microbial biofilm.

Selenium concentrations can fluctuate daily in FGD wastewaters due to variations in the coal types being burned; however, this fluctuation did not have a detrimental effect on selenium reduction. Selenium removal efficiencies >99% were consistently achieved during the pilot test as illustrated in Figure 4.

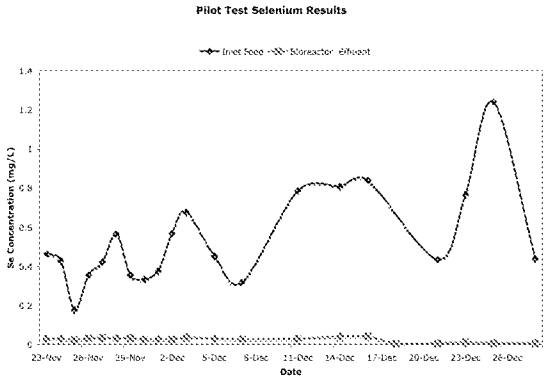


Figure 4.
Pilot Test Selenium Reduction Results

In addition to selenium, the system demonstrated effective removal of arsenic, copper, nickel, mercury and zinc throughout the test period. See Table 1.

.

Table 1.
Average Metal Concentrations
During FGD Pilot Test

Metals	Inlet Concentration in µg/L	Effluent Concentration in µg/L
Arsenic	27.63	15.20
Copper	320.86	16.50
Nickel	546.01	20.91
Mercury	2,47	0.08
Zinc	377.00	16.50

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DISCUSSION

Successful laboratory and pilot scale testing of FGD wastewaters indicate that the ABMet® process is suitable for selenium removal from these waters. Laboratory studies evaluating process operating variables demonstrated that the ABMet® technology can perform consistently over a range of operational scenarios. Specifically, selenium reduction was constant at native pH of 5.35 and a pH adjusted to 7.5. Additionally, DBA up to 2000 mg/L was not toxic to the microbes and the presence of DBA did not affect selenium reduction. Finally, selenium reduction was evident in water with chloride concentrations up to 15,000 mg/L, (the maximum concentration tested).

Effluent concentrations in the extended pilot scale testing exhibited selenium levels as low as 0.003 mg/L at a retention time of 5 hours. Influent temperatures to the pilot system ranged from 17 °C to 32.8 °C (62.6 °F to 91 °F). Results from the temperature testing in the lab demonstrated that selenium reduction and good biofilm health were maintained at temperatures up to 105 °F.

Selenium removal efficiencies >99% were consistently achieved during the pilot test. In addition to selenium, the system demonstrated effective removal of arsenic, copper, nickel, mercury and zinc throughout the test period. Mercury was consistently removed in the system to below 150 ng/L.

In extensive bench and pilot testing, successful biological selenium reduction was demonstrated in over eight FGD scrubber waters. Based on the success of the this testing, orders for 2 full scale facilities have been obtained for start up in 2007. A simplified full scale flow schematic is provided in Figure 5.

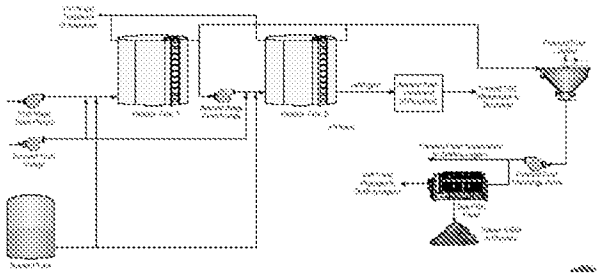


Figure 5.
Full Scale Process Flow Diagram for FGD Wastewater

Each facility will treat 1 or 2 Million Gallons/ Day (MGD) of FGD wastewater. Preliminary design criteria for full-scale implementation are displayed in the following table. See Table 2. Both four and eight hour retention time examples are listed. Full -scale design retention times will depend on site -specific treatment targets and inlet water chemistry.

Table 2.
Preliminary Recommended Full Scale Design Criteria

Parameter	Flow rate (gpm)		
	1600	640	150
System Retention time (hours)	4-8	4-8	4-8
Operating temperature (°F)	70-105	70-105	70-105
pH (su)	5.5 – 9	5.5 – 9	5.5 – 9
Total Reactor Volume (gallons)	1 M – 2 M	0.4 M – 0.8 M	94 K – 188 K

Pretreatment for the system may be required to remove TSS and reduce the solids loading of the system. It is anticipated that with an inlet feed TSS concentration of <20 mg/L, the lead reactors will need to be flushed on a biannual basis.

Post treatment may be necessary to re-aerate bioreactor effluent and remove residual organics before discharge. If DBA is present in the FGD water, it can be removed in the aerobic post treatment system. Post treatment may include fixed-film biological systems such as rotating bio-contactors and trickling filters, or wetlands.

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Electronic Patent Application Fee Transmittal

Application Number:	11996384
Filing Date:	22-Jan-2008
Title of Invention:	Apparatus and Method for Treating Fgd Blowdown or Similar Liquids
First Named Inventor/Applicant Name:	Jeffrey Gerard Peeters
Filer:	Scott Raymond Pundsack/Leslie Taggart
Attorney Docket Number:	218465-9

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Warnings:					
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11	Foreign Reference	JP2001062486A.pdf	500644 c1fc487c3d6084951355b31b6a47427b31679a76	no	7
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19	NPL Documents	BiologicalTreatmentofFGDWastewaters.pdf	4396335 3dc4f927b321ae5def03f7af8aca3de7586d1ae2	no	18
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21	Fee Worksheet (PTO-875)	fee-info.pdf	30492 1af668c6f86315390c29feb1b7d307fdc86bf163	no	2
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Application Number	11996384
Filing Date	2008-01-22
First Named Inventor	Jeffrey Gerard Peeters
Art Unit	1797
Examiner Name	Fred G. Prince
Attorney Docket Number	4320-957

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	Art Unit	1797
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	Attorney Docket Number	4320-957

	1	TWIDWELL, et al., "Technologies and Potential Technologies for Removing Selenium from Process and Mine Wastewater", Undated, Butte, Montana, U.S.A.	☐
	2	SCHEURMAN, "Selenium Reduction Issues in a Coal Fired Power Plant", Industrial Water Conference, Orlando, Florida, October 2005.	☐
	3	OREMLAND, "Selenate Reduction to Elemental Selenium by Anaerobic Bacteria in Sediments and Culture: Biogeochemical Significance of a Novel, Sulfate-Independent Respiration", Applied and Environmental Microbiology, September 1989, Vol. 55, No. 9, pp. 2333-2343.	☐
	4	STOLZ, et al., "Microbial transformation of elements: the case of arsenic and selenium", Int. Microbial (2002) 5, pp. 201-207.	☐
	5	OREMLAND et al., "In Situ Bacterial Selenate Reduction in the Agricultural Drainage Systems of Western Nevada", Applied and Environmental Microbiology, February 1991, vol. 57, No. 2, pp. 615-617.	☐
	6	HAZTECH NEWS, "Biological Process Proved Most Consistent in Demonstration for Removing Se from Water", The Newsletter of Hazardous Waste Treatment Technology, Vol. 16, 2001.	☐
	7	WAHLQUIST et al., "Effective Biological Water Treatment Through Biological Process Control", Tailings and Mine Waste Conference, Vail Co., 2003.	☐
	8	ADAMS et al., "Microbial and Cell-Free Selenium Bioreduction in Mining Waters", Ch. 24 of Frankenberger et al., Environmental Chemistry of Selenium, Marcel Dekker, Inc., 1998, pp. 479-499.	☐
	9	LOVLEY, "Dissimilatroy Metal Reduction", Annu. Rev. Microbiol. 1993, 47, pp. 263-290.	☐
	10	ADAMS et al., "Biotechnologies for Metal and Toxic Inorganic Removal from Mining Process and Waste Solutions", Randol Gold Forum 1996, pp. 143-146.	☐
	11	ADAMS et al., "Bioreduction of Selenate and Selenite", from A.E. Torma et al., Biohydrometallurgical Technologies, The Minerals, Metals and Materials Society, 1993, pp. 755-771.	☐

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	Examiner Name	Fred G. Prince
	Attorney Docket Number	4320-957

	12	MSE TECHNOLOGY APPLICATIONS, INC. et al., "Selenium Treatment/Removal Alternatives Demonstration Project", Mine Waste Technology Program, Activity III, Project 20, June 2001, Bute, MT, U.S.A.	☐

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Scott Pundsack/	Date (YYYY-MM-DD)	2010-03-08
Name/Print	Scott R. Pundsack	Registration Number	47330

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TECHNOLOGIES AND POTENTIAL TECHNOLOGIES FOR REMOVING SELENIUM FROM PROCESS AND MINE WASTEWATER

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Abstract

An extensive review of the literature has been performed to identify appropriate technologies for the removal of selenium from a variety of wastewater. The identified treatments include technologies now being utilized and technologies that show good potential for being implemented in the near future. The technologies are briefly discussed in this paper. A more detailed discussion is available to the reader in a noted reference. The emphasis of this presentation is removal of selenium from mine waters.

Several technologies appear to hold promise for application to mine waters for removing selenium to the low µg/L range, e.g., <10 µg/L. Success for achieving effective selenium removal will depend on the site-specific characteristics of the individual wastewater to be treated. Those technologies that are presently being used and those technologies that appear to hold promise for effective selenium removal include: ferrihydrite adsorption, HWFIX, emulsion liquid membranes, nanofiltration, tailored ion exchange, reductive cementation, and biological reduction.

Introduction

An extensive review of the literature has been performed to determine what technologies are presently being utilized, and what technologies may potentially be applicable, for the removal of selenium from industrial mine wastewater. The information presented in this paper emphasizes possible mine water treatment technologies. Detailed presentations of the review results are presented elsewhere[1]. A summary of the review is presented in this paper.

Summary of Selenium Removal Technologies

A summary of treatment technologies that are applicable or may be applicable for selenium removal from wastewater is presented in Table 1. Because of the large number of publications on selenium only a very brief summary for each technology will be discussed in this presentation. Literature sources and detailed discussions for other technologies are available to the reader in the reference Issues Identification and Technology Prioritization Report-Selenium[1].

Table 1. Summary of Treatment Technologies for Removing Selenium from Solutions

Technology and Comments
PRECIPITATION
Selenate, Selenite-Precipitation of selenates and selenites as a mine water treatment technology is ineffective because of their relatively high solubility as a function of pH.
Selenide, Elemental Selenium-The reduction of selenate and selenite species with the subsequent precipitation of metal selenides or elemental selenium appears to hold promise as a mine water treatment option.
ADSORPTION
Ferrihydrite-Ferrihydrite precipitation has been extensively investigated. Selenium (IV) is effectively removed at pH<~8. This technology is not effective for Se(VI). Reduction of Se(VI) prior to adsorption is required. The presence of other aqueous species in the solution may influence the removal of Se(IV), i.e, the order of adsorption (at pH 7) has been shown to be phosphate>silicate=As(V)• bicarbonate,carbonate• citrate=Se(IV)• molybdate• oxalate>fluoride= Se(VI)• sulfate.
This is U.S. EPA's BDAT for treating selenium bearing wastewaters. Long-term solids aging studies are recommended.
Alumina-Selenium (IV) adsorption is nearly complete at pH levels between 3-8. Aqueous silica adsorbs in preference to Se(IV) at pH 7 but is no problem at pH 4. Selenium (VI) adsorption by alumina is poor. Selenium (VI) adsorption drops off rapidly with increasing pH and is less than 50% at pH 7. Sulfate and carbonate adsorption significantly interferes with Se(VI) adsorption.
Application of selenium adsorption by alumina may be a problem in gold heap leach effluents because of the presence of dissolved silica and, in some cases, the presence of cyanide. Alumina may be a viable adsorbent for some mine waters.
Ferric Oxyhydroxide/Peat/Resins-HW-FIX is a USBM development that shows good promise for Se(IV) removal. The adsorption is not as effective for Se(VI). This technology shows promise for application to mine waters.
Activated Carbon-Activated carbon adsorption is widely used in treatment of groundwater and as point-of-use treatment of drinking waters for organic adsorption. Activated carbon is ineffective for adsorbing selenium from mine waters.
ION EXCHANGE AND MEMBRANE PROCESSES
Ion Exchange-IX is used for treatment of drinking waters and groundwaters for metals, arsenic and selenium removal. Selenium removal is accomplished by using a strong base anion ion exchange resin. Selenium (VI) is extracted much more effectively than Se(IV). The extraction of Se(VI) is decreased by sulfate.
Tailored resins show good selectivity for selenium in the presence of sulfate but only laboratory studies have been performed. Further laboratory studies (on mine waters) are required.
Liquid IX has been investigated on a pilot scale for treating gold heap leach solution effluents. The results were encouraging but the technology has been documented at only one site. Further laboratory and pilot test work should be conducted.

Technology and Comments

Reverse Osmosis-RO is extensively used for removing inorganic contaminants from drinking and groundwater. Publications on industrial application to mine waters were not found. RO may require extensive pretreatment of mine waters to remove solids and to lower the concentration of total dissolved solids.

Emulsion Liquid Membranes-Pilot studies have shown that Se(VI) is extracted rapidly even in the presence of sulfate at all pH values >2. Selenium (IV) extraction is influenced by the presence of sulfate, i.e., the rate of extraction is decreased. Sulfate is extracted in preference to Se(IV) unless the pH is >9.5. This technology shows good potential for application to mine waters but requires further test work to answer questions concerning the stability of the emulsion in acidic, multicomponent solutions.

Nanofiltration-Nanofiltration appears to hold potential for treating some low metal containing selenium bearing mine waters. Nanofiltration has been used commercially for sulfate removal from a variety of waters. Documentation or application to mine waters were not found in the published literature. Nanofiltration technology shows good potential for application to mine waters but requires further test work to answer questions concerning the presence of high total dissolved solids loads, and the stability of the membranes in acidic multicomponent solutions.

REDUCTION PROCESSES

Ferrous Hydroxide-The U.S. Bureau of Reclamation has developed a process for treating selenium surface and agricultural waters. However, the generation of large volumes of iron sludge and the relatively high cost of reagents makes its application to mine waters questionable.

Iron-The successful use of iron as a reductant is based on the reduction of aqueous selenium species in the presence of copper ions. The elemental iron reduces both selenium and copper to produce a copper selenide on the iron surface.

Further test work is required to determine the applicable pH range.

Biological Reduction-Bacterial reduction of selenium aqueous species to elemental selenium has been shown to be a potential candidate for treating mine waters. The bacteria that appears to offer great promise is *P. Stutzeri* which can reduce both Se(IV) and Se(VI) species.

Test work was conducted on mine waters using two different bioprocessing approaches. *Pseudomonas* bacteria were grown on carbon surfaces by pretreating the surfaces with nutrients and biopolymers, i.e., a biofilm was formed on the carbon surfaces. A second approach was to extract enzymes from the *Pseudomonas* bacteria which was then coated onto carbon surfaces or impregnated in gel beads. A mine water containing 620 µg Se(VI)/L was treated in a single-stage aerobic bioreactor. The live biofilms produced effluent waters containing<10 µg Se/L for about nine months without breakthrough. The enzyme preparations also produced effluents containing < 10 µg Se/L with breakthrough occurring after four months of continuous operation.

Further test work was conducted to investigate the possibility of simultaneously removing cyanide and selenium from mine waters. The results showed that simultaneous destruction of cyanide (102 mg/L) and reduction of selenium (from 31.1 mg/L to <10µg/L) could be successfully accomplished.

CONVENTION WATER TREATMENT

Lime Softening/Ferric Coagulation/Filtration-The Best Available Technologies (BAT) for treating selenium bearing drinking and groundwaters are listed by EPA to include ferric coagulation-filtration. Application of these unit operations to mine waters was not found in the literature. The achievement of selenium removal to regulated discharge concentrations by these technologies is doubtful.

Adsorption Processes

A large number of investigations have focused on surface adsorption as a means of removing metals, arsenic, and selenium from aqueous phase solutions, e.g., adsorption by ferric hydroxides, aluminum hydroxides, alumina, activated carbon, clays, etc. Adsorption phenomena on ferric surfaces has been the emphasis of most of the investigations.

Ferrihydrite/Selenium-Ferrihydrite adsorption of selenium has been selected as the Best Demonstrated Available Technology (BDAT) for selenium removal from wastewater solutions by the U.S. EPA[2].

Many investigators have considered the adsorption of selenium on ferrihydrite surfaces[3-21]. The

commonality between these studies is that:

- Se(IV) is adsorbed much more effectively than Se(VI);
- Se(IV) adsorption is a function of type of oxyhydroxide present (amorphorus ferrihydrite is best, crystalline FeOOH is considerably less effective);
- The initial concentration of Se(IV) in solution is very important (the higher the Se(IV) concentration the more effective the adsorption);
- The best pH for effective Se(IV) adsorption is 4-6 (85-90% removal), adsorption decreases slowly until about 7 (80-85% removal), then decreases drastically to about (20-40% removal);
- Se(VI) adsorption is poor (<10% removal) at all pH levels;
- Se(VI) adsorption may be strongly effected by the presence of sulfate, bicarbonate, and aqueous silica species.

Merrill, et al[15] and EPRI[8,22] found that the optimium pH for Se(IV) removal was 6.5 and optimum iron dosage was 14 mg/L for a water initially containing 40-60 µg Se/L. The effluent selenium concentration resulting from the treatment of 33 gallons/min in a continuous pilot facility was < 10 µg/L. WSPA[18] found fairly similar results for the treatment of a petroleum biotreater effluent (similar to gold heap leach effluent solutions, i.e., they contain cyanide as well as selenium and sulfate), e.g., after solution oxidation to destroy any selenocyanate species the optimal pH was 5-7; optimal ferric chloride dosage was 40-80 mg/L. The process treatment created 2.1-3.1 kg sludge/kg iron added.

Merrill, et al[15] estimated the cost of processing 26 million gallons/day (for arsenic, heavy metals and selenium removal) would be 35• ¢/1000 gallons. The estimate is based on a flowsheet consisting of ferric chloride addition to form ferrihydrite, rapid mixing, polymer addition for agglomeration, clarification, sludge thickening and dewatering, and disposal of dewatered sludge in a muncipal landfill. They suggest that the estimated processing cost is within • 30%. Updating this cost figure using the M&S index ratio gives a present day processing cost of 50• 15• ¢/1000 gallons.

Parida, et al[23] investigated the adsorption of Se(IV) onto various ferric oxyhydroxide surfaces. They found that amorphorus ferrihydrite adsorbed significantly more Se(IV) than the other phases. The order of adsorption was ferrihydrite (225 m^2/g)>δ-FeOOH (117 m^2/g)>γ-FeOOH (61.1 m^2/g)>β-FeOOH (77.8 m^2/g)>α-FeOOH (70.8 m^2/g), e.g., at 20 mg Se(IV)/L ,ferrihydrite was loaded to 48 mg Se(IV)/g); α-FeOOH was loaded to 10 mg Se(IV) /g). Essentially complete removal of Se(IV) from a 20 mg Se(IV)/L solution was attained by using 1.2 g ferrihydrite/L at a pH of 3. Whereas, α-FeOOH removed only about 25% of the Se(IV) under the same conditions. Adsorption was a relatively strong function of solution pH, i.e., adsorption fell rapidly as the pH was increased from 3.5 to 9.5. There was essentially no adsorption at pH 9.5.

Balistrieri and Chao[3] investigated the role of the presence of other anions on the adsorption of selenium. Their results are in agreement with the conclusions stated above. However, they found that if other anions are present they may preferentially adsorb to the ferric oxyhydroxide surface. The order of adsorption at pH 7 was found to be phosphate>silicate=As(V)• • bicarbonate/carbonate• citrate=Se(IV)• molybdate• oxalate>fluoride=Se(VI)• sulfate.

Hayes, et al[9] studied the adsorption of selenium oxyanions on α-FeOOH surfaces. Their conclusion was that Se(IV) adsorbs in a bidentate manner (inner-sphere adsorption which is much stronger than outer-sphere adsorption). Selenium (VI) adsorbs as an outer-sphere hydrated complex which is why it can be easily replaced by other solution anions such as sulfate. This conclusion has also been confirmed by others[4,9,24].

The question is always whether the wasteform product will be stable when stored outdoors in storage ponds exposed to air. It appears that there is, at least, a good possibility that such storage is appropriate. Long term aging stability, however, needs to be experimentally demonstrated. The question that still remains as to what happens when the amorphous wasteform crystalizes to the more thermodynamically stable phases of goethite (FeOOH) or hematite (Fe_2O_3). Large decreases

in surface area will result.

Aluminum Hydroxide/Alumina-Activated alumina has been studied for selenium adsorption by several investigators[25-29]. Trusell, et al[25] investigated the adsorption of Se(IV) and Se(VI) on activated alumina. Selenium (IV) was effectively adsorbed over the pH range 3-7 (for concentrations of 100-200 µg Se/L, one hour contact time. The loading capacity was 90 mg Se/L of alumina. Selenium (VI) adsorption was much less effective (loading capacity was 7 mg Se/L of alumina). They demonstrated that the order of selectivity for anions was $OH^{-1}>H_2PO_4^{-1}>F^{-1}>H_2AsO_4^{-1}>$Se(IV) (from batch studies) and that the order of other less adsorbed anions was $SO_4^{-2}>$Se(VI)$>HCO_3^{-1}>Cl^{-1}>NO_3^{-1}>H_3AsO_3^{\circ}$ (from column tests). Sulfate and bicarbonate had no effect on Se(IV) but greatly affected Se(VI) adsorption.

Trusell[30] demonstated alumina regeneration using 0.5% NaOH. A cost estimate was presented for treating 1 MGD containing 100 µg Se/L. The cost estimate was 23• /1000 gallons for Se(IV) and 78• /1000 gallons for Se(VI). This estimated cost when updated (by using the current 1999 M&S index) is 26• /1000 gallons for Se(IV); 88• /1000 gallons for Se(VI). Yaun[26] is in agreement with the Trusell results. He, however, demonstrated successful Se(VI) removal by reducing the pH of the solution to four or less. He also demonstrated that alumina could be regenerated repeatedly without loss of adsorptive power; that regeneration was effective using 0.25% NaOH.

The above studies were conducted on groundwaters not on mine waters. Investigators[27,28,31] have applied alumina adsorption to an actual gold mine effluent (FMC Gold's Paradise Peak Mine in Nevada). They demonstrated that selenium recovery is not very effective if aqueous silicon species are present. Mine waters that have appreciable silica present cannot be effectively treated by alumina adsorption. The alumina adsorption capacities for selenium and silica were determined to be 5.6 mg Se/ft^3 alumina,103 mg Si/ft^3 alumina, respectively. In the absence of silica the alumina adsorption capacity for selenium was 24.4 mg/ft^3 alumina.

Activated Carbon-Activated carbon adsorption is the most universally used technique to remove organic contamination from drinking water point-of-use supplies[32]. It is widely used in treatment of point-of-use waters for metals recovery. Activated carbon adsorption of selenium, either Se(IV) or Se(VI), is completely ineffective, e.g., Se(IV) or Se(VI) at concentrations from 30-100 µg/L showed < 4% removal using dosages of activated carbon up to 100 mg/L[33].

Other Absorbents-The USBM has performed studies utilizing peat impregnated with ferric oxyhydroxide[34-36]. Ferric oxyhydroxide/peat mixtures were formed into beads of polysulfone resin (designated HW-FIX). Loadings of 1,700 mg Se/L of resin were demonstrated on synthetic solutions containing 4800 µg Se(IV)/L, 4500 µg Se(VI)/L. The treated effluent contained from 10-20 µg/L Se(IV), and approximately 1000 µg/L Se(VI). The beads were stripped with 10 g/L NaOH. The strip solution was treated with iron to precipitate elemental selenium, thereby producing only a small amount of disposable selenium bearing waste.

Adutwum[37] investigated a process for adsorbing selenium onto a lanthanum oxide or lanthanum oxide/alumina substrates. The adsorption is effective for both Se(IV) and Se(VI). The loading capacity of the lanthanum oxide is 30-40 mg Se/g of oxide. Residence times are in the range 5-30 minutes. Sulfate is an interfering ion. If it is present in a concentration >1000 mg/L then the sulfate should be removed prior to exposure to the lanthanum oxide. The applicable pH range is 3-10. Cost estimates have were not presented.

Conventional Water Treatment

Ferric sulfate and alum coagulation-pilot scale studies (using EPA's 2 gallon/min plant) for treating waters containing 30-100 µg Se/L) have been conducted[38]. The conclusions of the study were: ferric sulfate and alum coagulation (at 25 mg/L) can achieve EPA's selenium maximum concentration levels (MCL) when the Se(IV) concentration is only slightly above the MCL and the pH of the water is 7 or less. However, the Best Available Technologies (BAT) for treating selenium bearing waters are still listed by EPA to include ferric coagulation-filtration (removals=40-80% for Se(IV); <40% for Se(VI)) and lime softening (removals=40-80% for Se(IV); <40% for Se(VI)). Alumina adsorption (removals>80%), reverse osmosis (removals>80%) and ion exchange (removals>80%) perform better and are also listed as

BATs[39,40].

Conventional water treatment technologies of ferric sulfate and alum coagulation are not viewed by the authors of this review as viable processes from treating mine waters that contain dissolved selenium. However, Patterson[41] summarized full scale selenium removal from coal mining, coal preparation, metallic ore mining and milling by conventional sedimentation processing. The predominate form of selenium in these waters is particulate. Sedimentation removals have been shown to be greater than 90% for selenium concentrations>20 µg/L.

Ion Exchange (IX)

IX is used widely for treating drinking waters, dilute metal bearing solutions, wastewaters, and groundwaters. However, its application for removing selenium has some successes and some failures[42,43]. Please see reference 1 for detailed discussions.

The Western States Petroleum Association[18] investigated the use of IX (strong base anion resins) for treating refinery wastewaters (sourwater and biotreater effluent, containing 11-4,870 µgSe/L). Their conclusions included:

- IX is very inefficient for selenium because other anionic species may load in preference to the selenium, especially sulfate. Selenium loaded well but after 200-600 bed volumes sulfate displaced the selenium. High sulfur and high total dissolved solids reduce the effectiveness of selenium extraction. Bed volumes to breakthrough of selenium ranged from 50 to 750.
- IX is not able to routinely produce effluents with<50 µg/L total selenium.
- Efforts to identify selective resins were unsuccessful.

Tailored chelating polymer resins have been investigated[44] for extracting selenium in the presence of high concentrations of sulfate. The resins investigated included Dow 2N (loaded with copper, 1.7 meq/g) and IRA-900 (no copper loading). The resins were evaluated for extraction in a solution containing 250 mg sulfate/L. The selectivity sequence at pH 9.5 was $SeO_4^{-2}>SO_4^{-2}>SeO_3^{-2}>NO_3^{-1}>Cl^{-1}$. Resin loading capacities and cost evaluations were not presented by the authors.

Virnig and Weerts[45] considered the use of liquid ion exchange as a way to treat spent gold heap leach effluent. The ion exchange reagent was CyanoMet R, a proprietary reagent. The reagent extracted anion complexes including gold, silver, nickel, copper, zinc, and selenium. Laboratory studies were conducted. The extracting reagent contained 30 weight percent CyanoMet R. There were three stages of extraction followed by two stages of strip (4 volume percent NaOH). The selenium removal from the influent feed (11 mg Se/L) was very good, e.g., the final raffinate solution contained 70 µg Se/L. Pilot studies were conducted at a Nevada gold mine. The feed rate was 1 gallon/minute, the reagent consisted of 15 % CyanoMet R in kerosene. The selenium removal from the influent feed (1.7 mg Se/L) was very good, e.g., the final raffinate solution contained 300 µg Se/Lfor the first pass; 36 µg Se/L for the final second pass.

Membrane Separations

Reverse Osmosis-RO is listed by EPA as one of the BATs for selenium removal[39,40], i.e., the removal effectiveness is quoted as being >80% irregardless of valence state. Reverse osmosis/membrane ultrafiltration processes require that the solutions to be treated contain very dilute concentration of solids. Therefore, pretreatments are normally required in order not to foul the separating membrane. Whereas, reverse osmosis is readily appliable to drinking waters it is doubtful that these separation techniques are applicable to acid mine waters except as a final polishing step after rather extensive pretreatment.

Emulsion Liquid Membranes- ELM technology is presently being developed by TDA Research for selenium removal[46]. This technology is based on the transfer of selenium from the aqueous phase to a liquid extractant phase contained inside droplets of organic. The exchange process is similar to that which occurs during conventional solvent extraction except an aqueous strip phase (0.1-5 µm diam) is present as an emulsion within organic droplets (0.1-2 mm diam). The organic droplets are, in turn, dispersed throughout the bulk aqueous phase. Selenium is transferred from

the bulk wastewater to the organic phase. Internal within the organic phase the selenium is tranferred to the aqueous strip solution. The organic emulsion is subsequently recovered from the bulk wastewater phase and the loaded aqueous strip solution is separated from the organic. Laboratory studies have shown that Se(VI) is extracted rapidly even in the presence of sulfate at all pH values >2. Pilot studies at pH 10.5 have shown that Se(VI) (at 1 mg/L) can be reduced to below 1 µg/L in 2 min.; that Se(IV) can be reduced to below 1 µg/L in 5 min.. Sulfate is extracted in preference to Se(IV) unless the pH is >9.5. In spite of this, extraction of Se(IV) in the presence of up to 1 mg sulfate/L at pH 10.5 was very effective. The concentration of selenium in the strip phase shows an enrichment factor of ~40.

Nanofiltration-Nanofiltration appears to be a technology on the horizon for treating some low metal containing selenium bearing mine waters. Nanofiltration has been used commercially for sulfate removal from seawater prior to injection into oil field reservoirs, for sulfate removal from concentrated brines, for organic compound removal from paper plant effluents, and for organic compound removal from groundwater. Publications demonstrating the application of nanofiltration to mine waters were not found but the technology has been applied to agricultural waters (high in Se(VI), sulfate and total dissolved solids) taken from San Joaquin Valley drainage[46].

Nanofiltration is based the use of membranes constructed of a porous inert layer of polysulfone and a negatively charged hydrophobic rejection layer (generally polyamide). These membranes reject multivalence anions, including sulfate. The technology is similar to reverse osmosis but the nanofiltration system is operated at pressures that are about one-third of that required for reverse osmosis. Kharaka[47] demonstrated >95% removal of selenium for waters (pH 6.3-8.5) containing 24-308 µg Se(VI)/L; 2,080-26,100 µg sulfate/L; and 780-38,800 µg TDS/L. Similar recoveries were demonstrated for Se(VI) concentrations up to ~1000 µg/L.

Reduction Processes

Various reductants can be used to produce elemental selenium or metal selenides. Reductants such as ferrous hydroxide, iron, zinc, aluminum have been proposed.

Ferrous Hydroxide-Murphy[52] patented a process for the Bureau of Reclamation (BOF) for treating selenium bearing waters. The process consisted of reducing selenium species to elemental selenium by using ferrous hydroxide as the reducing agent. The reduction is accomplished at a pH of 8.8-9.2 under reducing conditions. Magnetite and/or maghemetite formed. Interferences in the process are nitrate, dissolved oxygen (DO), and bicarbonate. Costs were estimated[53] at 31• /1000 gallons for low nitrate, DO, and bicarbonate. For high nitrate, DO, bicarbonate the costs were estimated to be 58• /1000 gallons. These costs when updated using the M&S index ratio to 1999 are 37• /1000 gallons (for the low nitrate, DO, and bicarbonate treatment) and 68• /1000 gallons (for the high nitrate, DO, and bicarbonate treatment).

Lien, et al[54] applied the BOR recipe to a silver tailing mine water (4 mg Se(VI)/L, pH 9). They used ferrous sulfate as the source of iron. They found that in order to attain a selenium effluence concentration of less than 100 µg/L, 25 g/L ferrous sulfate was required. The cost for the reagent alone would be about $5-$15/1000 gallons of water treated. Also, large quantities of iron bearing sludge would have to be handled and treated for disposal.

Iron Reductant-McGrew, et al[55] have developed a selenium recovery process using iron powder as the reductant. McGrew notes that mine waters are usually extremely high in sulfate compared to selenium (and compared to drinking waters and groundwaters), e.g., the ratios are often of the order 10^8 sulfate/selenium. Therefore, a process is required that is not influenced by sulfate. The success of this process compared to other proposed iron reduction processes is that the rate of Se(VI) reduction by iron to selenium is catalyzed by the presence of copper.

McGrew demonstrated their process on a gold mine heap leach effluent (Brewer Pit water, 257 µg Se/L). The water was tested (pH 3-3.5) using 1 to 10 g Fe/L, and copper levels between 10 and 81 mg/L. The optimum conditions resulted in selenium concentrations less than detection limit (<5 µg/L). Residence times of 10-20 minutes were required for a copper content of 10 mg/L at an iron dose of 10 g/L. The consumption of iron was ~3 pounds/1000 gallons. The product could be further treated for selenium recovery. Simple conventional hydrometallurgical equipment would be required, i.e., mix chambers, dewatering settlers, and clarifiers. This process has been applied at the Brewer mine since 1994.

Biological Processes

There are several patents and studies in the literature that propose potential biological processes for removing selenium[56-69].

Pseudomonas stutzeri bacteria/immobilized alginate enzymes-Adams, et al[56] have investigated selenium removal (on a bench scale) from agricultural and mining wastewaters by bacteria and immobilized alginate enzymes. *Pseudomonas stutzeri* isolates have been identified and grown from mining wastes. *Pseudomonas stutzeri* are known to reduce selenium from solutions under aerobic conditions. The authors treated solutions containing up to 1000 mg/L of Se(VI) and 285 mg/L sulfate over a wide range of pH. Selenium was removed from mine waters containing 620 µg/L to <10 µg/L in an 18 hr residence time. Enzymatic bioremediation processes have been used to treat mining solutions containing 600 µg/L to 30,000 µg/L selenium. Continuous selenium removal to <10 µg/L has been demonstrated for up to four month periods.

A *P. stutzeri* isolate that was acclimated to mining process waters was capable of removing 33 mg/L Se(VI) from mining waters containing 285 mg/L sulfate in a bioreactor in residence times as short as six hours. Test work was conducted on mine waters using two different bioprocessing approaches. *Pseudomonas* bacteria were grown on carbon surfaces by pretreating the surfaces with nutrients and biopolymers, i.e., a biofilm was formed on the carbon surfaces. A second approach was to extract enzymes from the *Pseudomonas* bacteria which was then coated onto carbon surfaces or impregnated in gel beads. A mine water containing 620 µg Se(VI)/L was treated in a single-stage aerobic bioreactor for a residence time of 18 hours. The live biofilms produced effluent waters containing<10 µg Se/L for about nine months without breakthrough. The enzyme preparations also produced effluents containing < 10 µg/L with breakthrough occurring after four months of continuous operation. Further test work using enzymes showed simultaneous destruction of cyanide. The above described bioprocessing techniques were also successful in removing nitrate from 53 mg/L to<0.1 mg/L at a rate of >3.3 mg/L/hr.

Koren, et al[57] also investigated the use of *Pseudomonas stutzeri* for selenium specie reduction to elemental selenium. The authors demonstrated that bacteria could survive and operate effectively in Se(VI) solutions containing over 3500 mg Se/L. The rate of reduction was ~32 mg Se/L/hr. The bacteria could also survive and operate effectively in Se(IV) solutions to approximately 1500 mg/L, then the rate of reduction dropped dramatically. Maximum reduction rates occurred in the pH range 7-9.5; optimal temperature was 25-35°C. The presence of sulfate, nitrate, and nitrite ions had no harmful effect on either Se(VI) or Se(IV) reduction.

Bacterial inoculum-Altringer, el al[58,59] investigated both chemical and biological treatment of three mine waters, e.g., tailings pond water from a silver mine (4.2 mg Se/L, pH 8.9), barren solution from a gold heap leach operation (30 mg Se/L, pH 8.4), and pregnant solution from a gold heap leach operation (1.7 mg Se/L, pH 8.8). Selenium reducing bacteria were not found in the mine waters so bacteria from the Kesterson reservior (San Joaquin Valley, CA) were separated and cultured (*Pseudomonadaceas* and *Enterobacteriaceae* families) in mine waters. The waters were first treated by oxidation to destroy cyanide (to<3 mg/L) and to oxidize the selenium to Se(VI). Solution pH levels were adjusted to ~8.5. The bacterial inoculum was added to the waters to produce 10^9 cells/mL. The tests were run in 2.1 L solutions, 25°C, 350 rpm stirring, 5 g/L peptone nutrient, and 40 cc/min N_2, 10 cc/min air (this gave a solution oxygen content of 2 mg/L). The effluents from the tests contained 160 to 1000 µg Se/L.

Clostridium Bacteria-Kauffman, et al[60] investigated microbiological treatment of uranium mine waters for selenium removal. Pilot scale column tests were performed for selenium and sulfate removal using *clostridium* and *desulfovibrio* bacteria containing local soils, respectively. Selenium(VI) removal from 1.6 mg/L to less than 50 µg/L was achieved in both laboratory and field test columns. Selenium break through (100-175 L/m^2/day) never occurred even after 7-months of continuous operation. Selenium was present in the column material in the elemental form.

Biological Anaerobic Reactors-Ergas, et al[61] demonstrated on a laboratory scale the successful utilization of anaerobic bacterial reactors for selenium removal from agricultural waters. The test work utilized an anaerobic biological bacterial growth reactor. The Se(VI) was reduced to

elemental selenium. Owens, et al[62] followed up this work in a pilot-scale study. Water from agricultural drainage sites (pH 8) in California was used for the study. The anaerobic reactors used in the study were two parallel 50 gallon tanks, each tank was fed the same water but a different nutrient source (either acetate or methanol). The results showed that Se(VI) was reduced through a two step sequence, i.e., Se(VI) was reduced to Se(IV) then to Se(O) in the anaerobic reactors. Influent selenium concentration was 453 µg Se(VI)/L. At the end of a 24-hr cycle the average concentration of selenium (at a pH of 8) was 280 µg/L (for the acetate reactor) and 265 µg/L (for the methanol reactor). Filtering of the product water through 0.22 µm filters resulted in a solution selenium content of 45 µg/L (for the acetate reactor) and 65 µg/L (for the methanol reactor). Nitrate was removed completely by both reactors.

Oremland[63-66] patented a process for treating selenium and nitrate bearing solutions. His process can also handle sulfate solutions. The proposed process includes an aerobic bioreactor (first stage) to remove nitrate. Nitrate has been shown to inhibit the reduction of Se(VI) at concentrations >0.5 millimoles nitrate/L. Nitrate can be removed to below 1 millimole/L by aerobic bioreactor treatment. The nitrate free water can then be treated in a second stage anaerobic reactor (pH 7-10) using selenium respiring microorganisms. The biomass from the first stage can be utilized in the second stage as the nutrient source for the microorganisms. A further development was to treat sulfate bearing waters in a bioreactor containing sulfate reducing bacteria. The product from this reactor was aqueous hydrogen sulfide (1-3 millimoles/L) which can then be fed into the selenium removal stage to further reduce the solution potential so that a better yield of elemental selenium can be obtained. The selenium concentration in the product water should be within the range 1-10 µg/L.

Summary

Several technologies appear to hold promise for application to mine waters for removing selenium to the low µg/L range, e.g., <10 µg/L. Success for achieving effective selenium removal will depend on the site-specific characteristics of the individual wastewater to be treated. Those technologies that appear to hold promise for effective selenium removal include: ferrihydrite adsorption, HWFIX, emulsion liquid membranes, nanofiltration, iron reduction and biological reduction.

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Selenium Reduction Issues in a Coal Fired Power Plant

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Keywords: Wastewater, flue gas desulphurization, FGD, coal power plant, metals reduction, selenium, NPDES, ICP

Summary: The paper will review a treatment study for reducing the Selenium level in the wastewater discharged from a wet limestone flue gas desulphurization unit. Covered in the paper are problems encountered with analytical testing procedures for determining low levels of Selenium in high TDS water and a successful work-around.

The paper includes data generated at a power plant burning high Selenium coal with a single module scrubber built by Alstom. The scrubber uses wet limestone technology with forced oxidation to manufacture commercial grade gypsum. Di-Basic Acid is used in the scrubber to improve limestone utilization. To control chlorides in the FGD sump and to facilitate washing the gypsum, a constant stream of water must be wasted as blown down from the system to a treatment plant. The effluent from this WWTP is discharged to a small creek thru an NPDES outfall. The function of the wastewater plant is therefore to remove heavy metals, reduce suspended solids and reduce BOD coming from the use of the DBA. Historically the plant had been able to comply with all of the discharge limits except Selenium. This paper will review the steps taken to bring Selenium under control and outline the technical issues that complicate Selenium control in high TDS wastewater.

INTRODUCTION

The subject account is a 700-megawatt Eastern coal fired facility. The plant burns a mixture of washed and unwashed coal. In 2001, they installed a single module wet limestone flue gas desulfurization scrubber. The plant produces gypsum that is sold for wallboard and and as such requires washed gypsum cake with a maximum chloride content. To control the chloride in the gypsum, the washings are blow down from the system to a wastewater treatment system.

To facilitate blowdown a 200,000 gallon per day wastewater treatment plant was installed at the time the FGD was built. The function of the wastewater plant was to remove metals, suspended solids, control BOD and maintain pH sufficient to meet their NPDES permit.

Blowdown from the FGD is taken from the decant water after the centrifugal separators that feed the gypsum crystals to their vacuum filter belts. In this configuration, FGD liquor is pumped to an array of 16 centrifugal separators. The heavy gypsum crystals are spun to the outer wall, settle, flow out the bottom opening, and are fed to the vacuum belt filters while the liquid containing lower solids passes out of the top. This liquid contains primarily fines and waste inerts from the limestone and is approx. 1% to 2% solids. Most of this liquid is returned to the scrubber while a portion is wasted to the WWTP feed tank (153,000 gallons). The rate of blowdown is controlled to maintain a target chloride concentration in the scrubber liquor of less than 15,000ppm as Cl.

From the WWTP feed tank the wastewater is pumped to a first stage neutralization tank where lime is added for pH adjustment. From here the water passes to a roughing 1st stage clarifier that removes the bulk of the solids. The 1st stage clarifier effluent, typically less than 200ppm SS, passes to the 2nd stage neutralization tank for added polishing treatment then to the 2nd stage solids contact clarifier. The effluent water from the 2nd stage goes to a final adjustment blend tank for trimming. This water is then pumped through plate flow heat exchangers and onto an integrated activated sludge

unit. The activated sludge process was installed to remove organic loading since the plant would be using dibasic acid to assist limestone utilization in the scrubber. Effluent from the activated sludge unit passes through gravity multimedia filters before discharge to the NPDES permitted outfall. (See drawing 1.)

Sludge from the 1st and 2nd stage clarifiers plus waste solids from the activated sludge unit are pumped to a conventional thickener. Sludge from the thickener is dewatered on 2 rotary vacuum filters with the cake going to on-site landfill.

System Startup

As with all new processes start-up issues and design oversights are reveled as the system is put into operation and this one was no exception. Many of the problems encountered could have been prevented and still more could have been corrected at construction, but were not. Many of these flaws had significant impact on function and made daily operation of the system difficult. These became more complicated because much of the design basis was taken from concepts and forecasted data not from actual previous plant operation. In short, this was design Version #1 of this particular equipment arrangement.

The plant was operated for 3 years as the owner, engineers and contractors struggled to meet operating design parameters. During this period, the system was able to meet the NPDES limits with the exception of selenium. The selenium limits were 0.8 ppm monthly average with 1.6 ppm maximum. During the initial operation the facility was consistently over the 1.6 ppm on the averages and the value was more typically 3 ppm.

In June of 2004, Applied Specialties, Inc. [ASI] was contracted to assist the plant in making permit limits. The information to follow is not a complete study since the project is on going. The information should serve and be used as a starting point for those planning or currently operating an FGD wastewater operation where selenium control is a problem or will be a requirement.

Initial Process Investigation

The plant was using a low level of ferric chloride feed into the 1st stage and was controlling the pH at 9.3 with lime. The pH in the 2nd stage was reduced 8.5 with hydrochloric acid and then reduced to 7.6 in the final with additional hydrochloric acid. Additional treatment products were used in the process. A cationic polyamine was added to the raw wastewater entering the 1st stage blend tank and a high molecular anionic polymer was added to the 1st stage clarifier center well. A cationic amino-methylated tannin polymer was added to the 2nd stage blend tank.

Many of the mechanical design oversights were adding to the operational difficulties and making some operations essentially impossible. Design errors that added to operating trouble included:

1. The turbine in the contact solids clarifier could not pass the required flow also provided no draft.
2. Failure to install a sloped floor in the clarifiers.
3. Slits in air scour distributors of gravity filters were larger than sand size.
4. Sludge pumps were oversized and not able to pump some of the more dense sludge.
5. NPDES effluent discharge line for off-spec effluent was combined with the sump return line to feed tank. This created a potential to feed return sludge to the outfall when the sump pumps operated.
6. No sample lines were installed on the clarifiers' sidewalls or the draft tube of the solids contact clarifier.
7. BOD load created by DBA in FGD was a calculated estimate while actual BOD (food) was much lower and insufficient to maintain the target design for MLVSS in the activated sludge system.

At the same time the operating chemical controls were adding to these problems. As an example, operating the 1st stage at a pH of 9.3 created a tremendous amount of excess solids in the system since calcium sulfate and magnesium hydroxide were both being precipitated. The volume of solids that were produced then overloaded the design capacity of the vacuum filters.

Selenium Reduction Process

Selenium chemistry is very similar to that of sulfur and many of the compounds have similar properties. The most common ionic forms are the –2 (Selenide), 0 (selenium metal), +4 (Selenite) and +6 (Selenate). In addition to these there are organo-selenium compounds and mixed sulfur-selenium compounds such as selenothionate. The presence of these compounds are discussed by Radian in their Patent. Selenium metal has 2 distinct forms and "red" form

makes its identification rather easy. It is the red form that gives many of the old scrubber evaporation ponds their distinct pink color. It is was also gives many of the bacteria that assimilate selenium their distinct pink color.

Based on literature searches, Selenite is absorbed on ferric flocs preferentially at pH's less than 6.5. On the other hand, Selenate is not that readily absorbed. In addition, elevating the pH to 9.0 or greater can reverse the adsorption of Selenite on these iron flocs.

Early sample testing indicated that the wastewater from the FGD contained primarily selenium in the Selenite form. As such, the operating practices were changed such that the 1st stage pH was lowered to 7.0 and the 2nd stage was lowered to 7.2 and the final was maintained without chemical feed (7.1pH).

At the initiation of the program, ASI installed portable IX units with anion resin to polish the plant effluent to get it within NPDES limits. The vendor used to supply the resin could not supply the IX vessels in the bisulfite form. Based on the vendor's in-house specialists' advice, chloride form was used. Earlier bench testing of the effluent had indicated that the bisulfite form of ***Mitsubishi SA-20*** would remove the selenium to under 400ppb even in the high chloride effluent (15,000ppm Cl).

After 2 weeks of attempted operation, the use of the anion vessels was terminated. The particular strong-based anion resin used by the mobile supplier could not remove the selenium in the higher chloride wastewater that the plant was generating (18,000ppm Cl). Continued bench testing with the ***SA-20*** demonstrated that the bisulfite form would work but that regeneration on-site would not be possible and that avenue of study stopped.

The focus was then shifted to coagulation and sedimentation practices to reduce the selenium.

Selenium Analysis Issues

During ASI program start-up an apparent sampling or analytical problem surfaced. The Se values from the ASI laboratory were typically running 700 to 900ppb higher than the client's contracted EPA certified local laboratory. This created a sidetrack of interests to uncover the cause. ASI was using a Thermal Electron Iris II ICP while the contract lab was using Graphite-AA. To determine a potential cause of this difference samples were also run using XRF. The XRF results supported the higher ICP values. Unfortunately XRF was not specifically listed as an EPA approved test method for determining Se in wastewaters and it was necessary to determine a valid method that was or uncover the reason why there was a difference.

To validate the ICP results, a sample of plant effluent water was filtered through 0.45-micron filter media. This water was then used as a matrix base. To this water, a series of known selenium spikes were made. These spikes were from 500 ppb to 4 ppm. To insure that the form of selenium did not interfere with the analysis the spikes were made in 2 (two) spike sets. One set was made using sodium selenite and the other set was made with sodium selenate (both reagent grade). Results from the ICP scans demonstrated a maximum variance of test verses calculated of less than 1% on 9 of the 10 spike samples with less than 3% on the 10th sample spike.

Table 1

Sample	Calculated	Actual test	delta
Base FGD water		0.6798	
0.5ppm Se (Se+4)	1.1798	1.1737	-0.517%
1ppm Se (Se+4)	1.6798	1.665	-0.881%
2ppm Se (Se+4)	2.6798	2.6824	0.097%
3ppm Se (Se+4)	3.6798	3.698	0.495%
4ppm Se (Se+4)	4.6798	4.653	-0.573%
0.5ppm Se (Se+6)	1.1798	1.1805	0.059%
1ppm Se (Se+6)	1.6798	1.698	1.083%
2ppm Se (Se+6)	2.6798	2.745	2.433%
3ppm Se (Se+6)	3.6798	3.682	0.060%
4ppm Se (Se+6)	4.6798	4.661	-0.402%

The results demonstrated that the ICP values were accurate and the method was not being influenced by background interference nor was it influenced by the form of selenium in the sample. It also demonstrated that the changes in nebulizer and software design for new generation ICP units have adequately removed the argon interference of earlier ICP designs.

To further validate the ICP reliability for Se analyses a sample was sent to Thermal Electron's development and customer service group. They ran samples on an ICP-MS unit, both with and without a hydride generator feeding the ICP. They also ran the sample on their GF-AA. The GF-AA results matched the GF-AA results from the contract service laboratory to within 5ppb on a 1.8ppm sample result while the ICP-MS matched the ASI ICP results to

within 10ppb on a 2.63ppm result. Please note there was an 803ppb difference in the 2 (two) methods and the difference in the ICP to Hydride ICP was 842ppb.

As a result most interesting results came from the use of a hydride unit in front of the ICP-MS. Here the Se values essentially equaled the GF-AA method (1.78ppm). Because the sample being fed to the hydride unit was undigested, the Se should only be from Selenite since only Se in the Selenite form generates a hydride gas that is pulled into the plasma flame. In addition the hydride generator essentially eliminates all potential background matrix interferences since only compounds capable of forming a hydride will be aspirated into the ICP nebulizer. The results indicate quite clearly a difficulty with GF-AA result validity that seems associated with Se forms other than Se+4.

Table 2

	ICP Data in sample	Hydride-ICP data in sample
	Sample A	Sample A
Results	Avg	Avg
Se @ 1960	**6.826 ppm**	**1.846 ppm**
	Sample B	Sample B
Se @ 1960	**9.544 ppm**	**6.83 ppm**
	Sample C	Sample C
Se @ 1960	**0.9828 ppm**	**0.6717 ppm**
	Sample D	Sample D
Se @ 1960	**2.63 ppm**	**1.788 ppm**

Sample A	WWTP Influent
Sample B	FGD Filtered Liquor
Sample C	Plant Effluent
Sample D	2ns stage OF

Comparing the isotope species of Se in the hydride run to that of the straight ICP yielded the same isotope ratios. This was further validation of the ICP as the preferred analytical tool for Se analyses in an FGD matrix.

This investigation strongly suggested a problem with the sample preparation and the GF-AA method for analyzing Selenium in a FGD matrix water Presented the data, the contract lab admitted there were errors and began running Hydride-AA in a side by side comparison to the GF-AA. The limiting factor for accuracy in the hydride method is the digestion steps since all Se must be converted to Selenite to be measured. The contract lab revised their digestion process and eventually migrated to microwave digestions. This seemed to correct some initial errors and once the digestion step was worked out, the contract lab results by the hydride method matched the straight ICP method.

This testing occurred prior to the plant's annual outage. During the outage the oxidation air disperses and mixers were all rebuilt with a new designed disperser installed. The blowers and piping were all rebuilt and cleaned. Once the unit came on line, the spread of selenium values between the ICP and the contract laboratory again began to differ.

In an attempt to uncover the possible cause of the difference, two reagent standards were made, one was 11ppm of Se from sodium Selenite and another was 11ppm Se from sodium Selenate. A sample of each, plus a 50/50 weight blend of each and a 50/50 blend of the Selenite standard and de-mineralized water were provided as blind samples to the contract laboratory. Since these samples were prepared with high quality de-mineralized water, secondary matrix issues were eliminated. Upon analyses, the contract lab was able to determine the sodium Selenite samples within a reasonable range for the 5.5ppm sample, but the deviation from the 11ppm was rather high. Their results were 9.87ppm for the 11ppm standard and 5.63ppm for the 5.5ppm Se from Selenite standard. Results for the Selenate-derived samples were very poor. Their result for the Se+6 standard was 0.9ppm and for the 11ppm Se and 5.88ppm for the 11ppm Se from the mixed Se+4/Se+6 sample This demonstrated that the contract outside lab was still not doing a proper digestion (oxidation reduction) prior to the Hydride-AA.

As an earlier follow-up to determine the ionic forms of the selenium in the FGD waters, samples were sent to ***Applied Speciation and Consulting, LLC.*** This is a specialty laboratory that determines the species of elements in a material as their primary business with selenium being one of the more important products. They determined that the selenium in the wastewater plant prior to the outage was primarily Selenite (Se+4).

Since it was found that the outside lab was able to reasonably determine Se+4, the fact that the ICP and Hydride-AA test results matched prior to the outage was not surprising.

After the start-up that followed the outage and with the Se results once again differing, the potential

causes for the difference needed to be determined. To this end a sample of FGD wastewater effluent was treated by cation resin and another was heavily treated with reactive iron bicarbonate at a pH of 5.0. The starting selenium value was 2.1ppm as Se. The cation resin removed approx. 0.267ppm Se while the iron media removed approx. 1.85ppm Se. Since the iron will only remove the anion forms of Se and the cation resin will only remove the cation form of Se, we generated strong evidence that we had approximately 0.3ppm of a cation Se and 1.8ppm of the Se was present as anion forms. The presence of the cation Se is somewhat surprising but work by Radian suggested the possible formation of selenothionate and similar molecules under various FGD operating conditions where a higher reducing environment had been created (poor oxidation).

In response to this, samples were taken across the wastewater treatment plant locations. These were again submitted to ***Applied Speciation and Consulting, LLC (ASC)*** for speciation of the Se and comparison of this speciation profile to their total Se values. The samples submitted to ASC were taken from the same samples submitted to 2 different outside laboratories as well as samples analyzed by ASI.

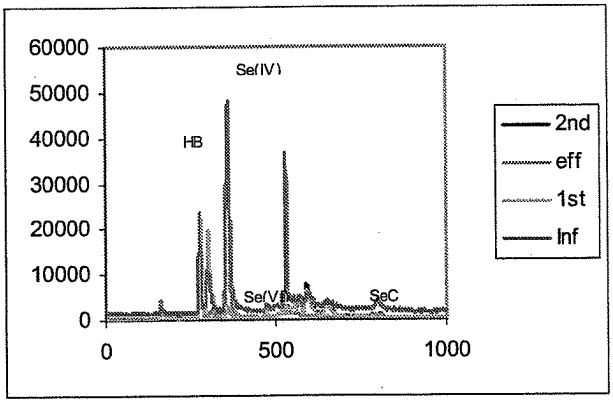
The intent of this sampling is to see the relative abilities of the various labs and their methods to accurately determine total Se values in an FGD water matrix. These comparison results should be available soon.

At this point, the reader should have a clear understanding that the accuracy of the analytical process for Se is of the utmost importance in treating for Se reduction. It should be obvious that a poor technique would lead the plant environmental personnel to a false conclusion on the efficiency of their treatment program. It cannot be stressed enough that the owner of any Se treatment process must first validate their analytical support.
The results from the cross system speciation will clearly prove the importance of analytical accuracy.

Up until now we have assumed that the primary Se species in the WWTP influent and effluent were Selenite and Selenate since both are favored by current literature. The speciation results demonstrated an entirely different picture. While Selenite was a predominant species in this particular FGD stream Selenate concentration was very small. The unexpected find was that there were no less than 8 anionic Selenium peaks in the samples 5 of which are not yet identified. Those identified are Selenite, Selenate and selenocyanate. Comparing the species across the system permits us to now examine the changes that each step of the process has on the specific selenium form.

The technique used to determine the Se speciation utilized an IC with an anionic column coupled to an ICP-MS. In this configuration the eluted peaks from the IC are educted into the ICP-MS unit and analyzed. This process confirms the presence of Se in the individual IC peaks. By comparing elution times of the IC peaks to a known library, the specific Se anions are determined.

It becomes obvious that the analytical difficulties of the Hydride-AA were due to the non-Selenite species and the digestion step but not specifically because of Selenate. At the same time it is possible that the errors of the GF-AA were due to the loss of specific Se species that were volatile and lost during the open digestion step used in the GF-AA preparation. Because most of the prior preparation work has focused on the determination of total Se from mixed solutions of Se+4 and Se+6, the digestion procedures for hydride-AA concentrated on converting Se+6 to Se+4 and eduction into the hydride generator. ASI started using a cold digestion for our ICP preparations where the preparation was performed in sealed tubes. In this process volatile specie losses are very unlikely and it is not necessary to make a conversion to Se+4 to generate an accurate total Se reading.



CONCLUSIONS

The most significant conclusion to be drawn from our work to this point is that prior analytical studies may be flawed by the assumption that selenium species in an FGD water system will mirror that of sulfur with the primary forms being Selenite and Selenate. As

we have discovered Selenite increases with decreasing oxidation but the Selenate specie does not seem to be influenced to any appreciable degree by the level of oxidation. Additionally Selenate concentration seems to be extremely low in comparison to the total Se concentration. Loss of oxidation seems to increase the formation of Selenite over the yet to be determined selenium forms recently discovered.

AA methods depend on digestion to determine the actual total Selenium in any sample. These digestion procedures add a significant potential error to the analyses that standard quality control checks are not going to easily uncover. Running triplicate digestions to insure a low standard deviation would be costly and very time consuming.

Cold digestion followed by axial or radial ICP would be a preferred method to determine total Se in an FGD matrix. Low levels of Se (sub 5ppb) would be best achieved by Trace ICP or ICP-MS using the ASI cold digestion method.

Determination of Selenite in a native sample can effectively be determined by either hydride AA or hydride ICP. Either method will generate an accurate Se+4 value since neither would require digestion where a laboratory procedural error could be introduced. Since ICP will provide an accurate total Se concentration, hydride ICP would then be the preferred Se+4 only test method thereby requiring only 1 piece of equipment and 1 experienced operator. The difference in Se values between hydride-ICP and straight ICP may not be all Se+6 in a FGD wastewater. In the current plant case study very little Se+6 exists.

Since our detailed work to this point has been limited to a single facility we will need to test a greater general sampling of FGD waters before the relationship between Selenate and Selenite can be better and more accurately defined.

We will require additional analytical study to determine the unknown Se species in the most recent speciation tests.

A number of treatment reagents will be tested to see how they affect the unknown and unexpected Se species in our recent tests. It will also reinforce that these species are common to this facility and not a one time sampling occurrence.

Work needs to be done to determine if these species are the result of specific fireside conditions at this test plant or they are uniform across various furnace conditions.

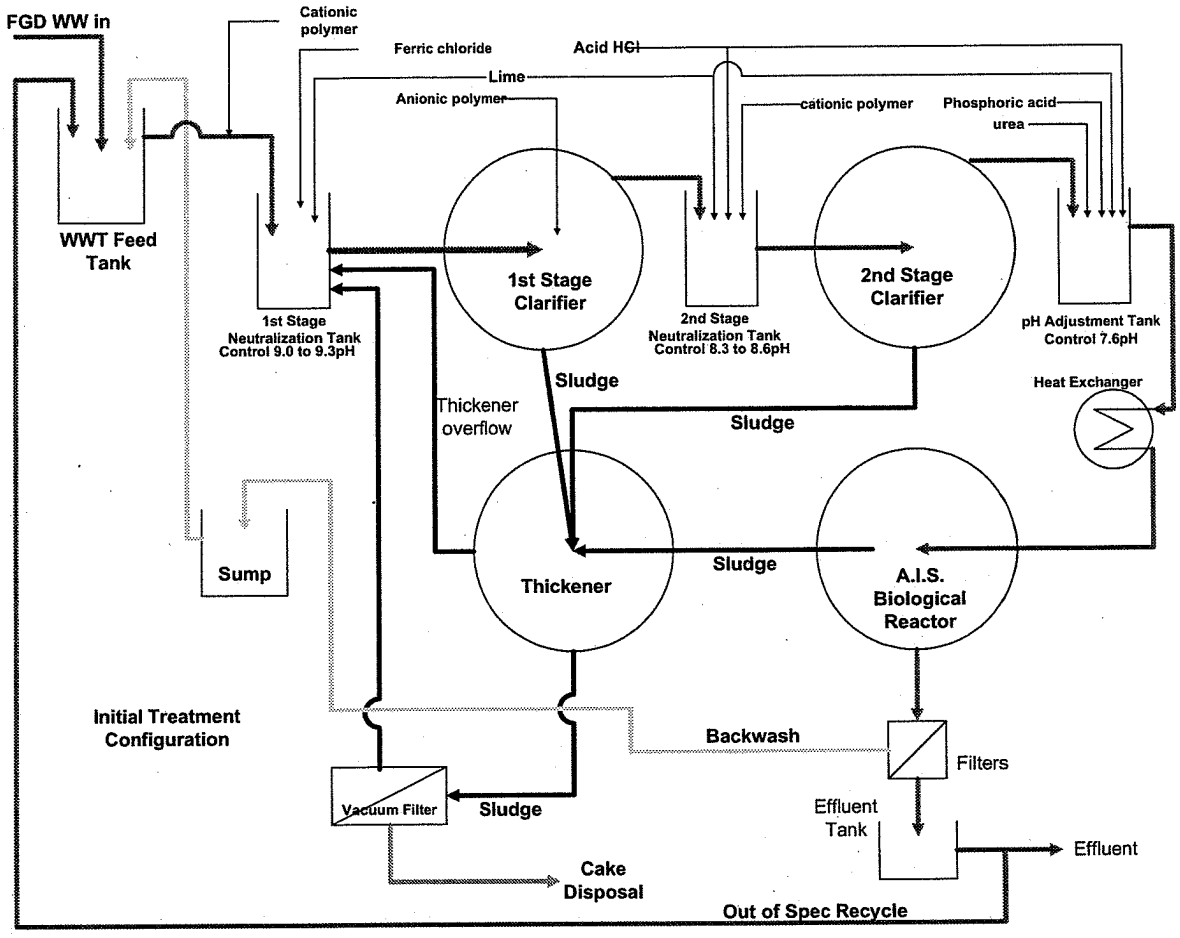
Special Consideration and Mention

The writer wishes to express a special thanks to Russell Gerads and his company Applied Speciation and Consulting, LLC for their speciation work. Without their unique tools much of the technical mystery would still be unknown and we would still be assuming that all the non-Se+4 is Se+6.

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Drawing 1


FGD WW in
Cationic polymer
Ferric chloride
Acid HCl
Lime
Anionic polymer
cationic polymer
Phosphoric acid
urea
WWT Feed Tank
1st Stage Clarifier
2nd Stage Clarifier
1st Stage Neutralization Tank Control 9.0 to 9.3pH
2nd Stage Neutralization Tank Control 8.3 to 8.6pH
pH Adjustment Tank Control 7.6pH
Heat Exchanger
Sludge
Thickener overflow
Sludge
Sump
Thickener
Sludge
A.I.S. Biological Reactor
Initial Treatment Configuration
Backwash
Filters
Vacuum Filter
Sludge
Effluent Tank
Effluent
Cake Disposal
Out of Spec Recycle

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Selenate Reduction to Elemental Selenium by Anaerobic Bacteria in Sediments and Culture: Biogeochemical Significance of a Novel, Sulfate-Independent Respiration†

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Interstitial water profiles of SeO_4^{2-}, SeO_3^{2-}, SO_4^{2-}, and Cl^- in anoxic sediments indicated removal of the seleno-oxyanions by a near-surface process unrelated to sulfate reduction. In sediment slurry experiments, a complete reductive removal of SeO_4^{2-} occurred under anaerobic conditions, was more rapid with H_2 or acetate, and was inhibited by O_2, NO_3^-, MnO_2, or autoclaving but not by SO_4^{2-} or FeOOH. Oxidation of acetate in sediments could be coupled to selenate but not to molybdate. Reduction of selenate to elemental selenium was determined to be the mechanism for loss from solution. Selenate reduction was inhibited by tungstate and chromate but not by molybdate. A small quantity of the elemental selenium precipitated into sediments from solution could be resolublized by oxidation with either nitrate or FeOOH, but not with MnO_2. A bacterium isolated from estuarine sediments demonstrated selenate-dependent growth on acetate, forming elemental selenium and carbon dioxide as respiratory end products. These results indicate that dissimilatory selenate reduction to elemental selenium is the major sink for selenium oxyanions in anoxic sediments. In addition, they suggest application as a treatment process for removing selenium oxyanions from wastewaters and also offer an explanation for the presence of selenite in oxic waters.

Bacterial mineralization of organic matter in anoxic sediments proceeds by reduction of electron acceptors like nitrate (denitrification), sulfate (sulfate reduction), and bicarbonate (methanogenesis). In addition, Fe^{3+} and Mn^{4+} can also be of significance in the oxidation of carbon compounds (14, 19, 22, 26). Although these dissimilatory processes oxidize deposited organic matter, they also have important environmental or geochemical effects such as the removal of nitrogenous fertilizer (denitrification), the production of hydrogen sulfide (sulfate reduction) or methane (methanogenesis), and the deposition of magnetite formed from iron reduction (23). Because of the relative abundance of the above oxidants in sediments, these processes have been well studied. However, oxyanions of less abundant elements may also serve as electron acceptors for bacterial respiration in anoxic sediments. Here, we report that selenate is one such oxyanion. Because it is present at only trace (nanomolar to micromolar) concentrations in the environment, selenate is unimportant as an oxidant for the mineralization of organic carbon when compared with more abundant (millimolar) electron acceptors. However, it is geochemically and environmentally significant that its dissimilatory reduction removes toxic selenate (and selenite) from water by forming nontoxic, insoluble elemental selenium [Se^0(s)].

Selenate and selenite are both constituents of agricultural wastewaters which drain from seleniferous soils, like those found in parts of the San Joaquin Valley in California (35). This phenomenon is apparently widespread in the western United States (42). These oxyanions were implicated as the cause of massive waterfowl mortalities in the Kesterson Wildlife Refuge, a man-made salt marsh which received the agricultural wastewaters of the San Joaquin Valley. However, selenate was not chemically conservative in this environment (35), and the sediments may have acted as a sink for selenium by processes mediated by microorganisms (O. Weres, A. R. Jaouni, and L. Tsao, Appl. Geochem., in press).

Because of their proximity as group VIA elements, sulfur and selenium share many chemical and biochemical properties (12, 40). In this regard, sulfate has been identified as an antagonist for selenate transport or toxicity in various microorganisms (7, 8, 37, 39). However, less is known about the biogeochemistry of selenium, although a microbial "selenium cycle" has been proposed which is analogous to that for sulfur (38). Recent investigations have revealed that with regard to demethylation of dimethylselenide by methanogens (33) and reduction of selenate to selenide by sulfate reducers (51), such a cycle is at least conceptually possible. However, an important caveat upon this latter reaction was that sulfate acted as a competitive inhibitor, which thereby precluded significant selenate reduction to selenide in sulfate-rich environments. We now report on our observations of a novel bacterial dissimilatory reduction of selenate which occurs by pathways unrelated to those for sulfate. We therefore conclude that Se and S have different reductive biogeochemical cycles. These results suggest the feasibility of a microbial water treatment process and, in addition, help to explain the commonly observed relative abundance of selenite ions in oxygenated seawater.

MATERIALS AND METHODS

Core profiles of SeO_4^{2-}, SeO_3^{2-}, SO_4^{2-}, and Cl^-. A core (1 m by 8.25 cm) was taken from an agricultural wastewater evaporation pond from a farm located in the San Joaquin Valley west of Fresno, Calif. This site was chosen because the high levels of selenate present greatly exceeded detection limits. Processing took place within 12 h of collection,

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† This paper is dedicated to the memory of Ivan Barnes.

during which time the core was stored at 6°C. The core was vertically extruded in 4-cm segments. Segments were placed in an N_2-pressured squeezer which expressed the pore waters (pH 9.5) through a 0.45-μm-pore-size sterile filter directly into a collection syringe. Pore waters were analyzed for sulfate and chloride with a Waters liquid chromatograph equipped with an IC-PAK A 10 polymethacrylate column. Selenium oxyanions were determined by flow through hydride generation atomic absorption spectrometry employing a closed-system HCl reduction for Se(IV) plus Se(VI) and no pretreatment for Se(IV). Selenate was arrived at by difference. Detection limit for Se(IV) plus Se(VI) was 30 nM; detection limit for Se(IV) was 2.5 nM (6, 35, 45).

Experiments with sediment slurries. Intertidal San Francisco Bay sediments were homogenized under N_2 with an equal volume of artificial bay water (ABW): (31) with or without 21 mM sulfate (pH of the ABW was 7.3). Sulfate-free slurries were prepared by homogenization in sulfate-free ABW, followed by centrifugation, rinsing, and resuspension of the compacted sediment in sulfate-free ABW. The homogenate was dispensed (15 ml) into serum bottles (122 ml) containing an additional 50 ml of ABW. Soluble substrates (glucose, sodium acetate, sodium lactate, sodium succinate), electron acceptors (Na_2SeO_4, $NaNO_3$, Na_2SO_4, FeOOH, MnO_2), or group VI analogs (Na_2MoO_4, Na_2WO_4, Na_2CrO_4) were added to the slurries at the final concentrations indicated in the text. Additional controls consisted of autoclaved sediment (250 kPa and 121°C for 30 min). MnO_2 and FeOOH suspensions were prepared as outlined elsewhere (20). Hydrogen was also employed as an electron donor by replacing the N_2 gas phase. Consumption of H_2 during the incubations was monitored by uptake from glass syringes (27). The slurries were crimp sealed under H_2 or N_2 and incubated in the dark at 20°C with rotary shaking. Subsamples were periodically withdrawn by syringe, centrifuged, filtered (0.45-μm pore size), and stored at 4°C until analyzed for selenium oxyanions as outlined above. Methane in the gas phase of the slurries was determined by flame ionization gas chromatography (27).

Respiration of [^{14}C] acetate by sediment slurries. Anaerobic (N_2 gas phase) sediment slurries were prepared (and incubated) as indicated above and dispensed into serum bottles (bottle volume, 34 ml; slurry volume, 16 ml). The ABW contained sulfate (10 mM) and acetate (5 mM), and in addition, selected samples received either Na_2MoO_4 or Na_2SeO_4 (1, 10, or 20 mM). All samples were injected with 5 μCi (200 μl) of [2-^{14}C]sodium acetate (specific activity, 50 mCi/mmol; ICN Pharmaceuticals Inc., Irvine, Calif.). The quantity of $^{14}CH_4$ and $^{14}CO_2$ in the headspace was monitored during the incubation by radio-gas chromatography (10). After 12 days of incubation, the total amount of $^{14}CO_2$ formed in the samples was measured by injection of 1.5 ml of 6 N HCl, followed by shaking for 1 h (200 rpm) before analysis by radio-gas chromatography.

Slurry experiments with ^{75}Se. Sediment slurries for radioisotope experiments were prepared as outlined above. Slurries contained 0.5 mM Na_2SeO_4 and received 0.2 μCi of carrier-free [^{75}Se]selenate (ICN Pharmaceuticals), and subsamples (2 ml) were periodically withdrawn and microcentrifuged. The radioactivities in the supernatant and the ABW-rinsed pellet (contained in the disposable plastic centrifuge tube) were individually counted on a Beckman model 8000 gamma counter (sample background, ~95 cpm). Subsamples of the gas phase (1 ml) were also periodically withdrawn, and their radioactivities were counted to determine the presence of alkylated selenium gases (e.g., [^{75}Se]dimethylselenide). To extract for [^{75}Se]selenide, pellet samples were acidified with 1 N H_2SO_4 and placed in an N_2 flushing-trapping train, and their radioactivities were counted (51). Experimental controls consisted of samples with air headspaces (O_2 consumed was replaced by syringe) and autoclaved samples under N_2, H_2, or H_2 plus 2 mM $Na_2 \cdot 9H_2O$.

To determine whether elemental selenium was the precipitation product of the experiments described above, we extracted ^{75}Se in sediments with solvents. Ten sediment slurry samples were prepared as described above, injected with [^{75}Se]selenate (0.1 μCi), and allowed to incubate under H_2. After 4 days, no significant counts remained in solution. Because red, amorphous Se^0(s) is soluble in CS_2 (48), this solvent was employed for extraction purposes. However, it was first necessary to wash the sediment pellets sequentially with solvents of decreasing polarity. The procedure was to centrifuge 1.5 ml of slurry, count the radioactivity in the supernatant, and suspend the pellet in 1.2 ml of deionized water. The slurry was centrifuged again, the radioactivity in the supernatant was counted, and the pellet was suspended in 1.2 ml of 95% ethanol. This procedure was repeated next with ethyl acetate. Finally, the pellet was suspended in an equal volume of CS_2, followed by an extraction interval (40 h at 4°C followed by 6 h at 20°C). The sample was then centrifuged, and the radioactivity in the supernatant was counted while the pellet was vortexed in new CS_2, centrifuged, decanted, and added to the previous CS_2 volume for counting. Pellet counts were also resolubilized by treatment with Na_2SO_3 (D. J. Velinsky, Ph.D. dissertation, Old Dominion University, Norfolk, Va., 1987). Pellet samples (1 ml) were put into tubes containing 4 ml of 1.25 M Na_2SO_3 plus 1.25 N H_2SO_4, sealed under N_2, and extracted for 48 h at 20°C, and centrifuged, followed by separate counting of the radioactivities in the supernatant and pellet.

An experiment was conducted to examine the oxidative resolubilization of ^{75}Se-precipitated counts associated with the sediment phase of slurries. Slurries were prepared as above and dispensed (25 ml/160-ml bottle sealed under H_2 with 1 μCi of [^{75}Se]selenate). After 9 days, most of the added counts were associated with the sediment pellet. Additions (~10 mmol/liter) of $NaNO_3$, MnO_2, FeOOH, or water (all from O_2-free stocks) were then made to the samples. The radioactivities in the supernatant and pellet fractions of centrifuged samples were counted over the course of a 22-day incubation by the procedures outlined above.

Isolation and physiological experiments with bacterial cultures capable of selenate respiration. For enrichments and isolations, the following medium was employed (grams per liter of deionized water): NaCl, 20.5; $MgCl_2 \cdot 6H_2O$, 0.5; $NaHCO_3$, 1.0; Na_2SeO_4, 3.8; K_2HPO_4, 0.225; KH_2PO_4, 0.225; $CaCl_2 \cdot 2H_2O$, 0.04; $(NH_4)_2SO_4$, 0.225; $MgSO_4 \cdot 7H_2O$, 0.09; sodium acetate, 2.72; cysteine hydrochloride, 0.06; $Na_2S \cdot 9H_2O$, 0.06; and the solutions of trace elements (9 ml) and vitamins (10 ml) of Wolin et al. (49). The final pH was 7.3. Liquid medium was dispensed (10 ml) under N_2-CO_2 (4:1) into Balch anaerobic culture tubes, crimp sealed, and autoclaved. Enrichments were established by inoculating tubes with sediment (0.2 ml) from the slurry samples which were exposed to acetate and selenate. After ~2 weeks of static incubation at 20°C, the inoculated tubes produced abundant red precipitate later identified as elemental selenium (see text). Enrichments were maintained with biweekly transfers. The enrichment was streaked onto roll tubes, and isolated red colonies were evident after 1 to 2 weeks of

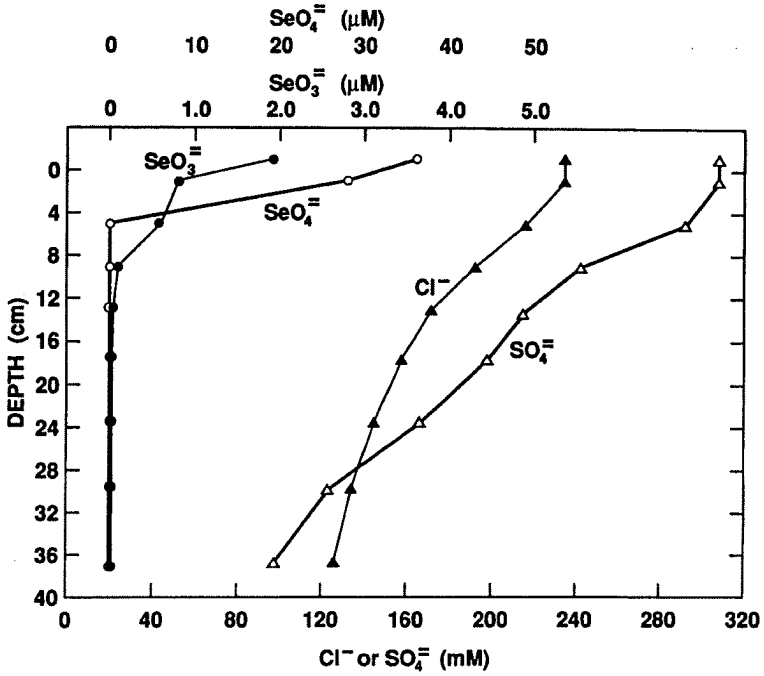


FIG. 1. Vertical profiles of dissolved selenate, selenite, sulfate, and chloride in a core taken from a San Joaquin Valley agricultural wastewater pond on 8 July 1988.

incubation. These colonies were picked and successfully transferred back to the liquid medium.

Growth of the isolate was determined by performing cell counts with acridine orange (15, 30). An experiment was conducted to demonstrate selenate-linked respiration of acetate. Culture tubes containing the above-described medium but with various concentrations of selenate (0, 0.5, 1.0, 2.0, 10.0, and 20.0 mM) and 2.5 μCi of [2-^{14}C]acetate were inoculated with 0.2 ml of a growing culture. After 28 days of incubation, tubes were injected with 1.5 ml of 6 N HCl, vigorously shaken, and, after 2 h, analyzed for $^{14}CO_2$ by radio-gas chromatography (see above). Similar growth and respiration experiments were also conducted on the initial enrichment culture with electron acceptors other than selenate (nitrate, trimethylamine oxide [TMAO], FeOOH, MnO_2, O_2). In addition, the effect of alternative electron acceptors (air, nitrate, TMAO, MnO_2, FeOOH) on the ability of enrichment cultures to remove [^{75}Se]selenate from solution was also examined. To determine whether the isolate was capable of denitrification, the acetylene-block assay was performed (3). Two culture tubes having medium lacking selenate but containing 10 mM nitrate were inoculated with 0.3 ml of a growing culture. One tube was incubated under N_2-CO_2, while the other had this atmosphere plus 2.6 ml of C_2H_2 (15% of the gas phase). After 48 h of incubation at 21°C, the quantity of N_2O in the headspaces of the tubes was determined by electron capture gas chromatography (32).

Scanning electron micrographs and X-ray diffraction. The procedures for the preparation of scanning electron micrograph-X-ray fluorescence micrographs of cultures have been described elsewhere (39a). X-ray diffraction analysis of dried precipitate from cultures was performed on a Nicolet X-ray diffraction unit model I-2.

RESULTS

Porewater profiles. Profiles of selenate, selenite, sulfate, and chloride in interstitial waters of a pond receiving agricultural wastewater are shown in Fig. 1. Selenate was abundant (36 μM) in the surface waters but was undetectable at depths below 2 cm. Selenite was about 20-fold less abundant than selenate and disappeared below 10 cm. In contrast, sulfate and chloride concentrations were reduced by only 68 and 54%, respectively, at the bottom of the core. Ratios of $(SeO_4^{2-} + SeO_3^{2-})/Cl^-$ were 1.65×10^{-3} in the surface water but rapidly decreased to 0.027×10^{-3} at the 4- to 6-cm depth interval. Ratios of SO_4^{2-}/Cl^- were constant (1.33 to 1.35) in the surface waters and upper 6 cm of sediment but progressively decreased with depth, reaching 0.79 at the bottom of the core (depth interval, 35 to 39 cm).

Sediment slurry experiments. In an initial experiment, selenate removal from slurries containing 21 mM sulfate proceeded more rapidly with H_2 than with N_2; however, after 7 days of incubation, both H_2 and N_2 slurries had removed >99.7% of the added selenate, while a more modest loss (~33%) was associated with an autoclaved control (Fig. 2). Selenite accumulated as a transient intermediate present at concentrations well below those of the initial selenate levels (<8 μM; data not shown). These results suggested that a bacterially mediated reduction of Se(VI) to a more reduced state than Se(IV) had occurred.

In a second experiment, the effect of several soluble electron donors (lactate, acetate, succinate, and glucose) on the removal of selenate (1.22 mM) from solution was examined (Table 1). After 5 days of incubation, unamended sediments removed 58% of the added selenate, while lactate- and acetate-amended slurries lost 100 and 95%, respectively. Succinate did not significantly speed the reaction, while

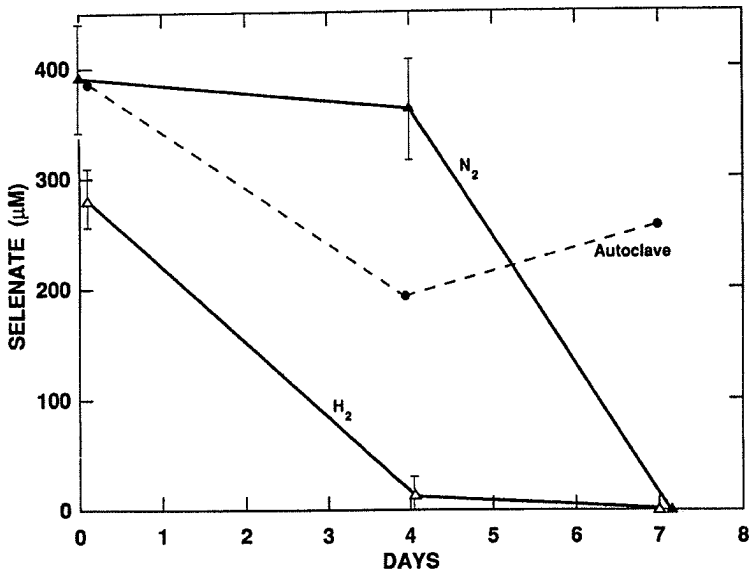


FIG. 2. Removal of added selenate from sediment slurries incubated under H_2 or N_2 or in an autoclaved control. Points represent the mean of three samples, and bars indicate 1 standard deviation (only one autoclaved control was run).

glucose appeared to have retarded selenate removal over that of the unsupplemented slurries. Significant quantities of selenite (0.160 to 0.272 mM) were present in the unamended, acetate, and succinate slurries at the end of the experiment.

The effects of several alternative electron acceptors on the removal of 5 mM selenate from sulfate-free slurries held under H_2 was investigated. Selenate removal was evident by day 4 in the unamended and FeOOH samples, and selenate disappeared completely by day 7 in these samples as well as those containing SO_4^{2-} (Fig. 3). Nitrate completely inhibited selenate removal during the incubation, and a partial inhibition (~72%) was achieved with MnO_2. A distinct color change to reddish brown was evident in all slurries except those amended with NO_3^- or MnO_2. This suggested that selenate was reduced to red elemental selenium, a fact we subsequently confirmed (see below). Significant levels of selenite were observed in some of the experimental flasks during the experiment. The highest values (0.76 to 1.3 mM) occurred by day 4 of the incubation in the unamended and the FeOOH-containing slurries and on day 7 in the sulfate-containing slurries (0.1 to 1.0 mM). No significant levels of selenite were observed in the slurries containing nitrate or MnO_2.

TABLE 1. Effect of various electron donors on selenate reduction in San Francisco Bay sediment slurries after 5 days of incubation[a]

Addition (10 mM)	SeO_4^{2-}[b] (mM)	SeO_3^{2-}[b] (mM)	SeO_4^{2-} lost (%)
None	0.510 (0.010)	0.160 (0.020)	58
Lactate	0.005 (0.001)	0.002 (0.000)	100
Acetate	0.058 (0.097)	0.272 (0.192)	95
Succinate	0.262 (0.380)	0.200 (0.143)	79
Glucose	0.817 (0.081)	0.001 (0.000)	33

[a] Initial levels of selenate and selenite were 1.22 and 0.001 mM, respectively.

[b] Results represent the mean of three samples, and values in parentheses indicate 1 standard deviation.

The effect of these various electron acceptors on the amount of selenate removed, methane produced, and H_2 consumed is shown in Table 2. In general, the quantity of H_2 consumed by the slurries was less than or equal to the total amount of electron-accepting equivalents present in the slurries. No significant levels of methane were produced in the slurries (with the exception of SO_4^{2-} plus WO_4^{2-}), indicating that the electron sinks for the H_2 oxidized were either the selenate or the alternative electron acceptors supplied. The only exception was the sulfate-amended slurries inhibited with tungstate. In this instance, selenate removal was totally inhibited and high levels of methane were present.

Sediment slurry experiments with [2-^{14}C]acetate. Slurries incubated with 10 mM sulfate formed $^{14}CH_4$ and $^{14}CO_2$ from [2-^{14}C]acetate (Fig. 4) and yielded very low $^{14}CH_4/^{14}CO_2$ ratios (0.0041). These values represented the metabolism of ~90% of the acetate. Incubation with 1 mM molybdate markedly inhibited $^{14}CO_2$ production (96%) but stimulated $^{14}CH_4$ formation 10-fold ($^{14}CH_4/^{14}CO_2$ ratio = ~1). Higher levels of molybdate had little further affect on $^{14}CO_2$ production but decreased $^{14}CH_4$ formation. The results for selenate contrasted with those for molybdate. Incubation with 1 mM selenate inhibited $^{14}CO_2$ production, although not to the same extent as that achieved with molybdate. However, 10 and 20 mM selenate resulted in significantly increased production of $^{14}CO_2$. Selenate only enhanced $^{14}CH_4$ production by ~35%, and there was no statistically significant effect of increasing selenate concentrations. The final $^{14}CH_4/^{14}CO_2$ ratio achieved with 20 mM selenate was 0.0083, and the quantity of $^{14}CO_2$ formed was 75% of that achieved in the absence of either molybdate or selenate.

Sediment slurry experiments with [^{75}Se]selenate. To identify the product(s) of selenate reduction in slurries, we conducted tracer experiments with $^{75}SeO_4^{2-}$. Loss of ^{75}Se from solution was associated with comparably increased levels of ^{75}Se in the sediments, and removal accounted for ≥95% of the added $^{75}SeO_4^{2-}$ (Fig. 5A). Similar results were achieved under an H_2 atmosphere, although the kinetics appeared to be slightly faster (Fig. 5B). No ^{75}Se was precip-

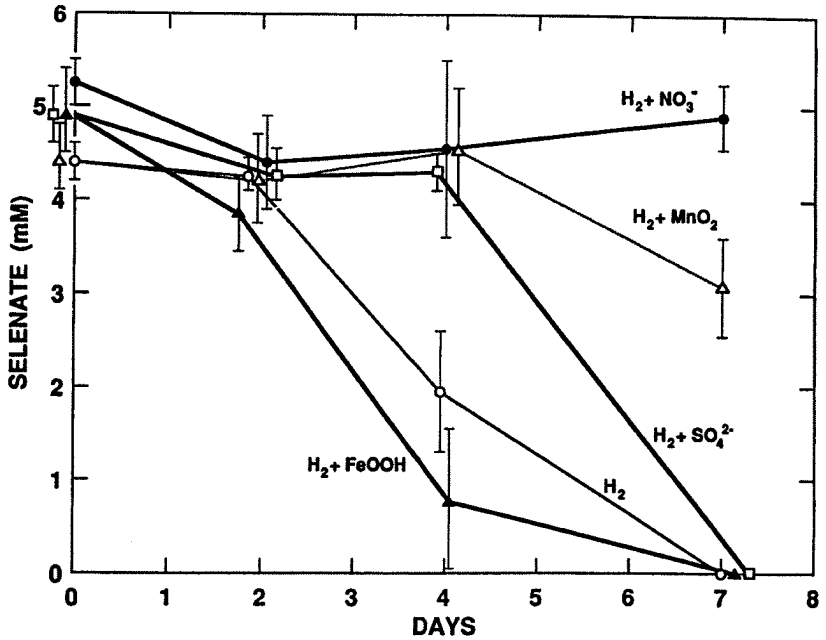


FIG. 3. Removal of added selenate from sulfate-free sediment slurries incubated under H_2 in the presence of various competitive electron acceptors. Points represent the mean of three samples, and bars indicate 1 standard deviation.

itated from solution in autoclaved (under H_2 or N_2) controls or in live slurries incubated under air (Fig. 5C). In addition, no precipitation occurred in autoclaved controls under air or in autoclaved controls under H_2 supplemented with 2 mM sodium sulfide (data not shown). No significant levels of ^{75}Se were detected in the gas phases of any of the slurries (Fig. 5A, B, and C), and in addition, ^{75}Se incorporated into the sediments in the "live" slurries was not acid volatile (Fig. 5A and B).

Removal of [^{75}Se]selenate from solution was inhibited by some but not all group VI oxyanions (Table 3). Chromate and tungstate effectively inhibited selenate removal. However, no inhibition occurred with molybdate.

Counts of ^{75}Se incorporated into the sediments could be extracted by the CS_2 extraction procedure (Table 4). Most of the counts (55%) were recovered in the CS_2, but significant quantities appeared in the ethyl acetate (15%) fraction and as a residual in the sediments (15%). Recovery was 92% for this procedure. Better recovery of $^{75}Se^0(s)$ was achieved by the sulfite oxidation method (Velinsky, Ph.D. dissertation). Re-

TABLE 2. Effect of alternative electron acceptors on selenate reduction, methane production, and hydrogen uptake by San Francisco Bay sediment slurries[a]

Addition (10 mmol/liter)	SeO_4^{2-} removed (mmol/liter)	CH_4 (mmol/liter)	H_2 (mmol/liter)
None	4.4	0.079	6.5
NO_3^-	0.4	0.0001	17.0
FeOOH	5.0	0.010	10.3
MnO_2	1.3	0.0004	21.0
SO_4^{2-}	4.9	0.115	7.2
SO_4^{2-} + WO_4^{2-}[b]	−0.7[c]	4.946	15.5

[a] Data represent the mean of three samples.
[b] Represents one sample only.
[c] Represents a nonsignificant increase of selenate.

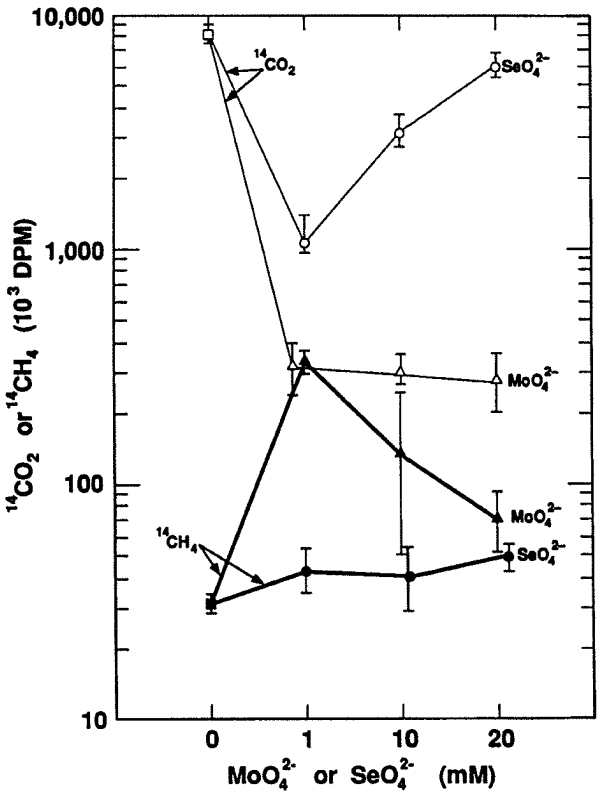


FIG. 4. Respiration of [2-^{14}C]acetate by sediment slurries containing 0, 1.0, 10.0, or 20.0 mM molybdate or selenate. Points represent the mean of three samples, and bars indicate 1 standard deviation.

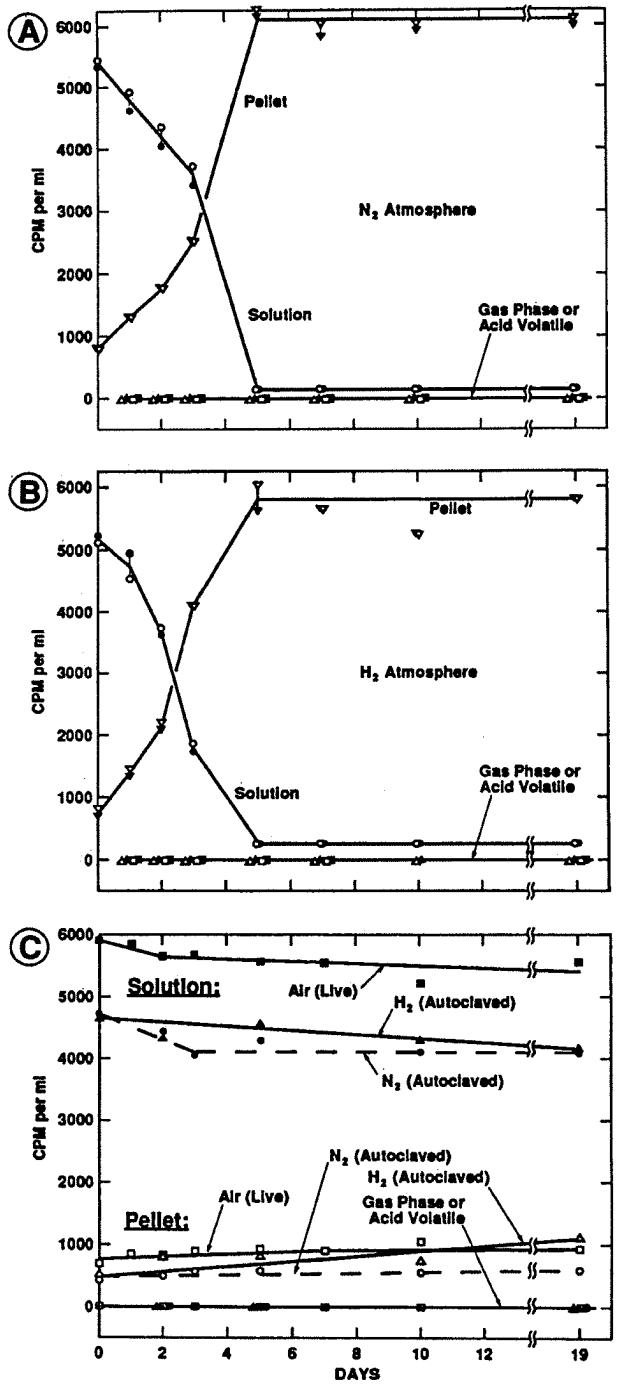


FIG. 5. Loss of ^{75}Se from solution and incorporation into sediments in slurries incubated under N_2 (A); or H_2 (B) or in autoclaved controls under N_2 or H_2 or live controls under air (C). All experimental conditions were run in duplicate; however, duplicates in panel C are not shown because of space limitations. All slurries initially contained 0.5 mM Na_2SeO_4.

TABLE 3. Inhibition of [^{75}Se]selenate reduction by group VI oxyanions[a]

Addition[b]	Counts in supernatant (dpm/ml)[c]
Na_2CrO_4	1,555
Na_2CrO_4	1,499
Na_2WO_4	1,364
Na_2WO_4	1,383
Na_2MoO_4	281
Na_2MoO_4	401

[a] Slurries were incubated in ABW plus sulfate (21 mM) under H_2 with 0.5 mM selenate.
[b] Concentration of inhibitors, 20 mM.
[c] After 12 days of incubation; total slurry mean disintegrations per minute per milliliter at start of experiment were 1,565 ± 58 (n = 6).

covery of Se^0(s) by oxidative solubilization was 74%, with 24% remaining in the sediments, which yielded a total recovery of 98% (Table 4).

The ability of various compounds to return the precipitated ^{75}Se into solution from the sediments is given in Table 5. In comparison with water controls, higher counts in solution occurred in the presence of FeOOH (about 3-fold) and nitrate (about 3.5-fold). However, incubation with MnO_2 produced only ~10% of the counts observed in the controls. Oxidative dissolution of ^{75}Se was small (~7%) when compared with the counts residual in the sediments.

Isolation of bacterial cultures and physiological experiments. Slow-growing enrichment cultures were obtained from inoculation of selenate-acetate medium with sediment from the slurry experiments. During growth, the enrichments produced either a red or black precipitate, the abundance of which increased with time. The more commonly observed red precipitate was soluble in CS_2 and could be solubilized by sulfite oxidation, as determined with ^{75}Se tracer (data not shown). The black precipitate was not soluble in CS_2 and was only slowly oxidized by sulfite treatment. These responses were consistent with that expected for red amorphous and black crystalline Se^0(s) (48). Analysis of both types of precipitates by scanning electron microscopy-X-ray fluorescence revealed amorphous (red) or crystalline (black) particles in which only Se was detected.

TABLE 4. Extraction of precipitated ^{75}Se from sediments with organic solvents or by sulfite oxidation[a]

Conditions	dpm/ml[b]	Recovery (%)
Total counts in slurry (before treatment)	1,507 (25)	
CS_2 extraction		
Distilled water	36 (4)	2
Ethanol	74 (8)	5
Ethyl acetate	228 (10)	15
Carbon disulfide	829 (40)	55
Counts remaining in pellet	232 (7)	15
Total recovered		93
Sulfite oxidation		
Supernatant	1,109 (45)	74
Pellet	369 (41)	24
Total recovered		98

[a] Extraction was performed after 4 days of incubation under H_2 when no significant counts (~9.7 ± 1.5 dpm) remained in solution.
[b] Values in parentheses indicate standard error of 10 samples.

TABLE 5. Dissolution of precipitated ^{75}Se in sediment slurries amended with various oxidants[a]

Addition (10 mmol/liter)	dpm/ml[b]	
	Supernatant	Total in slurry
Water	902	36,573
Water	1,035	36,194
Nitrate	3,666 (1,485)	37,154 (1,444)
MnO_2	112 (18)	35,445 (4,669)
FeOOH	3,686 (1,144)	40,474 (4,507)

[a] After 18 days of incubation.
[b] For duplicate or triplicate samples; parentheses indicate 1 standard deviation.

Because of the absence of significant quantities of other elements, the Se was presumed to be in the elemental state. X-ray diffraction analysis of the black precipitate revealed it to be composed of hexagonal Se^0(s). In some cultures, the initial red precipitate was observed to change into the black form over time (~2 weeks). No precipitate was formed in uninoculated media.

Incubation of the enrichment culture with [2-^{14}C]acetate (2.5 μCi per tube) for 28 days resulted in the formation of $^{14}CO_2$ for the following electron acceptors ($^{14}CO_2$ dpm per tube): none (0), 20 mM selenate (388,000), 10 mM nitrate (254,000), 10 mM TMAO (140,000), 20 mmol of MnO_2 per liter (10,300), 20 mmol of FeOOH per liter (131,000), and air (582,000). In another experiment, the enrichment culture was grown in medium containing 20 mM selenate with or without additional electron acceptors. Reduction of selenate was monitored as loss of [^{75}Se]selenate from solution. After 13 days of incubation, the percent selenate removed was as follows: regular selenate medium, 86%; with air, 8%; with 20 mM nitrate, 5%; with 40 mM nitrate, 3%; with 20 mM sulfate, 95%; with 40 mM sulfate, 83%; with 20 mM TMAO, 0%; with 40 mM TMAO, −6%; with 20 mmol of FeOOH per liter, 73%; and with 5 mmol of MnO_2 per liter, 17%.

An isolated red colony was picked from enrichment-streaked roll tubes and successfully transferred back to acetate-selenate medium. Cells consisted of gram-negative cocci (diameter, ~0.6 μm). Growth of the isolate (designated strain SeS) was rapid with nitrate or TMAO but considerably slower with selenate and only marginal under air (Table 6). After 6 weeks of incubation, however, selenate-grown cultures achieved densities comparable (~4×10^8 cells per ml) to those of nitrate- or TMAO-grown cultures.

A rough balance was achieved between the amount of selenate removed from culture fluid after growth, the final cell density, and the dry weight of the Se^0(s) precipitate. After 9 weeks of incubation, the dry weight of the washed red precipitate from a tube was 9.0 mg, while the residual selenate was 10.1 mM, indicating the removal of 9.9 mM selenate (~7.82 mg of Se per tube). With a final a cell density of 5×10^9 cells per 10 ml of medium and a cell volume of 0.113 μm^3 for spherical cells with a 0.6-μm diameter, the total cellular volume would be ~0.57×10^9 μm^3. Using a conversion factor of 5.6×10^{-13} g of C/μm^3 and assuming that C equals one-half of the cellular dry weight (5), this gives a total bacterial dry weight of 0.63 mg. Thus, the net Se weight of the precipitate (8.37 mg) divided by the expected weight (7.82 mg) yields a recovery of 107%.

TABLE 6. Growth of strain SeS with various electron acceptors

Addition[a]	10^6 Cells per ml at day:						
	0	2	3	4	5	6	11
None[b]	0.39				10		18
TMAO	0.44	15	79	105		580	
Nitrate	0.44	21	79	101		880	
Selenate	0.42				10		41
Air	0.27						3.9[c]

[a] TMAO, nitrate, and selenate were all added at 20 mM.
[b] Carry-over of selenate = ~0.4 mM.
[c] Taken after 14 days.

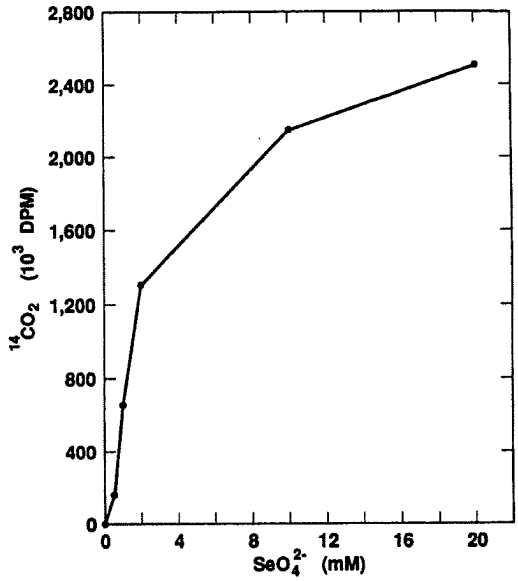


FIG. 6. Selenate-linked respiration of [2-^{14}C]acetate by strain SeS after 28 days of incubation in medium initially containing 20 mM selenate and 20 mM acetate.

Strain SeS respired selenate while growing on acetate. After 28 days of incubation at various selenate concentrations, progressively more red Se^0(s) was evident in tubes containing higher initial levels of selenate. Analysis for $^{14}CO_2$ indicated that more of this gaseous product was formed with increasing selenate concentrations (Fig. 6). The amount of $^{14}CO_2$ formed in the 20 mM selenate tube represented oxidation of 45% (9 mM) of the available acetate. When strain SeS was grown in nitrate medium, about 25-fold more N_2O was formed in the presence of C_2H_2 than in its absence (without acetylene, the isolate produced 0.1 and 3.5 nmol of N_2O at 0 and 48 h, respectively. With acetylene, the isolate produced 0.2 and 86.8 nmol of N_2O at 0 and 48 h, respectively.)

DISCUSSION

The interstitial profiles of selenate and selenite indicated that these oxyanions were removed by a process occurring near the sediment surface (Fig. 1). By contrast, removal of sulfate by sulfate reduction occurred at far greater depths. This pronounced spatial separation clearly indicated that two separate removal processes were operative in these sediments. Furthermore, the great abundance of sulfate ions (240 to 310 mM) in the upper core layers revealed that removal of selenium oxyanions was independent of sulfate, a point which contrasts with previous concepts with regard to

the biogeochemistry and microbiology of selenium which implied the involvement of sulfur-cycle organisms (12). The surface waters of these evaporation ponds contained abundant levels of nitrate (~2 mM) which, like the selenium oxyanions, also decreased rapidly with depth (R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, manuscript in preparation).

To better understand the process(es) responsible for the selenate-selenite removal, we conducted slurry experiments with well-studied San Francisco Bay mud (10, 27, 32). This material was chosen for sampling convenience and is thought to be representative of processes occurring in anoxic, saline sediments. However, we were also able to repeat our results with sediments from the evaporation ponds, as well as with materials from selenium-impacted subsurface soils (N. Dubrovsky, J. Neil, J. T. Hollibaugh, and R. S. Oremland; and R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, manuscripts in preparation).

Selenate removal in slurries was accelerated with H_2, lactate, and acetate and was inhibited by autoclaving (Fig. 2; Table 1). In addition, no significant acceleration occurred with succinate, and some retardation occurred with glucose. These results indicate that a microbial process was involved in selenate removal and that a measure of substrate specificity existed with regard to this process. The partial removal of traces (~1 μM) of selenate by biological activity in anoxic lake sediments has been observed by other workers (18). It was significant that H_2 and acetate were identified as potential substrates in our experiments because they are well recognized as key electron donors fueling terminal anaerobic processes like iron reduction, sulfate reduction, methanogenesis, etc. (19, 28, 47). Their addition to sediments results in the stimulation of these terminal processes (27, 31), as was observed for H_2 with regard to selenate (Fig. 2). Stimulation by lactate probably was a consequence of its ability to be quickly converted into acetate and H_2 in anoxic sediments.

Removal of high levels (5 mM) of selenate from slurries was unaffected by FeOOH or sulfate (Fig. 3). However, both nitrate and MnO_2 effectively inhibited selenate removal. Nitrate has been observed to interfere with selenate removal in soil column experiments (O. Weres, H. R. Bowman, A. Goldstein, E. C. Smith, and L. Tsao, Water Air Soil Pollut., in press). The removal process we observed was clearly reductive in nature because of the transient appearance of selenite in this and the preceding slurry experiment (Table 1). In addition, the marked appearance of a red coloration in the sediments implied the formation of amorphous, red Se^0(s). Because no significant selenite or red sediment coloration was present in nitrate- or MnO_2-containing samples, they served as controls for the observation of selenate reduction. All the hydrogen consumed in this experiment could be accounted for by the quantity of available electron acceptors, since no significant amounts of methane were formed (Table 2). The only exception to this was the case of the sulfate plus tungstate condition. However, because tungstate inhibits both sulfate reduction (29) and selenate reduction (Table 2), methanogenesis was stimulated by default (Fig. 7; see below). Two possibilities exist with regard to the mechanism(s) for inhibition of selenate reduction by nitrate and MnO_2: their action as competitive electron acceptors, or their ability to reoxidize reduced, precipitated Se from the sediments, or both. As we shall see below, experimental evidence clearly points to the former case.

Removal of selenate ions from solution resulted in their quantitative recovery as a precipitate in the sediments (Fig. 5). No significant quantities of gaseous Se or acid-volatile Se were detected when slurries were incubated under N_2 (Fig. 5A), H_2 (Fig. 5B), or in any of the controls (Fig. 5C). This indicated that reduced, alkylated gases like dimethylselenide (36) or selenide (e.g., H_2Se or FeSe) were not formed during the incubation. This latter point reinforces earlier observations made with these sediments in which only a tiny portion (≤~0.1%) of added [^{75}Se]selenate was converted to selenide in low-sulfate (<4 mM) slurries, even when they were amended with H_2 or lactate (51). The lack of selenate reduction in the controls (Fig. 5C) underscores that this was caused by an anaerobic, microbial process. This point was further stressed by the absence of any apparent selenate removal from autoclaved slurries incubated under highly reducing chemical conditions (H_2 atmosphere plus 2 mM sulfide). Thus, the observed selenate reduction was the result of direct biological activity and not due to the accumulation of reactive, bacterially formed reductants like sulfide.

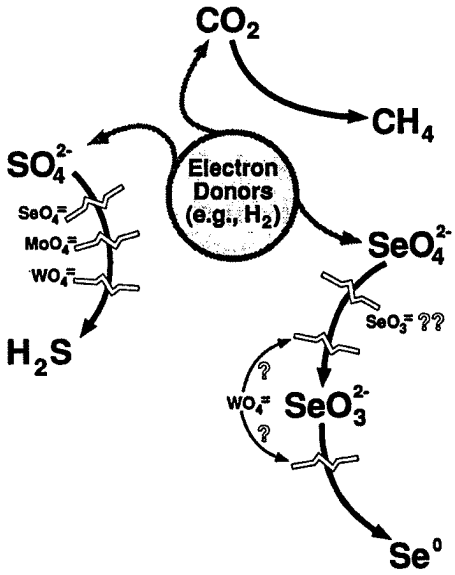


FIG. 7. Schematic representation of the possible modes of action of group VI inhibitors.

Since the precipitate was chemically more reduced than selenite and more oxidized than selenide, and because removal of high levels of selenate (5 mM) resulted in a red coloration in the slurries, elemental selenium was the logical product of the observed selenate reduction. This was confirmed in the extraction-oxidation experiments (Table 4). A significant quantity of counts remained in the sediment pellet in either the CS_2 extraction (15%) or the sulfite oxidation (24%). However, this can be explained by the presence of hexagonal, crystalline Se^0(s), a substance which is resistant to these extraction-oxidation procedures (48). The fact that this form of Se^0(s) was observed in some of the cultures (in addition to the red amorphous form) reinforces this explanation.

Sulfate did not inhibit selenate reduction in the slurry experiments (Fig. 3), in the enrichment cultures (see Results), or in [^{75}Se]selenate additions to slurries incubated with 0.1 to 10 mM sulfate and 0.1 to 10 mM selenate (data not shown). These results demonstrate that sulfate reduction was not the pathway for selenate reduction to Se^0(s). Thus,

the hypothesis generated from the selenate-sulfate core profiles (Fig. 1) has been confirmed.

The inhibitor results were, at first glance, contradictory but were nonetheless intriguing. Tungstate and chromate inhibited selenate reduction; however, no inhibition occurred with molybdate (Tables 2 and 3). Oxyanions of group VI elements inhibit sulfate-respiring bacteria (34), in part by destroying their intracellular ATP pools (43). However, a point often overlooked in inhibitor experiments with complex, mixed microbial populations is the unanticipated action of the inhibitor on nontarget organisms (29). Thus, although tungstate inhibits sulfate reduction, it also inhibits various molybdoenzymes which are not inhibited by molybdate. Relevant examples include the dimethyl sulfoxide reductase of *Escherichia coli* (4), which also reduces TMAO, nitrate, fumarate, hydroxylamine, and a variety of other compounds (46), as well as nitrate reductases (see review in reference 41). Thus, tungstate (and chromate) probably inhibited a similar enzyme involved in selenate reduction, while molybdate only interfered with sulfate reduction. These points are illustrated in Fig. 7. The fact that the isolated strain SeS grows on TMAO as well as nitrate (Table 6) suggests that some enzyme system similar to the dimethyl sulfoxide reductase or nitrate reductase is responsible for selenate reduction. However, from our current data we cannot predict the actual point of action of tungstate on selenate reduction. The compound may have inhibited the direct reduction of selenate or, alternatively, the subsequent reduction of selenite to Se^0(s), which could have resulted in the accumulation of toxic levels of selenite (Fig. 7). We observed a rapid accumulation of selenite (~0.022 μM) in the tungstate-inhibited slurry at the first sampling period (total incubation time, ~2 h), which at least suggests the latter case as a possible interpretation. However, other than implicating the involvement of a molybdoenzyme in the process of selenate reduction to Se^0(s), we cannot ascertain from our data the location of this enzyme in the overall pathway. This will be the subject for future investigations.

Clearly, selenate behaved differently from molybdate when these compounds were added to sediments to investigate acetate metabolism (Fig. 4). Molybdate lowered $^{14}CO_2$ production while stimulating that of $^{14}CH_4$, a result consistent with the stimulation of methanogenesis by inhibition of competitive sulfate reducers that has been reported by many investigators (29). However, the results for selenate, especially at the 10 and 20 mM levels, clearly indicated that it behaved as an oxidant for acetate metabolism to CO_2 rather than as an inhibitor of this process (although it certainly blocked the involvement of sulfate respirers). The fact that selenate did not significantly stimulate methanogenesis indicates that as for sulfate reduction or iron reduction (e.g., references 21 and 31), selenate-reducing bacteria have the potential to outcompete methanogens for common electron donors.

A small amount of precipitated ^{75}Se in sediments could be solubilized by incubation with nitrate or FeOOH (Table 5). It is possible that this occurred by an anaerobic oxidation of Se^0(s) analogous to that reported for sulfide by MnO_2 (2). However, only a small quantity of material (~7%) was "oxidized," and time course experiments indicated that these solubilized counts diminished rapidly by 3 weeks of incubation (data not shown). Thus, in terms of mass balances, reduction of selenate to Se^0(s) was the major reaction, while anaerobic reoxidation was only minor. Therefore, the inhibition of selenate reduction by nitrate and MnO_2 (Fig. 3) cannot be explained by rapid reoxidation. Furthermore, it is clear that, if anything, MnO_2 inhibited this oxidation (Table 5). Therefore, a more reasonable explanation is that nitrate and MnO_2 acted as preferred electron acceptors over selenate for bacterial respiration. Such phenomena have been well studied in sediments for a variety of processes like iron reduction versus methanogenesis or sulfate reduction (21) or sulfate reduction-denitrification versus methanogenesis (for reviews, see references 28 and 47). This interpretation is borne out by experiments with the enrichment culture in which selenate reduction (loss from solution) was inhibited by nitrate, MnO_2, and TMAO but not by sulfate or FeOOH, all of which sustained oxidation of acetate (see Results). Furthermore, the fact that strain SeS grew well on TMAO or nitrate (Table 6) and is a denitrifier (see Results) confirms the point that nitrate is preferred over selenate as an electron acceptor.

The fact that millimolar levels of selenate were reduced in slurry experiments (Fig. 3) and that these quantities of selenate could be linked to the metabolism of acetate in sediments (Fig. 4) indicated that isolation of a selenate-respiring culture was feasible. That strain SeS is capable of selenate-dependent growth was borne out in preliminary experiments (Table 6). The fact that a rough balance could be calculated between the amount of Se^0(s) formed and the quantity of selenate lost from solution indicated that a stoichiometric balance between reactant and product could be achieved. Finally, selenate-dependent acetate oxidation was demonstrated for the culture (Fig. 6), a fact which confirms that these organisms carry out a novel respiration. Although there were previous observations of what appeared to be Se^0(s) precipitation from selenate in various bacterial cultures (7, 9, 13, 17), these reports usually consisted of casual observations peripheral to the main thrusts of the investigations. Recently, Maiers et al. (24) reported Se^0(s) precipitation from selenate (maximum concentration, 1 mM) by enrichment cultures; however, it was not clear whether this occurred under aerobic or anaerobic conditions, and a respiratory linkage was not reported. However, a pseudomonad which respires selenate to selenite was recently isolated (J. Macy, personal communication). To our knowledge, that organism and strain SeS constitute the only bacteria identified thus far which are capable of performing this novel respiratory feat.

Although our physiological studies with SeS are only preliminary, a brief examination of the energy yields for anaerobic growth on selenate is instructive. We propose the following reaction using published values of ΔG_f^0 (44, 50):

$$4CH_3COO^- + 3SeO_4^{2-} \longrightarrow 3Se^0 + 8CO_2 + 4H_2O + 4H^+ \quad (1)$$

$$4(-369.3) + 3(-441.2) \longrightarrow 8(-394.4) + 4(-237.2)$$

Thus, $\Delta G^0 = -326$ kJ/mol of acetate. Because four protons are involved:

$$\Delta G^{0\prime} = \Delta G^0 + m\Delta G_f^*(H^+) = -1{,}463 \text{ kJ per reaction} \quad (2)$$

or −366 kJ/mol of acetate. Thus, the reduction of selenate to Se^0(s) with acetate is highly exergonic. However, because reduction of selenate to selenite with either H_2 or acetate is also highly exergonic (e.g., $G^0 = -162$ kJ/mol of H_2), growth with a selenite end product can also be achieved. This contrasts with an endergonic reaction associated with sulfate reduction to sulfite by H_2 ($G^0 = +20$ kJ/mol of H_2). At present, it is not clear whether strain SeS carries out a total reduction of selenate to Se^0(s) or a partial reduction to selenite followed by a later reduction of selenite to Se^0(s).

Other workers have observed that reduction of selenate to Se^0(s) is carried out sequentially by two organisms (J. Macy, personal communication). At present, we cannot confirm whether our strain SeS is pure, and these details will require future work. Bacterial Se^0(s) precipitation from selenite has been commonly reported for a variety of microbes including algae (16) and bacteria (25) and can be achieved chemically with strong reductants. However, the quantity of cysteine-sulfide in our medium (0.6 mM) was too little to precipitate selenite formed from the extent of selenate reduction to Se^0(s) which we observed (16 mM).

In summary, we discovered a novel bacterial respiratory process which profoundly influences the fate of selenium oxyanions. The dissimilatory reduction of selenate to Se^0(s) occurs independently of sulfate, and therefore the reductive Se cycle differs from that of sulfur. Indeed, that elemental Se is a product of the reductive Se cycle is in itself different from the S cycle because S^0(s) is neither a product nor intermediate in sulfate reduction but arises from partial oxidation of sulfide. It is significant, therefore, that Se^0(s) has been reported to be the major form of selenium in salt-marsh sediments (Velinsky, Ph.D. dissertation; Weres et al., in press). We observed the dissimilatory reduction of millimolar levels of added selenate (Fig. 5) and saw that this is quantitatively converted to Se^0(s) (Fig. 4; Table 4). In addition, we also performed experiments which indicated that selenate respiration occurs at nanomolar to micromolar selenate concentrations in sediments, and in situ assays have revealed that the upper few centimeters of the sediment column are the site for this activity (R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, in preparation). These observations indicate that selenate respiration is the major sink for selenium removal in anoxic sediments and therefore leave upon the possibility of an agricultural waste treatment process based on sequestering Se^0(s) by bacterial respiration. In addition, the occurrence of relatively high levels of selenite in oxygenated waters of the oceans has been explained by oxidation of Se incorporated into organic matter by assimilatory reduction (11). We now propose an alternative hypothesis, namely, that dissimilatory reduction of selenate to selenite occurs within the reduced microzones of marine particles (1).

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REVIEW ARTICLE

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Microbial transformation of elements: the case of arsenic and selenium

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Abstract Microbial activity is responsible for the transformation of at least one third of the elements in the periodic table. These transformations are the result of assimilatory, dissimilatory, or detoxification processes and form the cornerstones of many biogeochemical cycles. Arsenic and selenium are two elements whose roles in microbial ecology have only recently been recognized. Known as "essential toxins", they are required in trace amounts for growth and metabolism but are toxic at elevated concentrations. Arsenic is used as an osmolite in some marine organisms while selenium is required as selenocysteine (i.e. the twenty-first amino acid) or as a ligand to metal in some enzymes (e.g. FeNiSe hydrogenase). Arsenic resistance involves a small-molecular-weight arsenate reductase (ArsC). The use of arsenic and selenium oxyanions for energy is widespread in prokaryotes with representative organisms from the Crenarchaeota, thermophilic bacteria, low and high G+C gram-positive bacteria, and Proteobacteria. Recent studies have shown that both elements are actively cycled and play a significant role in carbon mineralization in certain environments. The occurrence of multiple mechanisms involving different enzymes for arsenic and selenium transformation indicates several different evolutionary pathways (e.g. convergence and lateral gene transfer) and underscores the environmental significance and selective impact in microbial evolution of these two elements.

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Introduction

Microorganisms are involved in a variety of element transformations including a change in valence (i.e. oxidation/reduction) or chemical form (i.e. solid, liquid, gas). Many of these transformations are key steps in biogeochemical cycles. For example, the conversion of dinitrogen gas to organic nitrogen (in the form of ammonium) by nitrogen-fixing bacteria is crucial for communities in low-nitrogen environments and an important intermediate in the global nitrogen cycle. To date, over 40 elements (in their elemental form or their compounds) are known to be affected by microbial activity. These include the "major" (i.e. carbon, nitrogen, sulfur, oxygen, hydrogen, phosphorus), "minor" (i.e. magnesium, iron, calcium, potassium) and "trace" (i.e. manganese, molybdenum, tungsten, copper, nickel, selenium, and zinc) elements that make up a cell's chemistry. In addition, there are elements needed for specific structure (i.e. skeletal) or function (i.e. catalytic site of an enzyme). Their transformation may be the result of assimilatory processes in which an element is incorporated into cell biomass, dissimilatory processes in which transformation results in the generation of energy, or detoxification [71].

Arsenic and selenium are two elements whose significance in microbial ecology has only recently been recognized [57, 58, 71]. They have some characteristics in common, situated next to each other on the fourth period of the periodic table (As with atomic number 33 and Se with atomic number 34). Their most common chemical oxidation states, however, are different. The primary oxidation states for arsenic are As(V), As(III), As(0), and As(-III), while those for selenium are Se(VI), Se(IV), Se(0), and Se(-II). Both have been called "essential toxins" because they are required in trace amounts for growth and metabolism but are toxic at

high concentrations. They have similar modes of toxicity and are used as antagonists (i.e. arsenic is used for treatment of selenium poisoning and vice versa) [18, 67]. Events such as the loss of wildlife in the Kesterson National Wildlife Refuge due to selenium contamination [61], and the continuing devastation to the people of Bangladesh due to arsenic in their drinking water [49, 50] have promoted the investigation of the role microorganisms play in the mobilization and speciation of these two elements. The purpose of this review is to provide a brief overview of the microbial processes involved in the transformation of oxyanions of arsenic and selenium. Further information can be found in several recent reviews [48, 57, 58, 71] and two book volumes [15, 16].

Arsenic transformation

While arsenic has an historically infamous reputation as a poison [6], its biological uses are less well known. Arsenate is a chemical analog of phosphate and can uncouple mitochondrial oxidative phosphorylation [26]. Arsenite has a high affinity for thiol groups and affects respiration by binding to the vicinal thiols in pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase [52]. It has recently been shown to affect the function of the glucocorticoid receptor [34]. The methylated forms of arsenic, such as monomethylarsonic (MMA) and dimethylarsonic acid (DMA), have been thought to be less toxic and the main route of detoxification in mammals [8]. Recent studies, however, have revealed that not all mammals have methyl transferases [80], and that the methylated species can also have deleterious effects. Paradoxically, organoarsenicals (e.g., arsenobetaine, arsenocholine) are common in marine organisms [4], and while arsenic is added to chicken feed (i.e. Roxarsone) as a prophylactic against coccidial infections [11], it also promotes growth [52].

Two mechanisms for arsenic detoxification have been described. The first mechanism is methylation with either arsenate or arsenite being methylated. In higher eukaryotes, glutathione reduces arsenate to arsenite, which then accepts a methyl group from *S*-adenosylmethionine producing MMA or DMA [52]. An 85-kDa methyltransferase has been identified as mediating the last step [52]. In some bacteria, arsenite is first oxidized to arsenate by a specific enzyme, arsenite oxidase, with the arsenate subsequently methylated [5,19]. The second mechanism, which has been found in bacteria, yeast, and some lower eukaryotes, is the ArsC system [21, 31, 32, 69]. ArsC is a small-molecular-mass protein (13–15 kDa) that mediates the reduction of As(V) to As(III) in the cytoplasm. While As(III) is more toxic, it can be excreted via an As(III)-specific transporter, ArsB [69]. Although originally described as being plasmid borne, chromosomal loci have been found in over 25 species of bacteria, yeasts, and protoctists. While at least three distinct ArsC systems are believed to exist, the system has been best described in bacteria. The *ars* operon of plasmid R733 from *Escherichia coli* comprises *arsA*, *arsB*, *arsC*, *arsD* and *arsR*, whereas the chromosomal locus has only *arsB*, *arsC*, and *arsR* [21, 45]. A cysteine residue near the N-terminal of ArsC binds the arsenate, which is transformed to As(III) with electrons donated by reduced glutathione. As(III) is then expelled from the cytoplasm through an ATP-dependent arsenite transporter formed by ArsAB [45]. The *ars* operon in plasmid pI258 of *Staphylococcus aureus* contains only *arsB*, *arsC*, and *arsD* [31, 32]. In this case, reduced thioredoxin provides the electrons to reduce As(V), and arsenite is expelled from the cell via an ATP-independent ArsB [31, 32].

The widespread ability to utilize the transformation of arsenic oxyanions for the generation of energy has only recently been elucidated [48, 57, 58, 71] . Sixteen species of prokaryotes are known to have the ability to use arsenate as a terminal electron acceptor (Table 1). These include representatives from the Crenarchaeota, thermophilic bacteria, low and high G+C gram-positive bacteria, and Proteobacteria (Fig. 1).

To date, only the respiratory arsenate reductase from *Chrysiogenes arsenatis* has been studied in any detail [38]. A periplasmic enzyme, it is comprised of subunits of 87 and 29 kDa. N-terminal sequence data suggest that both subunits contain an iron-sulfur cluster, and metals analysis has confirmed the presence of Mo, Fe, and S [38]. The protein sequence and the presence of two pyranopterin cofactors per molybdenum atom places this protein in the DMSO reductase subfamily of mononuclear molybdenum enzymes [25]. It is most closely related to polysulfide reductase and formate dehydrogenase F [38]. The enzyme has an apparent K_m of 300 μM and can use benzyl viologen as an electron donor [38]. Initial investigations of the arsenate reductase from the haloalkaliphilic gram-positive bacterium *Bacillus selenitireducens* revealed similar characteristics. N-terminal sequence analysis indicates a 50% sequence identity and 85% similarity of both ArrA and ArrB subunits (Afkar and Stolz, unpublished results). In contrast, the putative arsenate reductase from *Sulfurospirillum barnesii* is membrane bound, consists of a single subunit (48 kDa) and has no metal associated with it (Newman et al., submitted manuscript). It has a comparable K_m of 200 μM, and NADH can also be used as an electron donor. Enzymological and immunological analyses further indicate that *S. barnesii* and related *Sulfurospirillum* species (*S. arsenophilum*, *S. deleyianum*) do not have a *Chrysiogenes*-type arsenate reductase.

The oxidation of arsenite to arsenate is used both for detoxification and for energy generation. Over thirty strains representing at least nine genera of arsenite oxidizing prokaryotes have been reported and include α-, β-, and γ-Proteobacteria, Deinocci (i.e. *Thermus*), and Crenarchaeota. Physiologically diverse, they include heterotrophic and chemolithoautotrophic species (Table 2).

While the majority of these organisms use oxygen, a γ-proteobacterium isolated from Mono Lake, Califor-

Table 1 Arsenate, selenate, and selenite respiring prokaryotes. *TEA* Terminal electron acceptor

Species	Phylogeny	TEA	Reference
Pyrobaculum aerophilum	Crenarchaeota	Arsenate, selenate	[29]
Pyrobaculum arsenicum	Crenarchaeota	Arsenate, selenate	[29]
Thermus sp. HR13	Thermus	Arsenate	[20]
Chrysiogenes arsenatis	Chrysiogenes	Arsenate	[42]
Salana multivorans	Gram +, high G+C	Selenate	[78]
Bacillus selenitireducens	Gram +, low G+C	Arsenate, selenite	[73]
Bacillus arsenicoselenatis	Gram +, low G+C	Arsenate, selenate	[73]
Bacillus sp. JMM-4	Gram +, low G+C	Arsenate	[65]
Bacillus sp. HT-1	Gram +, low G+C	Arsenate	[24]
Clostridium sp. OhILAs	Gram +, low G+C	Arsenate	(A. Dawson, J. Lisak, and J.F. Stolz, unpublished)
Desulfitobacterium sp. OhF2	Gram +, low G+C	Selenate	(T. Kuchan, J. Lisak, and J.F. Stolz, unpublished)
Desulfitobacterium sp. GBFH	Gram +, low G+C	Arsenate	[51]
Desulfotomaculum auripigmentum	Gram +, low G+C	Arsenate	[47]
Selenihalanaerobacter schriftii	Halanaerobacter	Selenate	[74]
Bordetella petrii	β-Proteobacteria	Selenate	[77]
Thauera selenatis	β-Proteobacteria	Selenate	[41]
Aeromonas hydrophila	γ-Proteobacteria	Selenate	[36]
Citrobacter sp. TCA-1	γ-Proteobacteria	Selenate	[24]
Shewanella sp.	γ-Proteobacteria	Arsenate	(Venkateswaran, K., D. Newman, and K.H. Nealson, unpublished)
JSA	γ-Proteobacteria	Selenate	(T. Sakaguchi, E.Tamiya, and K.Yokoyama, unpublished)
TSA	γ-Proteobacteria	Selenate	(T. Sakaguchi, E.Tamiya, and K.Yokoyama, unpublished)
AK4OH1	γ-Proteobacteria	Selenate	[37]
Ke4OH1	γ-Proteobacteria	Selenate	[37]
Desulfomicrobium sp. Ben-RB	δ-Proteobacteria	Arsenate	[43]
Wolinella succinogenes	ε-Proteobacteria	Selenate	(M.A. Rasmussen and T.L. Giblin, unpublished)
Wolinella succinogenes R-1	ε-Proteobacteria	Arsenate, selenate	[24]
Sulfurospirillum barnesii	ε-Proteobacteria	Arsenate, selenate	[40, 55,72]
Sulfurospirillum arsenophilum	ε-Proteobacteria	Arsenate	[2,72]

nia, strain MLHE-1, oxidizes arsenite under anoxic conditions using nitrate as the terminal electron acceptor [59]. The physiological role of arsenite oxidation in the most well studied organism, *Alcaligenes faecalis* (which has recently been shown to belong to the genus *Achromobacter* [64]), has been subject to debate. While considered a heterotroph in some reports, studies have shown the presence of a periplasmic electron transfer chain consisting of arsenite oxidase, azurin (a copper-containing protein), cytochrome *c*, and cytochrome *c* oxidase [5,60]. The significance of this is that its arsenate oxidase has been purified [5] and its crystal structure determined [14]. A mononuclear molybdenum enzyme belonging to the DMSO class, it is structurally similar to the periplasmic nitrate reductase (NapA) from *Desulfovibrio desulfuricans* [14]. Comprising two subunits, the catalytic subunit (~85 kDa) contains molybdenum bound to two pterin cofactors and a [3Fe-4S] cluster. The associated subunit (~14 kDa) presumably functions as an electron shuttle and has a Rieske-type [2Fe-2S] cluster, a feature that is unique among molybdenum enzymes [14]. In addition, unlike other members of the DMSO reductase family, the molybdenum center is not coordinated by any endogenous protein ligand. Whether these will be common characteristics of all arsenite oxidases remains to be determined.

Arsenic is an important factor in biogeochemical cycling. While not considered an abundant element, it can reach significant concentrations in hydrothermal, sulfidic, evaporitic, and iron-hydroxide-rich environments [79]. Arsenate-respiring bacteria affect the speciation and mobilization of arsenic as they have been shown to release arsenite from sediments [3, 33] and ferrihydrite [81], as well as grow on arsenate-containing minerals (i.e. Scorodite) [47]. The discovery of chemolithoautotrophic arsenite oxidizers suggests that they could contribute to the overall carbon budget of microbial communities in arsenic-rich environments [27, 56, 59, 63, 64]. Arsenate respiration has been linked to the mineralization of ~14% of the photosynthate fixed during meromixis in Mono Lake, California [56]. The rates of arsenate respiration were so significant that they indicated a need for microbial oxidation and led to the discoveries of the anaerobic arsenite oxidizer and a robust arsenic cycle in the lake [56,59].

Selenium tranformation

Selenium is similar to arsenic in many respects. Selenium is an analog of sulfur and substitutes for sulfur in thiols. In high doses it causes respiratory distress, is

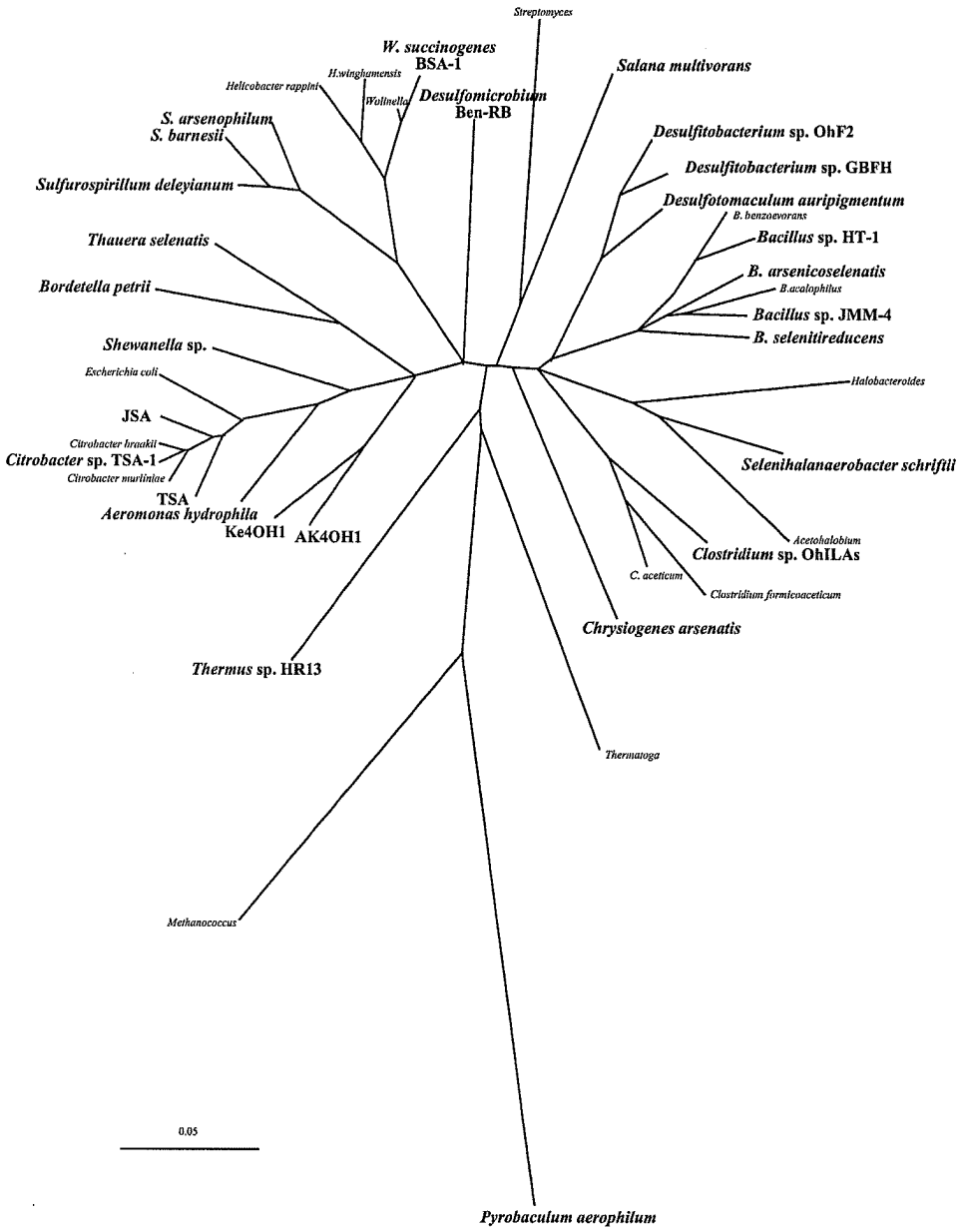


Fig. 1 Phylogenetic tree of prokaryotes that respire arsenic and selenium oxyanions constructed using distance matrix analysis (PAUP) [74]. Organisms not in bold type and in a smaller font are non-arsenic and non-selenium respiring species that were added to help define specific branches

teratogenic, and has clinical manifestations (e.g., blind staggers, alkali disease) [23, 28]. Trace amounts, however, are essential and it is often added to livestock feed [23]. The common biological forms are selenocysteine (the twenty-first amino acid) and selenomethionine. Selenocyteine is encoded by its own tRNA and provides the selenium in glycine reductase, formate dehydrogenase, and NiFeSe hydrogenase [17, 23, 46]. Selenium is assimilated in yeast and plants via the sulfur assimilation pathway [12,23]. Selenate is activated by ATP sulfurylase and subsequently converted to selenomethione [12]. While a similar mechanism has been proposed for prokaryotes, some studies have suggested specific mechanisms for the uptake of selenate and selenite [9, 10]. The reaction of selenite with glutathione produces selenodiglutathione. Selenodiglutathione and its subsequent reduction to glutathioselenol are key intermediates in the transformation of selenium [76].

Several different mechanisms are known for the detoxification of selenium. As with arsenic, methylation via a methyltranferase is a common mode of removal in eukaryotes [22] and prokaryotes [23]. Again, the reactivity of glutathione and other reduced species (i.e. dithiothreitol) with selenium creates a number of different intermediates. Selenocystine can be reduced to hydrogen selenide by reduced glutathione via selenocysteine-glutathione selenenyl sulfide [66]. Another strategy for detoxification is the reduction of selenite to elemental selenium. Selenium deposits may collect in the cytoplasm [7, 35], periplasm, or extracellularly [73, 74]. The phototrophic bacterium *Rhodospirillum rubrum* has

Table 2 Representative arsenite oxidizing prokaryotes. *TEA* Terminal electron acceptor, *NR* non-respiratory

Species	Phylogeny	TEA	Reference
Achromobacter "Alcaligenes" faecalis	β-Proteobacteria	NR	[60]
Agrobacterium albertimagni AOL15	α-Proteobacteria	NR	[62]
MLHE-1	γ-Proteobacteria	Nitrate	[59]
NT-26	α-Proteobacteria	Oxygen	[64]
Pseudomonas putida	γ-Proteobacteria	NR	[1]
Pseudomonas arsenitoxidans	γ-Proteobacteria	Oxygen	[30]
Thermus aquaticus	Thermus	NR	[19]
Thermus thermophilus	Thermus	NR	[19]
Thermus sp. HR13	Thermus	NR	[20]

been shown to expel elemental selenium across the plasma membrane and cell wall [35].

The use of selenium oxyanions, primarily selenate, as terminal electron acceptors is also widespread among prokaryotes, with at least two species of Crenarchaeota and fifteen species of bacteria (Fig. 1, Table 1). The respiratory selenate reductase from *Thauera selenatis* has been purified and characterized, and the genes encoding it cloned and sequenced [39, 68]. A heterotrimeric enzyme composed of subunits of 96, 40 and 23 kDa, it contains Mo, Fe, and S and has a visible spectrum indicative of a cytochrome b_{558} [68]. Located in the periplasm, it has a K_m for selenate of 16 μM. The *ser* operon contains four open reading frames, *serA*, *serB*, *serC*, and *serD* [39]. serA is the molybdenum-containing catalytic subunit. A member of the DMSO class of mononuclear molybdenum enzymes, it is most closely related to the membrane-bound nitrate reductase (NarG) of *Haloarcula marismortuii* (Stolz, unpublished data). serB contains four iron-sulfur cluster-binding sites while the serC is the putative cytochrome b_{558}. Similar to the function of narJ, serD may be a chaperone protein [39]. The enzyme has been crystallized recently, but its structure has yet to be elucidated [44].

Selenate respiring bacteria may also influence the speciation and mobility of selenium in the environment. Microbially driven selenate reduction, which has been found in a wide range of sediment types, results in the precipitation of elemental selenium [53, 54, 70]. While the remobilization of selenium through oxidation does occur, the rates are three to four orders of magnitude less than the reductive part of the cycle [13]. Thus unlike arsenic, microbial activity actually removes selenium from the environment.

Conclusions

Arsenic and selenium represent two examples of element transformation by prokaryotes. Prokaryotes with the ability to methylate, oxidize, or reduce inorganic and organic species of arsenic and selenium are widespread in nature and not confined to contaminated environments. This suggests that arsenic and selenium are more important in microbial ecology than previously recognized. The occurrence of multiple mechanisms for arsenate reduction is an indication of convergent evolution, whereas the presence of similar terminal reductases in distantly related species is suggestive of horizontal gene transfer. In addition, the phylogenetic diversity of species that can metabolize these two elements suggests that they are significant selective agents in microbial evolution.

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In Situ Bacterial Selenate Reduction in the Agricultural Drainage Systems of Western Nevada

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Dissimilatory in situ selenate reduction to elemental selenium in sediments from irrigated agricultural drainage regions of western Nevada was measured at ambient Se oxyanion concentrations. Selenate reduction was rapid, with turnover rate constants ranging from 0.04 to 1.8 h^{-1} at total Se concentrations in pore water of 13 to 455 nM. Estimates of removal rates of selenium oxyanions were 14, 38, and 155 μmol m^{-2} day^{-1} for South Lead Lake, Massie Slough, and Hunter Drain, respectively.

Respiratory reduction of selenate to elemental selenium is a mechanism whereby bacteria in sediments effect a removal of this toxicant from overlying waters (4). The process is an important sink in agricultural evaporation ponds in which selenate is abundant (~36 μM) (5). However, selenium at nanomolar levels occurs in other irrigated regions of the western United States (9) and still poses a threat to the biota. An example is the Stillwater Wildlife Management Area in western Nevada (3). Recently, we have found that diverse sediments carry out a rapid potential reduction of selenate (8). This raised the question of whether significant in situ reduction occurs in sediments of systems exposed to nanomolar concentrations of selenium oxyanions. Here we report that rapid in situ selenate reduction occurs in such sediments.

Sediments were collected from three sites in or near the Stillwater Wildlife Management Area, Nevada: Massie Slough, Hunter Drain, and South Lead Lake (3, 8). The South Lead Lake and Massie Slough sites contained considerable (i.e., 1 to 2 m deep) standing water, while Hunter Drain was a sluggish trickle of water (depth, ~0.5 cm) draining agricultural fields before seeping into the subsurface. The sediments were sampled in March 1990 by subcoring larger cores for bioassays and for collection of interstitial fluids as described previously (4, 5). Water temperatures were 9 to 16°C. Details on the assay have been reported (5, 8). Briefly, in the field subcores were injected with [^{75}Se]selenate and incubated at room temperature (20 to 26°C) for up to 2 h, during which time samples were sacrificed to obtain a time course. Subcores were extruded and washed free of $^{75}SeO_4^{2-}$, and the remaining $^{75}Se^0$ in the pellet was counted. Total dissolved selenium (selenate plus selenite plus organoselenium) was determined by atomic absorption spectroscopy (6). Because this procedure uses persulfate, it oxidizes organoselenium compounds to selenate. Therefore, selected samples were also run without persulfate to determine the amount of dissolved organoselenium. Selected samples were also spiked with 51 nM selenate to determine the extent of analytical interference from either dissolved organic carbon or nitrite (2, 7). Ion chromatography and autoanalyzer techniques were employed for the determination of other ions (4, 5).

Selenate reduction occurred without a lag at all three sites. Depth profiles indicated that reduction of $^{75}SeO_4^{2-}$ decreased with depth (Fig. 1). This decrease was most pronounced in South Lead Lake (Fig. 1A) and Massie Slough (Fig. 1C). In comparing the surface layers (0 to 5 cm) with the deepest layers (10 to 15 cm), rate constants decreased 21-fold, 1.3-fold, and 15-fold for South Lead Lake, Hunter Drain, and Massie Slough, respectively (Table 1). Thus, selenate turnover times were most rapid in the surface layers. We observed similar rate constants for these sites (0.68 to 0.84 h^{-1}) when we assayed the surface layers in August 1989, when the water temperature was 22 to 28°C (data not shown).

Hunter Drain had the highest concentrations of selenium oxyanions, and there were no appreciable amounts of dissolved organoselenium (Table 1). In contrast, Massie Slough and Lead Lake pore waters had selenate levels which were near the detection limit (13 to 25 nM), with equivalent amounts of dissolved organoselenium (Table 1). Hunter Drain also had the highest levels of nitrate, chloride, and sulfate (Table 1). This was probably a feature of the hydrology of the region, in that flow in Hunter Drain consists of seepage of saline groundwater (from proximate fields), while Massie Slough and Lead Lake receive surface and subsurface water from various sources and in greater quantity (7).

Multiplication of the rate constants by the porosities and the selenate-plus-selenite pool sizes yielded rates of selenate reduction in relation to depth in the sediments (Table 1). The highest overall rate of selenate reduction was in the surface layer of Hunter Drain (50 nmol $liter^{-1}$ h^{-1}), and the depth-integrated reduction rate was 155 μmol m^{-2} day^{-1}. In South Lead Lake virtually all the activity was confined to the surface and selenate concentrations were very low, yielding a rate of 9 μmol m^{-2} day^{-1}. Massie Slough, although displaying the most rapid turnover times for selenate, had much lower selenium oxyanion concentrations than Hunter Drain and thus yielded an intermediate integrated reduction rate of 39 μmol m^{-2} day^{-1} (discounting any contribution from the 10- to 15-cm horizon).

In comparison, the rate for the San Joaquin Valley evaporation pond was 301 μmol m^{-2} day^{-1}, with 71% of the activity occurring in the upper 2 cm (5). This value was comparable to that for Hunter Drain, but an order of magnitude greater than those for South Lead Lake and Massie Slough. However, the evaporation pond kinetics were characterized by a low turnover rate constant (0.089 h^{-1}) combined with a high level of selenium oxyanions (6.5 μM) at the surface, but the pattern reversed itself at depths below 2 cm. In contrast, Hunter Drain displayed similar rate

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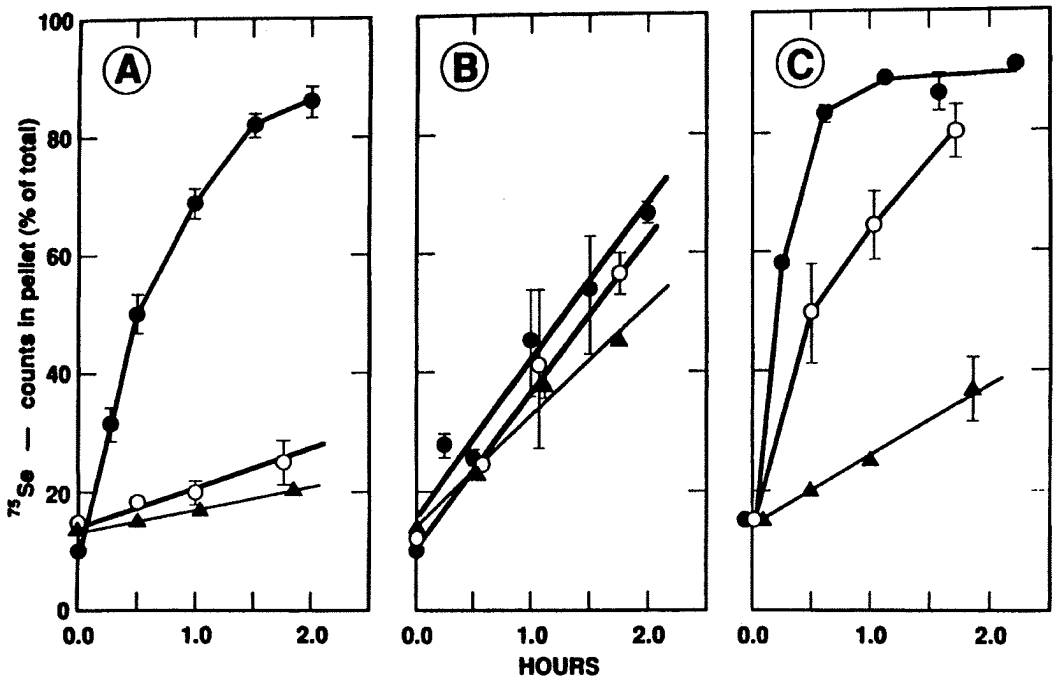


FIG. 1. Selenate reduction, as percentage of isotope added, in sediment profiles from South Lead Lake (A), Hunter Drain (B), and Massie Slough (C). Experiments were conducted during March 1990. Symbols: ●, 0 to 5 cm; ○, 5 to 10 cm; ▲, 10 to 15 cm. Symbols indicate the means of three samples, and bars indicate ±1 standard error. The absence of bars indicates that the error was smaller than the symbols.

constants at all depths, which, when combined with high selenium oxyanion concentrations at those depths, resulted in a significant integrated selenate reduction rate.

The persistence of selenium oxyanions in the pore waters from Hunter Drain, South Lead Lake, and Massie Slough (Table 1) is perplexing. The kinetics of selenate reduction (Fig. 1) suggest that selenium oxyanion concentrations should have rapidly decreased with depth in the sediment, as was observed in the static San Joaquin Valley pond (5). However, a hydrologic factor was probably involved whereby input of oxyanions from shallow groundwater would obliterate any chemical gradients established by bacterial removal processes. This was observed for nitrate in near-shore marine sediments (1). The presence of nitrate and selenate in the pore waters of all the sites reinforces this concept (Table 1), suggesting that the hydrologic supply rate of these oxyanions was comparable to their biological removal rates.

TABLE 1. Concentrations of dissolved constituents, porosities, selenate reduction rate constants, and reduction rates for surface and pore waters from South Lead Lake, Hunter Drain, and Massie Slough during March 1990

Source	Depth (cm)	Chloride (mM)	Sulfate (mM)	Nitrate (μM)	Selenate (nM)	Organo-selenium (nM)	Selenite (nM)	Porosity ($g\ g^{-1}$)	k (h^{-1})	Rate of SeO_4^{2-} reduction (nmol $liter^{-1}$ h^{-1})
South Lead Lake	0 (water)	21.0	5.9	18.5	13	BDL[a]	BDL			
	0–5	21.3	5.8	23.4	13	13	BDL	0.58	0.82	6.18
	5–10	21.3	5.9	63.1	25	ND	BDL	0.56	0.08	1.12
	10–15	22.5	4.7	79.1	25	ND	BDL	0.51	0.04	0.51
Hunter Drain	0 (water)	ND[b]	76.9	ND	456	88	215			
	0–5	646.1	78.6	433	354	BDL	101	0.42	0.26	49.7
	5–10	646.1	78.6	631	304	BDL	76	0.41	0.27	42.1
	10–15	627.0	82.1	691	367	ND	76	0.44	0.19	37.0
Massie Slough	0 (water)	12.0	3.9	33	51	ND	BDL			
	0–5	13.3	4.2	14	25	26	BDL	0.51	1.80	23.0
	5–10	12.3	4.1	21	25	13	BDL	0.51	0.72	9.2
	10–15	ND	ND	ND	ND	ND	ND	ND	0.12	ND

[a] BDL, Below detection limits (13 nM).
[b] ND, Not determined.

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BIOLOGICAL PROCESS PROVED MOST CONSISTENT IN DEMONSTRATION FOR REMOVING Se FROM WATER

A biological process was the most economically attractive technology demonstrated for removing selenium from an artesian flow during a field test for the EPA/DOE Mine Waste Technology Program, which is managed by MSE Technology Applications, Inc., Butte, Mont. The Biological Selenium Removal Process (BSeR™) of Applied Biosciences Corp., Salt Lake City (HTN, April 15&29, 1999, p. 41), also performed most consistently, an interim report says, noting that all but four BSeR effluent samples contained < 10 ug/l selenium, and 70% had below the detection limit of 2 ug/l.

Three technologies -- ferrihydrite adsorption, catalyzed cementation and the BSeR – were demonstrated at the Garfield Wetlands-Kessler Springs site of Kennecott Utah Copper Corp. Ground water there contains < 50-10,000 ug/l selenate; selenium levels of 200-2,000 ug/l are present in the 250-500 gpm artesian flows; and naturally occurring total dissolved solids in the water can be greater than 5,000 mg/l. The artesian flow currently is routed to KUCC's process water circuit. The demonstration goal was to lower selenium to below the national drinking water standard of 50 ug/l.

The BSeR process utilizes anaerobic solids bed reactors in which proprietary selenium-reducing microbes are grown in biofilms that are specially developed for operational longevity. The GW-KS ground water was pumped into the system at a rate of 1 gpm and passed through a series of 500-gallon bioreactors containing carbon/biosolids/biofilm or carbon/biofilm, depending on the test protocol. An agricultural grade molasses was used as a base for a proprietary nutrient supplement. Following treatment, the water passed through a slow sand filter and was discharged. The process produces a fine precipitate of elemental selenium, most of which can be backflushed from the bioreactors. The mean selenium concentration in the BSeR effluent was < 2 ug/l at residence times of 5.5 and 8 hours; 4.9 ug/l at 11 hours; and 8.8 ug/l at 12 hours, the report says. The estimated cost of scaling up the technology to treat 250-300 gpm is $188,000 for capital outlays; $0.75/1,000 gallons for inoculum and $0.54/1000 gallons for nutrient. Cost estimates for the three technologies are correct to +/-30%.

(Continued)

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SELENIUM REMOVAL PROCESSES (Cont.)

The ferrihydrite process, which is considered by EPA to be the best demonstrated available technology and was optimized for the demonstration by MSE, involves precipitating ferrihydrite and concurrently adsorbing selenium onto the ferrihydrite surface. At GW-KS, the water flowed into the treatment system at a rate of about 5 gpm and ferric chloride reagent was introduced in front of a static mixer. The water then entered a tank where a lime slurry was injected and pH was adjusted as needed. A flocculent was added and the water moved to a 1,000-gallon thickener for a residence time of about 200 minutes. Solids periodically were removed and processed by a filter press. The treated water was neutralized, filtered and discharged. Treatment at iron levels of 4,800 mg/l (Fe:Se ratio of 3200:1) resulted in a mean selenium effluent concentration of 90 ug/l and minimum concentration of 35 ug/l, MSE reports. TCLP testing of the filter cake was inconclusive. Capital costs of a scaled up system are estimated at $1,026,835, and reagent costs at $15.17/1,000 gallons.

The catalyzed cementation process, developed by Larry Twidwell of Montana Tech with assistance from MSE, removes heavy metals from solution by cementation onto an iron surface. In a system designed to process 1 gpm of GW-KS ground water, a catalyst and sulfuric acid were blended into the water by static mixers. The water then flowed to a reactor containing fluidized iron particles; through a cone-bottom tank where the iron particles were collected for recycling; and a second reactor where a lime slurry and oxidizer were added to complete the reaction. A flocculent was added to assist with solids separation in a thickening tank, where the residence time was about 15 hours. The cleaned water was filtered before discharge. Thickener solids were processed in a filter press and the collected liquid was returned to the thickener. Because the mean selenium concentration in the effluent was 834 ug/l and the minimum level was 193 ug/l, the system was switched to an increased oxidation condition, the report notes. This lowered the mean selenium concentration to 35 ug/l and the minimum level to 26 ug/l. Filter cake samples contained selenium below the TCLP threshold value of 1 mg/l. Capital costs are estimated at $1,083,285 for a full-scale system, and operating costs at $8.11/1,000 gallons.

Applied Biosciences also reports that it has implemented its biological arsenic removal (BAsR™) process at full scale and achieved operating costs of $.50/1,000 gallons in both pond and pump-and-treat applications. To biotreat organic pollutants, AB has introduced a proprietary mix of microorganisms, enzymes and nutrients, and developed unique blends of microorganisms and enzymes to meet site requirements. AB researchers will present BSeR process scale-up and EPA test results at the Battelle In-Situ and On-site Bioremediation symposium in San Diego, June 4-7. Contact at AB is Tim Pickett, (800) 280-7852, ext. 600; fax, (801) 468-1897; e-mail, tpickett@bioprocess.com. Contact at MSE is Helen Joyce, (406) 494-7100.

THE PERFORMANCE OF ADI MEDIA G2™ marketed by ADI International Inc., Fredericton, N.B., has been certified by Environment Canada's Environmental Technology Verification program with regard to the treatment of arsenic in chlorinated drinking water. ETV evaluation of the adsorbent, which is a chemically-coated, naturally-occurring material (HTN, April 23, 1998, p. 51), was conducted with water having a pH of 5.8-7.0. During the first cycle of operation, pentavalent arsenic levels of up to 41 ug/l were lowered, on average, to <5 ug/l (95% confidence) despite the presence of an average of 1.2 mg/l of naturally-occurring iron, according to the ETV program. The system, which had a design empty bed contact time of 15 minutes, processed 5.0×10^6 gallons of treated water without media regeneration. ADI Media G2 is a chemically-coated, naturally-occurring material that can adsorb copper, lead and other metals and is relatively unaffected by commonly interfering substances, such as chlorides and sulfates, the company says. A combination of natural minerals and water treatment chemicals, ADI Media G2 has a high porosity that provides an extremely large surface area to volume ratio, the firm notes, so it offers superior surface reaction chemistry and a very large number of reaction sites. The media performs over a pH range of 5.5-8.0 and can be regenerated in-place. Contact is Eric Winchester, (506) 451-7407; fax, (506) 459-3954; e-mail, elw@adi.ca.

Effective Biological Water Treatment through Biological Process Control

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ABSTRACT

Biological methods are gaining wider acceptance in the mining industry for the treatment of metals and other inorganics common to mining waste. While lower costs relative to conventional treatment have made biological treatment an attractive option, the performance of some biological systems has been less than predictable. In addition to performance complications, many biological methods make no provision for contaminant collection and disposal. Applied Biosciences has developed the ABMet™ water treatment process specifically to address these issues. The ABMet™ process is an engineered and highly controllable biological process for the removal of heavy metals, metalloids, and other inorganic compounds like cyanide, nitrate and ammonia. The contaminant removal activities of site-optimized microbial cultures in a bioreactor environment are precisely controlled through a regulated nutrient delivery system. ABMet™ systems are able to achieve non-detect effluent levels for contaminants such as arsenic, chromium, selenium, and zinc and are designed to remove these contaminants from the system as well as from the target stream. Data from operating full-scale plants, pilot plants and bench-tests will be presented.

Introduction

Biological water treatment methods for the removal of heavy metals and other inorganic contaminants have grown in popularity during the last decade. Low costs and minimal sludge generation have convinced many environmental managers in the mining industry

to experiment with biological systems, but performance has often been unpredictable. This paper outlines the experience of Applied Biosciences Corporation over the last several years in developing and implementing biological systems that meet very strict effluent guidelines and that do so reliably. The key to successful implementation of biological water treatment is process control: specifically *biological* process control. Mechanical and electronic methods widely available for the control of water treatment processes are well understood and the benefits of automated versus manual control when compared to the costs are judged differently in different treatment contexts. But the benefits of controlling the biological processes that are the foundation of successful water treatment are less well understood by mine operators. The benefits and costs of controlling these biological processes are the topic of this paper.

While many inorganic contaminants are present in various mine effluent waters, the following include those contaminants most commonly encountered in the past work of Applied Biosciences: arsenic, selenium, zinc, TDS (as sulfate), nitrate, cyanide, copper, and mercury. These are the contaminants most likely to survive in soluble form in trace amounts, even after pH adjustment for acidic streams. Some of these contaminants, especially nitrate, selenium and arsenic, are also commonly present in mining effluent or ground water that is neutral or near-neutral to begin with. These three contaminants will be the targets of the treatment systems discussed in this paper.

Biological Treatment

The strengths of biological treatment are its abilities to 1) remove contaminants with minimal sludge generation, 2) accomplish treatment using an inexpensive and biodegradable nutrient source as the reagent, and 3) remove contaminants to low levels – to below 0.100 mg/l for most biological systems and to below 0.005 mg/l for advanced biological systems such as those developed by AB.

The weaknesses of conventional biological treatment have been 1) long hydraulic retention times that require large foot-print systems, 2) susceptibility to changes in water

chemistry, 3) inconsistent effluent levels for target parameters, 4) weak performance in cold water, 5) the production of more bio-available or toxic forms of target contaminants, and 6) the gradual accumulation of concentrated contaminants inside the system.

Researchers and engineers at AB have developed an approach to biological water treatment that capitalizes on the specific strengths of biological treatment and minimizes the specific weaknesses.

A Prioritized Approach to Water Treatment

Priority 1 – Effectiveness

When implementing any water treatment solution in any context, whether biological or some other technology, the first priority is effectiveness. If the goal of water quality regulation is a healthy environment and the specific goal of a water treatment system is regulatory compliance than the treatment solution must meet the effluent limit guideline. Conventional biological methods have not always been able to reach the low limits (often below 0.005 mg/l) now being required.

Priority 2 – Reliability

Any water treatment solution that is effective at treating to the effluent limit guideline must be able to do so reliably, day after day, until treatment goals are met, or in some cases, in perpetuity. The performance of conventional biological treatment systems – from wetlands to modified activated sludge – has often been erratic, with effluent contaminant levels fluctuating in and out of compliance.

Priority 3 – Affordability

A water treatment system that reliably meets effluent limits must do so affordably. "Affordability" is a relative term, but in most mining contexts, any compliance option

that exceeds $1.00 or $2.00 per 1000 gallons treated (net present value) becomes cost-prohibitive. In the context of mine closure anything above $1.00 becomes cost-prohibitive and for abandoned mines, any expense is seen as too high. AB biotreatment systems target a cost window of $0.60 to $1.60 per 1000 gallons treated, including all capital and O&M costs.

Priority 4 – <u>Environmental Impact</u>

No true solution to water contamination can simply trade one environmental liability for another. While wetlands, for example, may be very affordable and sometimes effective there is some question as to their long-term environmental benefits. Toxic sludge accumulates in sediments. Wildlife can be exposed. The liability remains on-site, even if not in the effluent. A fairly recent discovery with biological systems has been the tendency of these systems to produce more bioavailable forms of the very contaminants they are supposed to be removing. 30 or 40 ppb selenite that leaves a biological system is more toxic to wildlife than the 500 ppb selenate that it was intended to remove.[1] Arsenic present in oxygen depleted ponds exposed to sunlight has been shown to develop into methylated compounds that are much more difficult to remove from water than arsenate and arsenite.[2]

The primary attraction of biological water treatment methods has been affordability. From passive, wetlands-type "systems" on the low-cost end to engineered activated-sludge-type systems on the other end, and gravel-bed systems in between, biological treatment is inexpensive relative to traditional chemical and mechanical methods for inorganics removal. The problems with biological treatment have been related to effectiveness, reliability, and to collateral environmental impacts.

Treatment Goals

The biological treatment solution that is going to satisfy all four treatment priorities is the solution that for the least expense can do two things:

1) remove the target contaminant from the stream, and
2) remove the collected contaminant from the system.

Wetlands and gravel-bed systems (or variations with plastic media) are not designed to accomplish both these tasks. A modified activated sludge system that relies on absorption for removal as much as on chemical reduction to insoluble forms will have difficulty in consistently accomplishing both goals.

Biological Process Optimization and Control

Based on this approach guided by treatment priorities and treatment goals, Applied Biosciences has developed the ABMet™ water treatment process. The ABMet™ process, like other biological methods, relies on the microbial reduction of soluble contaminants to insoluble forms that then precipitate from solution.[3] ABMet™ is a highly "engineered" biological process in the sense that it is based on much more deliberate control of the biological processes that accomplish the target chemical reductions than is typical of most biological methods. Controlling the microbiology allows us to control the process, which in turn, allows us to control the results. In addition, ABMet™ systems are designed with back-flushing capabilities for the periodic removal and recovery of collected contaminants.

Controlling the process requires control of the biofilm that is at the heart of the process. This control consists primarily of ensuring that:

1. the right microbes
2. existing in the proper densities

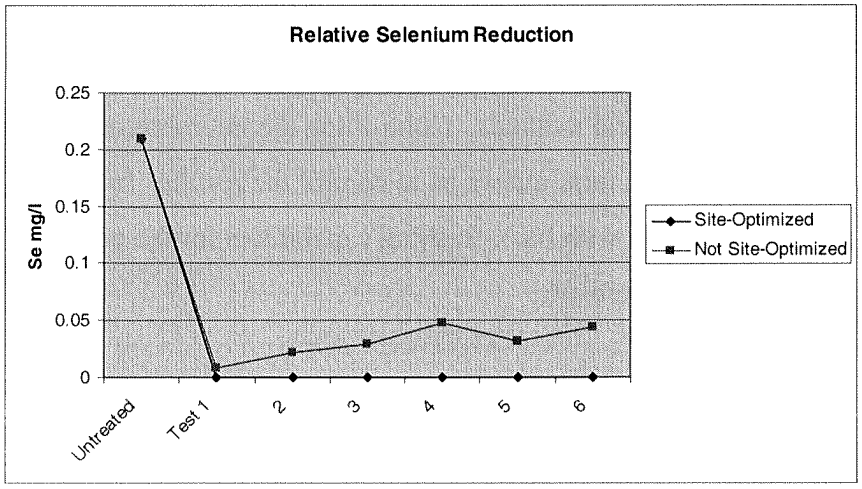
3. in a high-surface area environment
4. are fed just enough
5. of the proper nutrient
6. to encourage the target chemical transformations
7. to the target level
8. in the shortest possible time-frame.

The ABMet™ process accomplishes these tasks by focusing on three fundamental process components, pursuing "optimization" for each one:

1. site-optimized microbial cultures
2. site-optimized nutrient blends
3. a process-optimized matrix for biofilm stability

Figure 1 illustrates the difference in biological process performance that results from site-specific optimization.

Figure 1



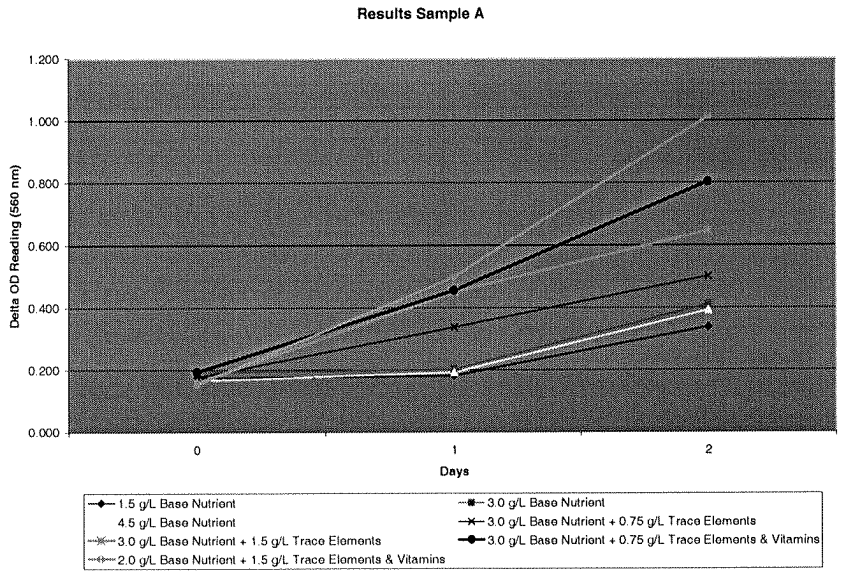
Water samples from a Montana mine site were pumped through a bench-scale ABMet™ system that contained a microbial mix that had been previously optimized for a mine site

in British Columbia. Despite an initial drop in selenium effluent levels the system demonstrated a gradual rise in selenium levels. A second test run with a new microbial mix optimized for this specific mine site's water demonstrated effective removal to below the detection limit of 0.002 mg/l Se for the length of the test.

Biological systems are able to benefit, at least at a basic level, from any number of carbon-rich substrates: ethanol, methanol, acetic acid, biosolids, natural fibers, or molasses. The ABMet™ process uses a molasses based nutrient blend that includes a balanced C:N:P:S ratio and trace vitamins and minerals, all adjusted based on site-specific water chemistry.

Figure 2 illustrates the difference in microbial growth, measured by optical density, resulting from changes in nutrient composition in a water sample from a South Dakota mine site.

Figure 2



Higher densities of the desired microbes perform more of the desired chemical transformations in less time. Encouraging healthy growth of the proper microbes translates into a system that is more efficient and less expensive to build and operate.

Controlling the microbial population, in part through nutrient composition and nutrient delivery, allows the system operator to control performance.

Bench-Testing Data

Figures 3 and 4 illustrate results from bench-testing done on water samples from the San Joaquin Valley in California where selenium is a problem in agricultural run-off. Following culture and nutrient optimization, water samples were run through bench-scale ABMet™ bioreactors to remove selenium and nitrate.

Figure 3

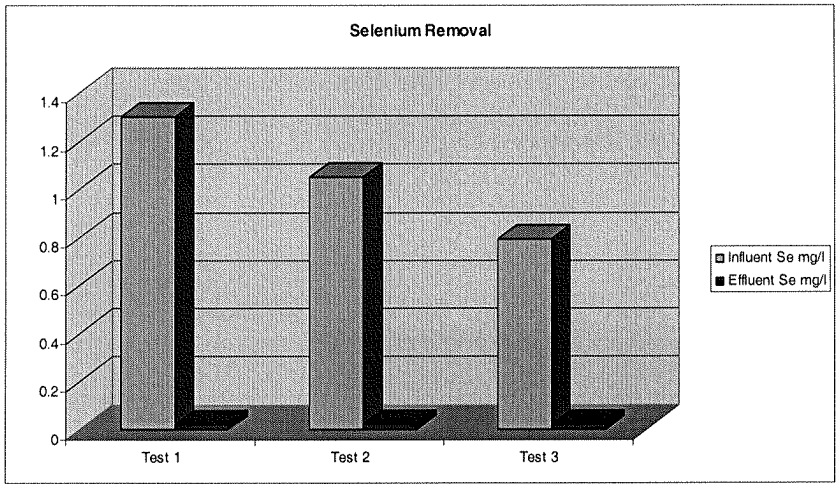


Figure 4

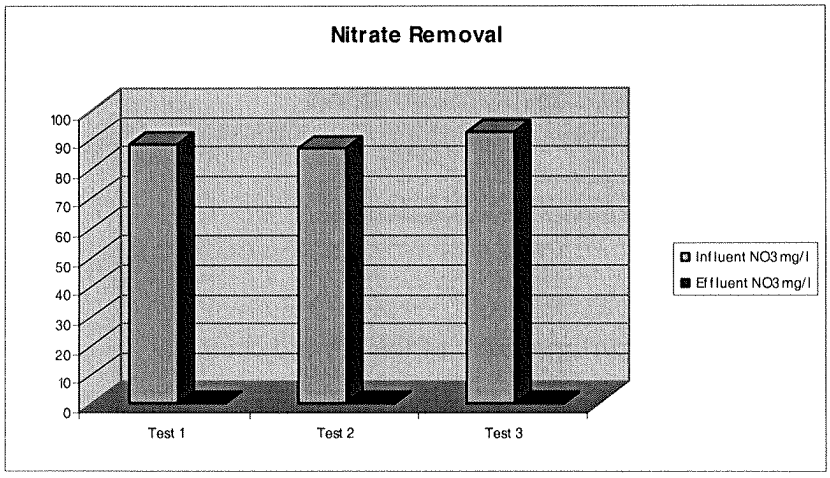
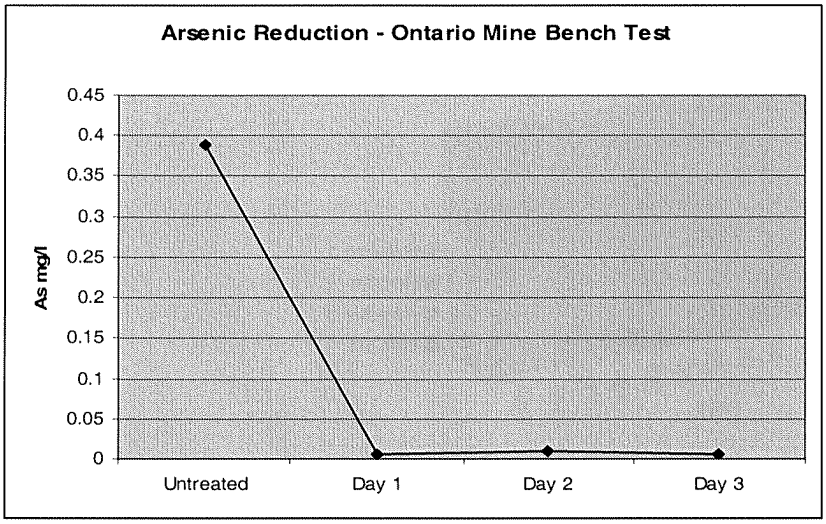


Figure 5 illustrates optimized arsenic removal in a bench-scale test using water samples from a mine site in Ontario. Data from this test are being used to design a 150 gpm pilot plant at that site in the Summer of 2003.

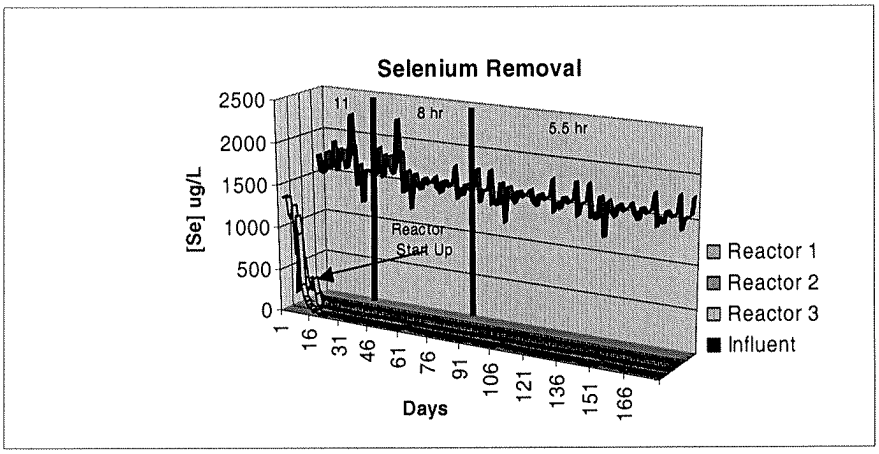
Figure 5



Influent arsenic levels of 0.388 mg/l were reduced the first day to below the detection limit of 0.005 mg/l. Day 2 saw a rise in effluent arsenic to 0.009 mg/l due to nutrient depletion. After feeding the reactor system at the end of day 2 the effluent arsenic levels dropped quickly to below detection. Optimizing the feeding schedule of an ABMet™ system is key to its effectiveness.

Figure 6 contains selenium removal data from a pilot plant that was operated at Kennecott Utah Copper in Utah for six months in 1999. This test was conducted under the EPA's Mine Waste Technology Program.

Figure 6



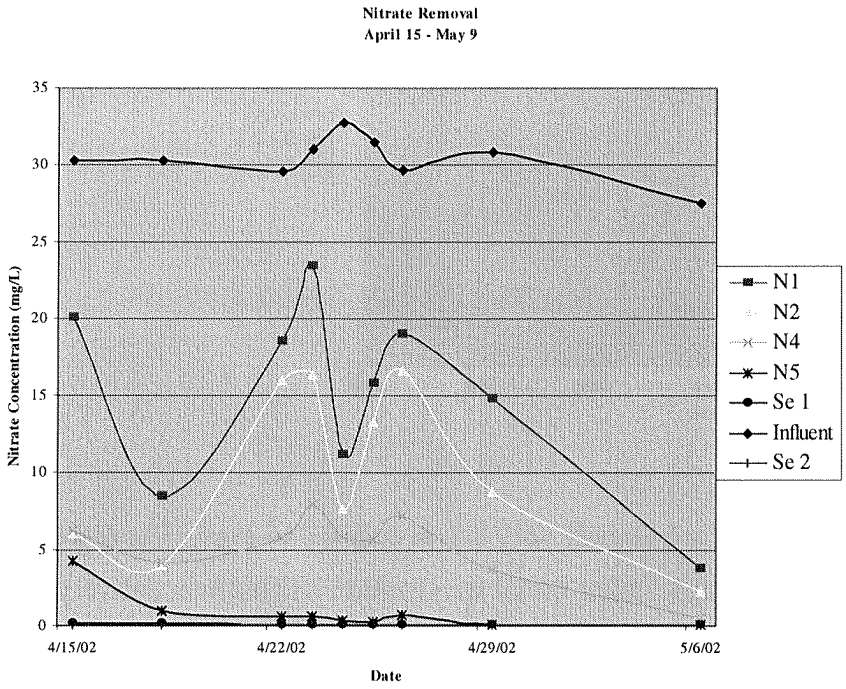
After an initial stabilization period of about two weeks, selenium was removed to below the detection limit of 0.002 mg/l within the first bioreactor.

Full-scale Implementation

During the winter of 2001-2002, a full-scale ABMet™ treatment plant was built at a mine site in South Dakota to treat nitrate and selenium. Bench testing for this site also removed trace amounts of arsenic (0.065 mg/l in the influent) but arsenic removal was not included in the full-scale design. This plant was designed to replace an existing biological nitrate removal system that was not consistent in meeting compliance targets and that also had to be heated in the winter. The goal of the ABMet™ implementation was to treat both selenium and nitrate and to do so at ambient water temperatures. Cultures and nutrient were optimized for this water stream during the laboratory phase of the project in the Fall of 2001.

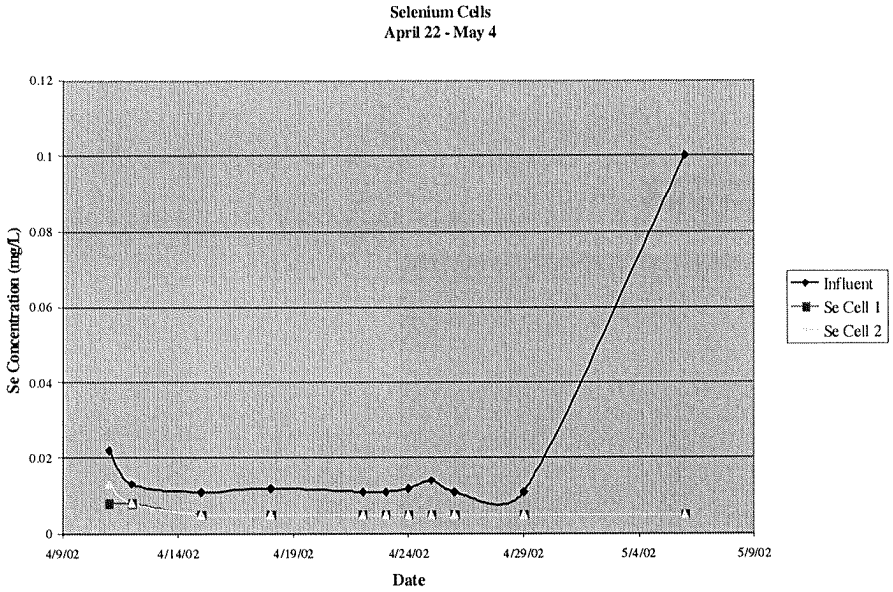
Figures 7 and 8 illustrate nitrate and selenium removal during the first few weeks of operation.

Figure 7



The influent to this plant is separated into two parallel streams, with each stream passing through two nitrate cells and one selenium cell (N1 to N4 to Se1 for one stream, and N2 to N5 to Se 2 for the other). The total flow averages 100 gpm and peaks at 300 gpm. After plant stabilization, effluent levels for nitrate were below the permit limit of 10 mg/l after the first nitrate cell in each train, and were at non-detect levels at the end of the complete treatment train.

Figure 8



Average selenium influent levels are already very low at only 0.014 mg/l. The discharge limit for South Dakota is 0.010 mg/l. After initial stabilization and nutrient delivery optimization, effluent Se levels were consistently below the detection limit of 0.005 mg/l.

Figures 9 and 10 present data from the first few months of plant operation.

Figure 9 – Nitrate Removal

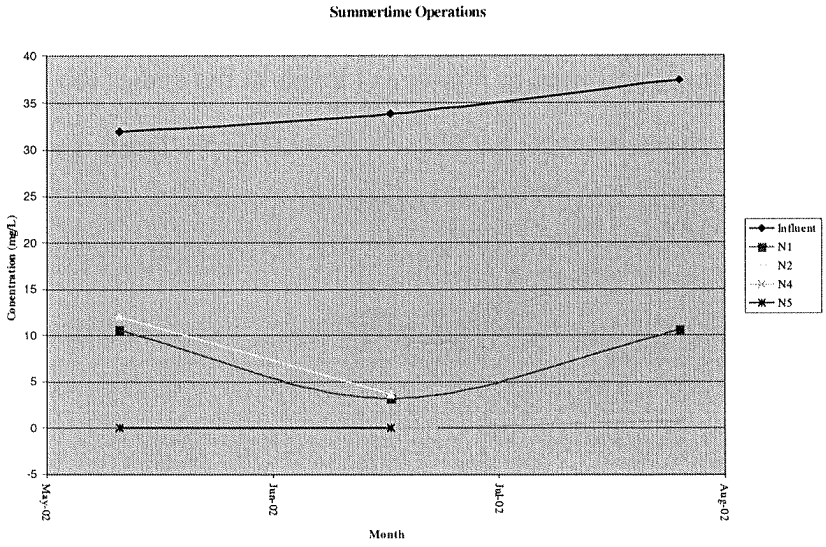
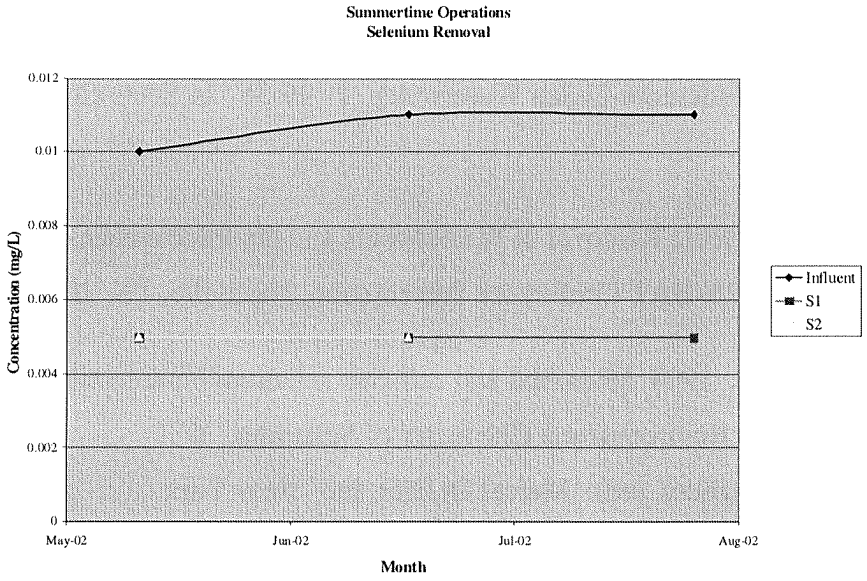


Figure 10 – Selenium Removal



Again, nitrate is below discharge requirements after the first nitrate cell and below detection after the second cell in each train. Selenium levels are consistently at non-detect levels below 0.005 mg/l. Costs of treatment over the first 18 months of full-scale operation have averaged $0.14 per 1000 gallons treated for nutrient cost. Other O&M costs are estimated at less than $0.20 per 1000 gallons.

Conclusion

The benefits of controlling the biological processes that are the foundation of biological treatment are made clear in data from bench, pilot, and full-scale treatment systems. To remove contaminants to very low levels requires that site-optimized microbial cultures and site-optimized nutrient blends be combined in a well-designed system that protects the health and activities of the biofilm.

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Microbial and Cell-Free Selenium Bioreduction in Mining Waters

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I. INTRODUCTION

This chapter discusses factors important for removal and recovery of selenium from mining waste and process waters. Selenium reduction is presented as an example of microbial metal/metalloid reduction with potential application to the treatment of high volume wastewaters. Mining and mineral processing wastewaters are generally high in volume and low in pollutant concentrations. Process solutions are usually lower volume and have a higher metal concentration, while acid drainage sites, which vary considerably in volume, have a lower pH and a high metal content. While acid drainage is not covered in this chapter, it represents a serious, widespread, complex, and ultimately long-term environmental problem.

The U.S. Bureau of Mines estimated that in the United States alone, there is approximately 50 billion tons of waste rock existing from past mining activities, with another 2 billion tons being generated each year (National Research Council, 1995). Associated with this potential source of metal contamination are billions of gallons of metal-contaminated wastewaters requiring treatment; much of this material contains selenium. Potentially adding to the problem is the increased use of biooxidation technologies, which allow for the processing of low grade, acid-producing sulfide ores. Often different from wastewaters, mining process waters can contain high concentrations of multiple metal and inorganic contaminants, such as cyanide, that are toxic to wildlife and microorganisms. Treatment technologies for the removal of dissolved metals and other inorganics are needed for mining and other high volume process and waste waters.

Biological and microbiological methods are more frequently being considered to remove and recover metals from contaminated aqueous solutions. Current

applications range from large-scale removal of metals from sewage, industrial effluents, and mining waters, to smaller scale metal recovery processes. Large-scale processes are generally unsophisticated, nonspecific, and often do not remove metals to desired levels. Metal removal and recovery processes from solutions may be based on one or more microbial pathways or attributes including (1) binding of metals to cell surfaces or biomolecules within cells, (2) translocation of metals into cells via active and passive methods, (3) formation of metal-containing precipitates through reaction with cellular polymers and produced materials, such as hydrogen sulfide, phosphates, and carbonates, (4) biotransformation of metals through oxidation/reduction/volatilization methods, and (5) metal removal and recovery through use of cellular components such as ligands and enzymes. Literature and test data show that selenium biotransformation depends at least somewhat on microorganisms, nutrients, oxygen, pH, temperature, and cocontaminants and can occur under both aerobic and anaerobic conditions.

Metals and metalloids, including selenium, can be used as electron acceptors by microorganisms and are reduced in the process. In general, microbial metal/metalloid reduction may represent either a detoxification mechanism or the end step in the respiration process, resulting in selenium biotransformation. Examples of laboratory and pilot-scale tests demonstrate selenium reduction using various reactor systems with live microbes as biofilms, as immobilized live cell preparations, and in cell-free systems.

II. BACKGROUND

Selenium (Se), a naturally occurring element, atomic number 34, atomic weight 78.96, lies between sulfur and tellurium in group 16 and between arsenic and bromine in period 4 of the periodic table of the elements. Selenium is the thirtieth most abundant element and is widely dispersed in igneous rock; in hydrothermal deposits it is associated isothermally with silver, gold, antimony, and mercury; it appears in large quantities, but in low concentrations, in sulfide and porphyry copper deposits; and it is associated with various types of sedimentary rock (Hoffman and King, 1997). Selenium is a common water contaminant throughout the world and is a problem contaminant in at least six western U.S. states. Selenium is commonly found in mining wastewaters in concentrations ranging from a few micrograms per liter up to 12 mg/L, and in process solutions at concentrations exceeding 33 mg/L. While most of these operations are currently zero-discharge facilities, eventual treatment prior to closure will necessitate removing selenium to meet discharge requirements.

Inorganic selenium is most commonly found in four oxidation states (Se^{6+}, Se^{4+}, Se^{0}, and Se^{2-}). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are highly water

soluble, while elemental selenium (Se^0) is much less soluble in water. The most reduced form, hydrogen selenide (H_2Se), occurs as a toxic gas but is readily oxidized to elemental selenium in the presence of air. Elemental selenium can be oxidized to selenite by microorganisms or converted chemically in alkaline or mildly acidic conditions; further oxidation results in the conversion to selenate. Well-aerated surface waters, especially those with alkaline conditions, represent a highly oxidized condition that contain the majority of selenium as selenate. The relative proportions of selenite and selenate depend on water redox potentials and pH. Selenite is reduced to elemental selenium under mildly reducing conditions, while selenate reduction occurs under stronger reducing conditions (Maiers, 1988; Hughes and Poole, 1989; Ehrlich, 1990).

Selenium forms covalent compounds with most other substances and is necessary in small amounts for most life-forms. Selenium is a chemical analog of sulfur and can interfere with normal cellular metabolism, particularly those aspects involving sulfur (Anderson and Scarf, 1983; Hoffman and King, 1997). Selenium is a teratogen in mammals and birds, and birth defects in several species have been attributed to it (National Research Council, 1976). Selenium poisoning, which can cause death and/or mutations in fish and waterfowl, has been documented extensively over the last decade (Bainbridge et al., 1988; Frankenberger and Benson, 1994). A highly publicized case of selenium poisoning occurred in California's Kesterson Wildlife Reservoir, resulting in the closure of this facility (Bainbridge et al., 1988).

Cost-effective, efficient, off-the-shelf technology is not available for selenium removal from large-volume waters. While reported physical–chemical methods for selenium removal will decrease selenium concentrations to acceptable levels, the treatment costs are prohibitive for large volumes. Conventional treatments include lime precipitation (Koren et al., 1992), chemical reduction (Marchant et al., 1978), activated alumina adsorption (Trussel et al., 1980), ion exchange (Maneval et al., 1985; Boegal and Clifford, 1986), and reverse osmosis (Wilmoth et al., 1978), many of which result in a mixed-metal waste product that can increase treatment/disposal costs.

III. BIOLOGICAL TREATMENT TECHNOLOGIES

Numerous biological technologies have been tested for selenium removal from water (Case et al., 1990; U.S. DOI and California Resources Agency, 1990). For example, a combined microalgal–bacterial treatment was demonstrated to effectively remove selenium from Kesterson agricultural waters (Gerhardt et al., 1991). Bench-scale processes for selenium and general metal removal from mining and other waters using *Pseudomonas stutzeri* and other microorganisms have been demonstrated (Adams et al., 1993, 1996a, 1996b). Mixed cultures of indigenous

bacteria demonstrated a 96% selenium removal at bench scale (Altringer et al., 1989; Larsen et al., 1989). Pilot tests using San Joaquin agricultural waters and *Thauera selenatis* removed 98% of the selenium in an anaerobic bioreactor and produced effluent selenium concentrations of less than 5 μg/L (Cantafio et al., 1996). A pilot plant study was conducted in which *E. coli* was used to treat a base metal smelter, weak acid effluent containing 33 mg/L Se. A rotating biological contactor (RBC) with a 9.2 m^2 disk surface and 30 L capacity was used as the bioreactor. Initial test results indicated that 97% of the selenium was removed from the contaminated solution with a 4-hour retention time. Tests on other mining process waters using a bench-scale RBC, *Pseudomonas stutzeri*, molasses (1.0 g/L), and a 6-hour retention time removed 97% of the selenium (Adams et al., 1993).

Microbial volatilization of selenium through biomethylation shows promise for an in situ treatment of selenium-containing waters and sediments. Biomethylation by several fungal and bacterial species has been demonstrated (Fleming and Alexander, 1972; Barkes and Fleming, 1974; Frankenberger and Karlson, 1995; Flury et al., 1997). This microbial reaction converts toxic selenium oxyanions to less toxic gaseous dimethyl selenide, which is released into the atmosphere. Rat toxicity testing demonstrated dimethyl selenide to be about one five-hundredth as toxic as selenite (McConnell and Portman, 1952). While selenium methylation does not pose the same ecological threat as methymercury compounds, synergistic toxicity of dimethyl selenide with mercury has been reported (Parizek et al., 1971).

IV. SELENIUM REDUCTION BIOCHEMISTRY

To completely understand microbial metal/metalloid reduction, it is important to have a complete picture of the biochemistry behind these transformations. However, the biochemistry of most systems for transforming microbial metals and metalloids has not been completely characterized (Stadtman, 1974). In some systems, selenium and other metal transformations appear to be coupled with the cytochrome system (Rosen and Silver, 1987; Beveridge and Doyle, 1989; Ehrlich, 1990). Also, specific selenium-active enzymes may play a role in selenium reduction. For example, the selenium-reducing organism *Thauera selenatis* appears to reduce selenate to selenite using a selenate reductase (Rech and Macy, 1992), and selenite reduction to elemental selenium appears to be catalyzed by a periplasmic nitrite reductase (Demoll-Decker and Macy, 1993). Additionally, Macy et al. (1989) report selenate reduction in a *Pseudomonas* species as part of a novel anaerobic respiration model.

Although there has been a tremendous amount of research on microbial selenium transformation, much disparity exists between transformation efficiencies observed under carefully controlled laboratory conditions and those observed

in the field. Often, there appears to be no correlation between the two sets of observations. This is probably due to the broad range of environmental and site factors involved in field applications of bioremediation technologies.

V. SITE CHARACTERIZATION, BIOASSESSMENT, AND BIOTREATABILITY ANALYSIS

Whether used as a primary treatment technology, a pretreatment, or as a polishing step, successful application of biological treatments involve site characterization, bioassessment, biotreatability testing, and bioremediation monitoring. Often these important steps are over-looked or considerably shortened because of initial assessment costs. These steps are necessary to answer the following frequently asked questions: Can bioremediation work for my selenium problem? How fast will it work? What volumes and flow rates can be processed? How much will it cost?

Responsible site evaluation, system design, treatment system development, construction, and operation requires the combined expertise of many disciplines. Input from microbiologists, chemists, geologists, hydrologists, engineers, and experienced field remediation specialists are necessary for successful execution and closure of most bioremediation projects. Site characterization, assisted through site maps, historical documentation, and sampling must determine, as best possible, the horizontal and vertical extent of the contamination. In most cases this can be achieved by answering three basic questions:

1. What form do the contaminants of interest and any cocontaminants take, and what is their concentration?
2. What contamination carrier, soils or liquids, must be treated, and what characterization is required to define the soil chemistry and site hydrology?
3. Is the contamination above, below, or in the water table, and what is the degree of contamination of these areas?

Site bioassessment for a proposed remediation system involves determination of the indigenous microorganisms and bioavailability of the contaminants, as well as identification of interferences caused by cocontaminants, ions, or required treatment conditions; site environmental conditions, including pH, redox, temperature, dissolved oxygen, and available carbon, nitrogen, and phosphate concentrations; and nutrient or amendment stability. At mining sites, other essential trace elements required for successful remediation, such as sulfur, potassium, magnesium, calcium, manganese, iron, cobalt, copper, molybdenum, and zinc, sometimes are present at high concentrations.

Following site bioassessment, more detailed biotreatability studies are needed to identify factors and parameters involved in determination of whether to consider bioremediation for a particular site. This process requires slightly different approaches for in situ versus ex situ or bioreactor treatment. For in situ treatment, it must be decided whether site adjustments or amendments of nutrients, oxygen, and/or microbes can result in transformation of selenium under the environmental conditions present. This approach requires a more thorough site characterization and bioassessment, and biotreatability testing often is more difficult. For ex situ application, the objective is to determine the optimum conditions for selenium and other target compound transformations. This testing can use reactor systems of various types, sizes, and configurations to allow microbes, nutrients, and oxygen concentrations to be controlled, monitored, and optimized more readily.

Both in situ and ex situ biotreatability testing require monitoring system performance to determine the rate and extent of metal/metalloid transformation and to predict the formation of undesirable intermediate products. Biotreatability tests do not have to determine or define the ultimate end points or finite end products, but they should define general rates and possible end products. This testing should also define key factors and parameters to monitor during remediation, (i.e., microbial populations, contaminant and nutrient concentrations, site conditions of pH, redox, dissolved oxygen).

VI. BIOAUGMENTATION AND BIOSTIMULATION

Whenever bioremediation is applied to a specific site, the question arises as to whether the bioremediation design should employ microorganisms indigenous to the site or to introduce new microbes. Biostimulation employs methods to enhance and use indigenous microbes, while bioaugmentation introduces new microbes with needed biotransformation capabilities. While both approaches have merits, generally indigenous microbes have an advantage for in situ application, and introduced species often outperform native microbes in bioreactors and some land application treatments. Indigenous microorganisms often are acclimated to the contaminated site, cocontaminants, and environmental conditions, while introduced species often are not. Careful consideration should be given to which approach is best for each contaminant mix and set of site characteristics. The evaluation should assess contaminant degradation or transformation by an indigenous microbial community or by several introduced microbes acting in concert.

A promising approach, taken by these authors and others, is development of microbial cultures for a specific site through microbial coculture under actual site or bioreactor treatment conditions. Coculture, in this instance, refers to

developing a new culture by culturing site microbes and known selenium-reducing microbes together in the system waters or soil slurries of interest. Often, relatively short culture times produce a microbe or a community of microorganisms with desirable properties for the site of interest. Another promising approach is use of adapted microbes or microbial populations isolated from similar selenium-contaminated sites. However, the increased use of bioremediation may possibly result in requirements for testing and registration of acclimated or enhanced microbes and microbial populations.

VII. SELENIUM-REDUCING MICROORGANISMS

Selenium-reducing microorganisms, used in experiments described herein, were isolated from selenium-contaminated mining process and waste waters and agricultural drainage(s) throughout the western United States. They were tested in their native state and subjected to a series of adaptation, acclimation, and culture enhancement methods. These microbes were again reisolated and characterized using standard microbial tests, biochemical tests, and fatty-acid-based microbial identification (Microbial ID, Inc.). Various microorganisms, including *Alcaligenes xylosoxydans*, other *Alcaligenes spp.*, *Escherichia coli*, *Enterobacter* spp., and *Pseudomonas* spp., isolates, were obtained from agricultural drainage waters. *Hydrogenophaga pseudoflava*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas stutzeri*, *Pseudomonas putida*, Pseudomonas spp., *Bacillus* spp., *Cellulomonas flaginia*, and numerous other microbes were isolated from mining waters.

VIII. SELENIUM ANALYSIS

Selenium analysis was conducted on filter-sterilized (Nalgene 0.20 μm) culture supernatant. Samples were stored at 4°C until analyzed. Total selenium and selenite were determined using a hydride generation atomic absorption spectrophotometry procedure (Manning, 1971). Selenite was determined directly by hydride generation. Total selenium was determined by oxidizing all selenium in the sample to selenate in a potassium persulfate–nitric acid digestion, reduction to selenite with HCl, and determination of selenite as total selenium by hydride generation. Selenate was calculated as the difference between total selenium and selenite.

IX. MINING WATERS

Mining waters are generally atypical, varying greatly in types of contaminant, contaminant levels, pH, ionic content, and so on. This makes bioremediation of

mining solutions a site-specific task. Table 1 shows examples of several selenium-contaminated mining waters used in some of the selenium bioreduction experiments described. Cyanide is often present in the mining of heap leach process solutions. For most experiments, residual cyanide was destroyed by treatment with bleach and/or hydrogen peroxide before treatment to remove selenium. Cyanide destruction is necessary because at concentrations above a few milligrams per liter it either kills selenium-reducing bacteria or severely inhibits bacterial growth and selenium reduction.

X. NUTRIENT SELECTION

Figure 1, a comparison of recent and past tests, shows selenium removal by different microbes, nutrients, and aeration levels in a mine water containing 0.62 mg/L selenium at pH 7.96. Tests used 250 mL Erlenmyer flasks containing 50 mL of filter-sterilized wastewater and a 10-fold dilution of log phase microbial cells (2.0×10^8 cells/mL) as an inoculum. Optimum aeration levels depend on the microorganism, the mining water, and the nutrient supplement. Under conditions of aeration (shaker flasks, 125 rpm), peptone–glycerol–yeast (PGY) and peptone supplements with *Ps. stutzeri* and *A. xylosoxydans* resulted in significantly lower selenium levels than did ammonium sulfate and dextrose supple-

TABLE 1 Examples of Selected Contaminant Concentrations in Actual Mine Waters, 1997

	Mine water contaminant concentration (mg/L)[a]					
Contaminant	S-1	S-4	G-4	G-5	U-2	U-3
Arsenic	3.5	<1.0	1.5	5.7	<1.0	<1.0
Copper	2.0	3.0	35.7	0.67	<1.0	1.6
Cyanide	120.0	87.0	159.7	102.0	0	0
Iron	1.31	0.25	13.0	7.90	1.37	3.23
Mercury	<1.0	<1.0	3.7	1.7	<1.0	<1.3
Nitrates	N/A	1.4	54.1	258.0	<1.0	27.8
Selenium	0.60	14.0	15.9	31.1	1.58–4.21	.04
Sulfates	155.2	35.6	75.1	540	11.2	250
Uranium	0	0	0	0	10.1	3.5
Zinc	3.6	14.0	37	29.3	5.7	<2.9
pH	8.0	9.7	11.0	10.6	7.9	3.5

[a]Sample 1–6.
Source: Confidential.

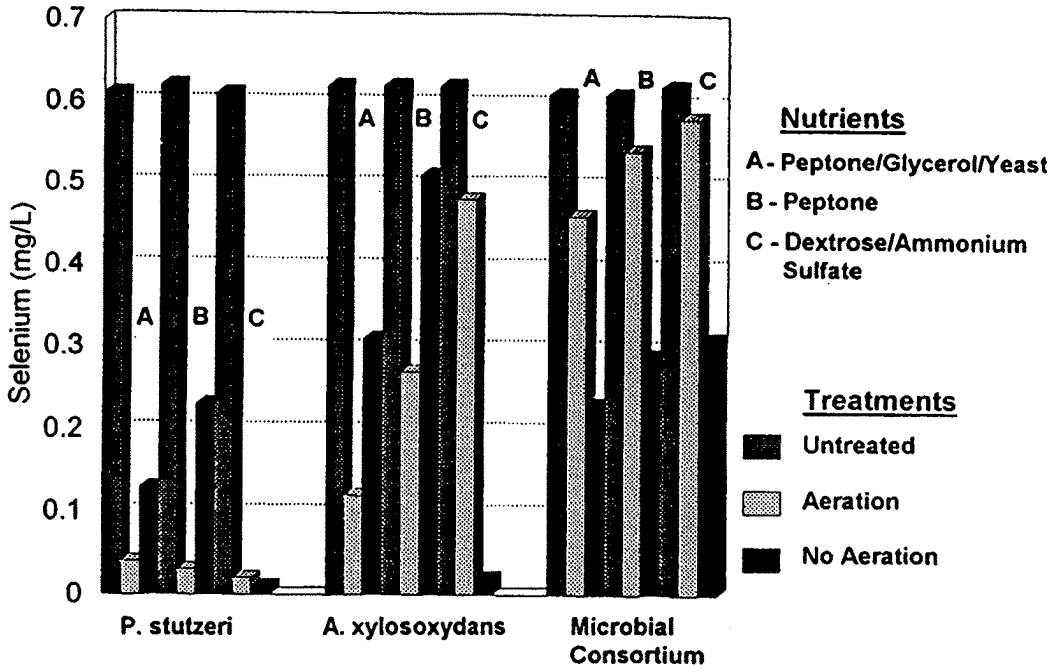


FIGURE 1 Selenium reduction by two microorganisms and a microbial consortium using different aeration treatments and nutrients. A, B, and C are the nutrient supplements tested under different aeration conditions.

ments. However, when oxygen levels were low (static flasks), the ammonium sulfate and dextrose supplement performed best. Selenium reductions to 0.02 mg/L or less were achieved with *Ps. stutzeri* and *A. xylosoxydans*. The microbial consortium, containing *A. xylosoxydans*, produced less dramatic results.

Additional quantitative assessments of selenate and selenite reduction by *Ps. stutzeri* and other isolates were performed in peptone-based media containing 10.0 mg/L selenium, followed by incubation at 24 °C with shaking at 125 rpm. Stoichiometric reductions of selenate and selenite were observed during late log and early stationary phases of cell growth. In related studies, Altringer et al. (1989), using mixed cultures of indigenous bacteria and precisely controlled mixtures of air and nitrogen, achieved 96% selenium reduction at bench scale.

Table 2 summarizes data obtained from studies comparing selenium reduction in the presence of mine waters 1 and 2, containing 1.48 and 6.2 mg/L selenate, respectively, different carbon sources, and microorganisms. *A. xylosoxydans* and the microbial consortium were indigenous to mining water 2, while *Ps. stutzeri* was not indigenous to either mining water. None of the organisms were indigenous to mining water 1. Cell growth and numbers were approximately equal when additions were made to the different carbon sources and mine water 1 at pH 7.9 and mine water 2 at pH 9.2. All microbes were used from cultures in log phase,

and microbial concentrations were at 2×10^8 after dilution. Three replicate samples were tested, without aeration, at 24°C, for 24 and 36 hours with the listed carbon source and additives. Selenium reduction by a particular microbe or consortia of microorganisms has distinct optimum and often different nutrient requirements.

Figure 2 shows selenium reduction by the microorganisms used in Figure 1 and Table 2. Dextrose and ammonium sulfate (1 g/L each) nutrients were added to mine water 1. All microbes were used from cultures in log phase, and microbial concentrations were approximately 2×10^8 after dilution. In cultures started with a 10-fold dilution of cells in late log phase, the reduction to elemental selenium was complete within 6 to 18 hours. Generally, with higher cell dilutions of 50- or 100-fold, reduction to elemental selenium required 24 to 36 and 36 to 72 hours, respectively.

Screening tests, using added selenium concentrations ranging from 10 to 10,000 mg/L, were conducted with various selenium-reducing bacteria. Reduced cell growth and cell yields were noticed with some unadapted selenium-reducing isolates at selenium concentrations of 100 mg/L or higher. Inhibition at lower selenium concentrations was not observed in the adapted *Ps. stutzeri* or the *A. xylosoxydans* isolates, but was noticed in a consortium containing *A. xylosoxydans*.

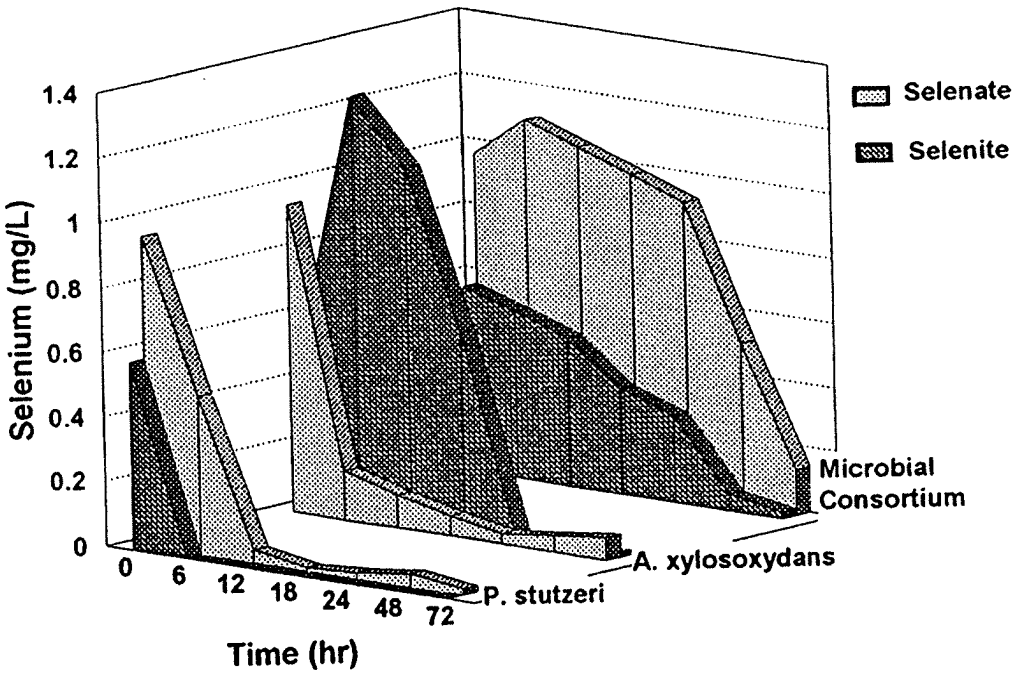


FIGURE 2 Selenium reduction in mine water 1 by two microorganisms and a microbial consortium using dextrose and ammonium sulfate. All tests were started using a 10-fold dilution of late log phase cells (approximately 2×10^8 cells/mL).

Most microorganisms were severely inhibited in both growth and selenium reduction at selenium concentrations of 1000 mg/L or less. One *Ps. stutzeri* isolate was only slightly inhibited in growth and selenium reduction at selenium concentrations of 1000 mg/L or less. In a similar study, Barnes et al. (1991) reported that selenium concentrations greater than 0.5 mg/L caused a significantly longer lag phase, slower growth rates, and lower cell yields compared to lower concentrations of selenate using a *Ps. stutzeri* isolate.

Testing also examined selenium reduction by various microorganisms at different temperatures. Selenium reduction by *Ps. stutzeri* was observed at temperatures of 25 to 35°C. Selenium reduction decreased approximately 60% at 20°C, compared to maximum selenium reduction observed at 30°C. However, selenium reduction by different *Ps. stutzeri* isolates varied up to 50% at 20°C. No temperature–nutrient relationship was observed. Barnes et al. (1991) suggested that the difference in selenium reduction at lower temperatures by a *Ps. stutzeri* isolate might indicate that proteins involved in selenium reduction are plasmid-encoded and/or temperature-sensitive.

XI. ANION INHIBITION OF SELENIUM REDUCTION

Table 3 shows results of the authors' recent screening tests conducted in a peptone medium (pH 7.6) to investigate inhibition of selenium reduction by nitrate, nitrite, sulfate, and sulfite. Test data for two microorganisms are presented. Various microorganisms were incubated in triplicate on a rotary shaker (125 rpm) at 25°C in flasks containing initial selenium concentrations of 50 mg/L. Selenate and selenite reduction by various microbes was inhibited to different degrees by the anions. Similar results are reported by Adams et al. (1993) and in tests with other *Ps. stutzeri* isolates (Barnes et al., 1991). Barnes et al. reported complete inhibition of selenate reduction at nitrate concentrations of 5 mM, and partial (75%) inhibition was observed at 1 mM nitrate. These researchers suggested that when nitrate levels approach that of the selenate present, cells use nitrate for respiration in preference to selenate. This conclusion is also supported by an in situ study of aquatic sediments conducted by Steinberg and Oremland (1990). However, Adams et al. (1993), observed that anion inhibition of *Ps. stutzeri* selenate or selenite reduction was isolate-dependent; only slight inhibition was observed with some isolates. Barnes et al. (1991) also reported no inhibition of selenate reduction by sulfur, an observation noted by the authors in testing *Ps. stutzeri* mining site isolates. This observation also is supported by Oremland et al. (1989) and by Steinberg and Oremland (1990). However, sulfite ions were reported to significantly reduce selenite reduction by one *Ps. stutzeri* isolate (Adams et al., 1993).

Table 2 Comparison of Microorganisms and Nutrients for Selenium Reduction in Two Mine Waters,[a] 1996

	Mine water 1												Mine water 2							
	Ps. stutzeri				*A. xylosoxydans*				Consortium				*Ps. stutzeri*				Consortium			
Carbon source	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Water									+	+			+	RR	+	R	+	+		
Dextrin	+	RR	+	R					+		w	w	+	RR	R	R	+		R	R
Glycogen	+	RR	+	R	+	+		w	+	R	w	w	+	RR	w	w	+	R	R	R
Tween 40	+	RR	+	R	+	+			+	R			+	RR	w	w	+	R	w	w
Tween 80	+	RR		RR							+		+	RR	R	R			R	w
Fructose									+	+							+	+		
Galactose	+	RR	+	RR					+	+	+		+	RR	+	RR	+	+	+	w
Glucose	+	R	+	+									w	R	+					
Inositol									+								+			
Lactose	+	R	w	R					+	+	+		+	R	w		+	+	R	R
Maltose	+	R	+	RR					+		+		+	R	w	R	+			
Mannitol									+		R						+	w	R	RR
Mannose																				

Sorbitol											+									
Sucrose																				
Methyl pyruvate	w	+		R	+	w			w	w			+	w	w		+	w	R	RR
Monomethyl succinate	+	+			+	+	+	+	+	R	+	+	w	w			+	R	R	R
Acetic acid	w	w	+	+	+	+		w	+	+			w	+			+	+		
Citric acid	+	R	+	R	+	+		w	+	+			+	R	w	w	+	w		
Formic acid		w		w						+				+		w	+	+		
α-Hydroxybutyric acid					w	w			w	w							w	+		
Lactic acid	+	+	+	R	+	R			+	w		w	+	+	R	RR	+	w	+	w
Malonic acid	w	+	+	R									w	w	w					
Propionic acid	w	w	+		+	+			w	+			w	w	+	+	+	+		
Alanine	w	R	+	R	+	R	w	R	+	R		R	+	R	+	R	+	R	w	w
Aspartic acid	+	RR	+	R	+	+	w	R	+	+	+	R	+	RR	w	w	w	w	w	w
Glutamic acid	+	RR	+	RR	+	R	+	R	+	R	+	+	+	RR	+	R	+	R	w	w
Glycerol	w	R	+	R									w	R	+	RR				

[a]A, normal saline (0.85%); B, normal saline (0.85%) plus 50 mg/L Se as sodium selenate; C, normal saline (0.85%) made with wastewater; D, normal saline (0.85%) made with wastewater plus 50 mg/L Se as sodium selenate. *Results key*: blank, no cell growth, no selenium reduction; w, light microbial growth/no reduction to elemental Se; +, good microbial growth/no reduction to elemental Se; R, low to moderate reduction to elemental Se; RR, moderate to high reduction to elemental Se.

TABLE 3 Microbial Selenium Reduction Inhibition Achieved by Various Anions, 1996

Microorganism	Selenium species	Anion	Concentration (M)	Decrease in Se reduction (%)
E. coli	SeO_3^{2-}	SO_4	10^{-2}	6
		NO_3	10^{-2}	37
		NO_2	10^{-2}	43
		SO_3	10^{-3}	45
E. coli	SeO_4^{2-}	SO_4	10^{-2}	8
		NO_3	10^{-2}	17
		NO_2	10^{-2}	22
		SO_3	10^{-3}	67
Ps. stutzeri	SeO_3^{2-}	SO_4	10^{-2}	1
		NO_3	10^{-2}	9
		NO_2	10^{-2}	5
		SO_3	10^{-3}	85
Ps. stutzeri	SeO_4^{2-}	SO_4	10^{-2}	3
		NO_3	10^{-2}	1
		NO_2	10^{-2}	3
		SO_3	10^{-3}	10

XII. ENHANCED/IMMOBILIZED MICROBIAL AND CELL-FREE BIOREACTORS

Without the requisite bioassessment and biotreatability studies, both in situ and ex situ selenium reduction processes can be quickly overshadowed by inadvertent stimulation of indigenous bacteria. Normal biofilms developed for selenium reduction and removal can be quickly overgrown as the bioreactor system is exposed to waters containing indigenous microbes and renewed nutrients. Overgrowth of the selenium-reducing population in a bioreactor can be delayed by optimizing the bioreactor and nutrient selection for the chosen selenium reducers. However, once nutrients have been added, time, nutrients, and indigenous microbes slowly erode the biofilm, and consequently the selenium-reducing capability. Selenium-reducing capability can be extended by several practices: encapsulation of the selenium-reducing microbes, use of selenium-reducing enzymes and/or cell-free preparations, and by more permanent immobilization of the biofilm on the support surface.

Enhanced biofilms of *Ps. stutzeri* have been developed on activated carbon by pretreatment of the carbon with selected nutrients and biopolymers. Biofilms established in such a manner have supported single microbes and microbial consortia for selenium reduction for periods up to 9 months. Figure 3 compares

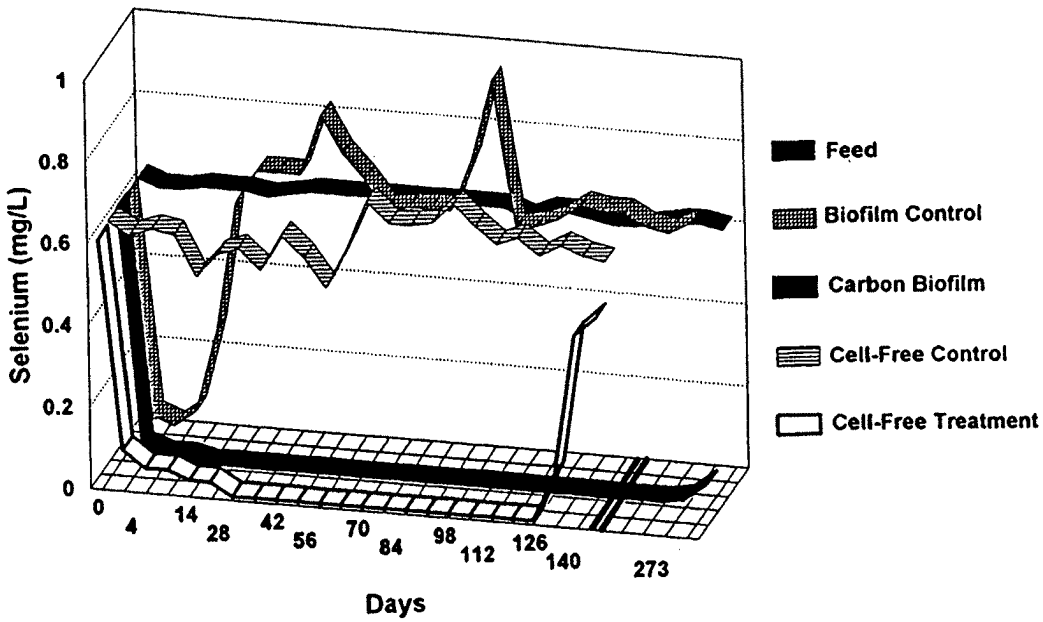


FIGURE 3 Selenium reduction by an enhanced/immobilized biofilm on activated carbon and a calcium alginate immobilized, cell-free preparation. Tests used a single-pass bioreactor with a retention time of 18 hours. Mine water feed, sterilized carbon, and empty calcium alginate bead controls are shown.

an immobilized cell-free preparation with an enhanced/immobilized carbon biofilm of *Ps. stutzeri* using a mine water containing 0.62 mg/L selenium as selenate. Tests used a single-pass bioreactor with a retention time of 18 hours. Cell-free extracts were produced by disrupting the bacterial cells and immobilizing the lysate in calcium alginate beads (Adams et al., 1993, 1996a). The immobilized enzyme preparation performed for approximately 4 months, removing selenium to below 0.01 mg/L. The enhanced carbon biofilm preparation removed selenium to below 0.01 mg/L for 9 months without supplemental nutrients.

Cell-free systems hold considerable potential for the removal of various inorganics and metal/metalloid contaminants. As mentioned earlier, cyanide is often a cocontaminant with selenium in heap leach mining process solutions and severely inhibits selenium reduction. Figure 4 shows test results from cell-free preparations of *Pseudomonas pseudoalcaligenes*, *Pseudomonas stutzeri*, and cyanide-oxidizing and selenium-reducing microorganisms combined and immobilized in calcium alginate beads. Tests were conducted in 1 in. diameter columns operated in single-pass, upflow mode with a retention time of 9 to 18 hours, at ambient temperature (25°C), using a process solution containing 102 mg/L cyanide and 31.1 mg/L selenium, at pH 10.6. Simultaneous cyanide removal and selenium reduction were demonstrated for 2 weeks (process water limitation). Cyanide

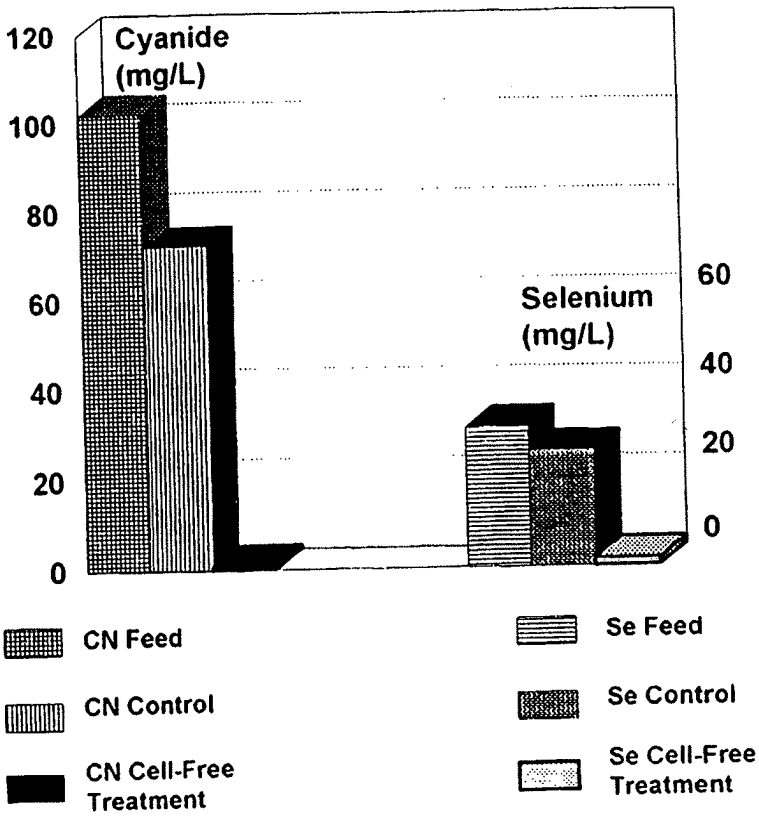


FIGURE 4 Simultaneous cyanide oxidation and selenium reduction by cell-free preparations immobilized in calcium alginate beads. Tests used a single-pass bioreactor (retention time, 9–18 h). Process solution cyanide and selenium concentrations and empty calcium alginate bead controls are shown.

levels decreased from 102 mg/L to below 1 mg/L, and selenium concentrations decreased from 31.1 mg/L to 1.6 mg/L.

XIII. SCANNING ELECTRON MICROSCOPY ANALYSIS OF SELENIUM PRECIPITATES

Depending on the mining water used and the oxygen content of the water, either red amorphous or dark brown to black crystalline selenium precipitates are formed. However, the dark brown to black crystalline selenium precipitates have been noticed only in systems with low dissolved oxygen. Preliminary analysis of the selenium precipitates by scanning electron microscopy (SEM) indicates a relatively pure selenium by-product from bioreactors of both types (i.e., those using live cells immobilized in calcium alginate and those using cell-free prepara-

tions). SEM scans similar to the one in Figure 5 were obtained from both immobilized live cells and immobilized cell-free preparations of *Ps. pseudoalcaligenes* and *Ps. stutzeri* used to treat complex mining waters containing uranium, copper, arsenic, and other metals. Selenium was the predominant metal/metalloid detected. Silicon is from the glass slide mount, and gold was deposited on the sample through the sputter-coating process. Calcium present in the sample is from the immobilization polymer. These preliminary data indicate the selectivity of the microbes examined for selenium reduction in complex waters. Production and recovery of a high purity selenium product from wastewaters could partially offset treatment costs.

XIV. CONCLUSIONS

Selenium contamination is a widespread environmental problem throughout the western United States and elsewhere. Although selenium is naturally occurring, anthropogenic activities (mining and agriculture) have greatly contributed to the

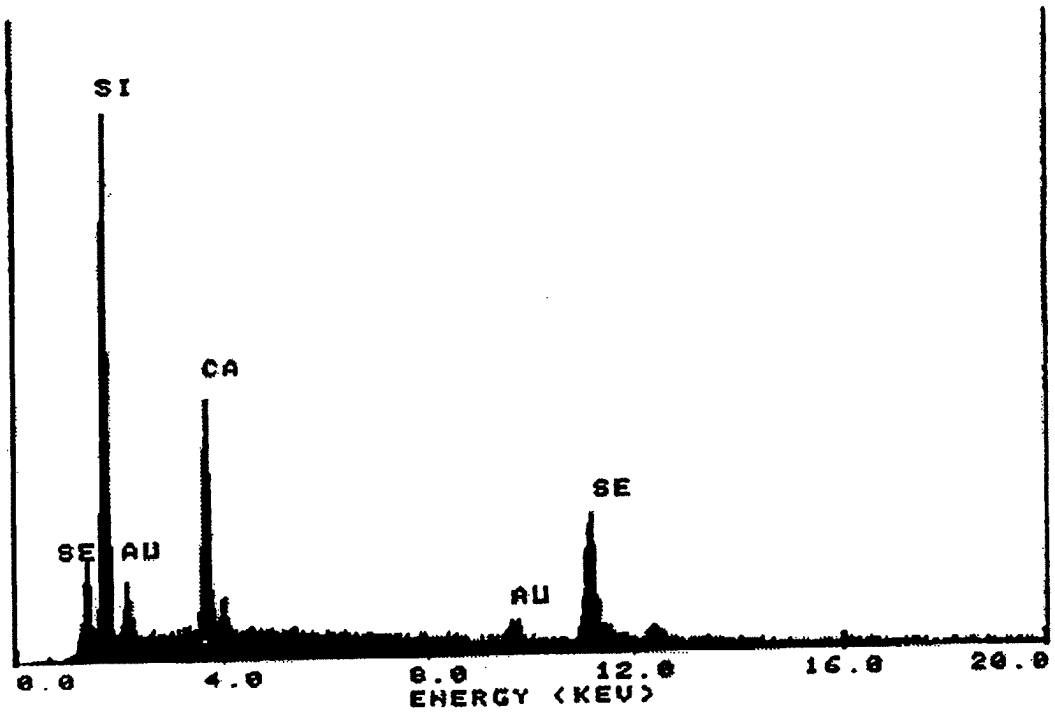


FIGURE 5 SEM analysis of microbial selenium reduction precipitate formed in calcium alginate beads when treating a mining water containing 15.7 mg/L selenium.

problem. Economical off-the-shelf technologies are not available for large-volume treatment of selenium-contaminated waters.

Conventional physical–chemical treatment technologies are generally non-specific, and too costly for the complete treatment of large-volume, complex waters, such as are produced by the mining industry. Selenium bioremediation processes have the potential for providing a low cost treatment and have been demonstrated in laboratory and field tests by the Center for Bioremediation. Based on these tests, estimated treatment costs range from $0.10/1000 gallons using only agricultural grade nutrients, to $1.50 ± $0.50/1000 gallons for immobilized or encapsulated cells for difficult-to-treat situations.

Site- and water-specific characterization, bioassessment, and biotreatability analyses are required for successful biotreatment field application. Selenium-reducing microorganisms, or microbial consortia, have distinct requirements dictated by each specific site or water to be treated. Additional considerations, such as interfering ions, redox conditions, temperature, and overgrowth by indigenous microorganisms, must also be addressed. Enhanced/Immobilized live cell and cell-free bioreactors were found to be effective for selenium removal from actual wastewaters for extended periods.

Cell-free systems show promise for the treatment of complex waters and waters with cocontaminants toxic to living systems. Immobilized, cell-free preparations were used to simultaneously remove selenium and cyanide from an actual process solution. Advantages of cell-free systems over live systems include potential for greatly increased kinetics, absence of a requirement for nutrients, and elimination of the effects of toxic process solutions. Cell-free bioreactors can be engineered to be resistant to microbial overgrowth and degradation.

These and other passive biotreatments, such as biomethylation of selenium in soil and water systems, show great potential. Field testing of these biotechnologies, to demonstrate cost-effective treatment of large water volumes to meet discharge or drinking water criteria, will validate this potential.

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DISSIMILATORY METAL REDUCTION[1]

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KEY WORDS: iron, manganese, uranium, selenium, chromium

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ABSTRACT

Microorganisms can enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation. Some microorganisms can conserve energy to support growth by coupling the oxidation of simple organic acids and alcohols, H_2, or aromatic compounds to the reduction of Fe(III) or Mn(IV). This dissimilatory Fe(III) and Mn(IV) reduction influences the organic as well as the inorganic geochemistry of anaerobic aquatic sediments and ground water. Microorganisms that use U(VI) as a terminal electron acceptor play an important role in uranium geochemistry and may be a useful tool for removing uranium from contaminated environments. Se(VI) serves as a terminal electron acceptor to support anaerobic growth of some microorganisms. Reduction of Se(VI) to Se(0) is an important mechanism for the precipitation of selenium from contaminated waters. Enzymatic reduction of Cr(VI) to the less mobile and less toxic Cr(III), and reduction of soluble Hg(II) to volatile Hg(0) may affect the fate of these compounds in the environment and might be used as a remediation strategy. Microorganisms can also enzymatically reduce other metals such as technetium, vanadium, molybdenum, gold, silver, and copper, but reduction of these metals has not been studied extensively.

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INTRODUCTION

Microorganisms that use metals as terminal electron acceptors, or reduce metals as a detoxification mechanism, have an important influence on the geochemistry of aquatic sediments, submerged soils, and the terrestrial subsurface. Furthermore, it is becoming increasingly apparent that microbial metal reduction may be manipulated to aid in the remediation of environments and waste streams contaminated with metals and certain organics. The purpose of this review is to give a brief overview of the physiology and ecology of microorganisms that reduce environmentally significant metals for non-assimilatory purposes.

Fe(III) REDUCTION

Microbial reduction of Fe(III) to Fe(II) has been studied not only because of its influence on iron geochemistry but also because Fe(III) is one of the most abundant potential electron acceptors for organic matter decomposition in many aquatic sediments and subsurface environments (71). Until recently, much of the Fe(III) reduction in sedimentary environments was generally considered to result from nonenzymatic reactions. However, we now know that in the anaerobic, nonsulfidogenic environments in which Fe(III) reduction

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is most important, dissimilatory Fe(III) reducers enzymatically catalyze nearly all of the Fe(III) reduction (71, 85).

Fe(III)-Reducing Microorganisms

In most sedimentary environments such as aquatic sediments, submerged soils, and aquifers, the oxidation of organic matter coupled to the reduction of Fe(III) requires the cooperative activity of several metabolic types of dissimilatory Fe(III) reducers (71) (Figure 1). For example, a great diversity of microorganisms that can metabolize sugars or amino acids with the reduction of Fe(III) have been described (32, 33, 36, 70). However, in all the cases examined, Fe(III) reduction is a trivial side reaction in the metabolism of these organisms (71). The primary products of the metabolism of the fermentative Fe(III)-reducing microorganisms are typical fermentation acids, alcohols, and H_2. Microorganisms that can completely oxidize sugars and amino acids to carbon dioxide with Fe(III) as the sole electron acceptor are unknown. Numerous attempts to isolate or enrich for such organisms have been unsuccessful (72). Even if microorganisms that completely oxidize glucose to carbon dioxide with Fe(III) as the sole electron acceptor exist, their

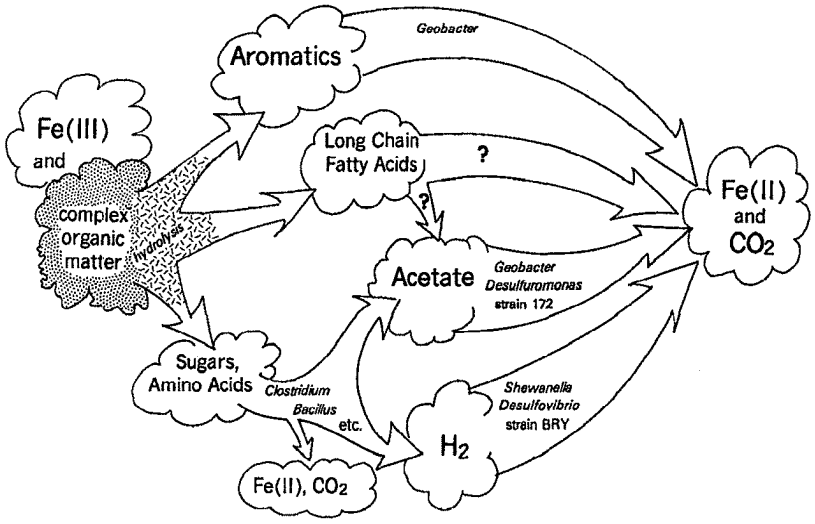


Figure 1 Model for oxidation of organic matter in sediments coupled to dissimilatory Fe(III) reduction showing examples of the microorganisms in pure culture known to catalyze the various reactions. Fermentation of sugars and amino acids has been simplified to designate the production of only the two major fermentation products, acetate and H_2. However, other short chain fatty acids are produced in lesser amounts. These include propionate and formate, which may be directly oxidized to carbon dioxide by organisms such as *Geobacter metallireducens* (propionate) or *Shewanella putrefaciens* (formate), as well as lactate, which is oxidized to acetate and carbon dioxide by organisms such as *S. putrefaciens*.

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metabolism does not appear to be important in Fe(III)-reducing sediments as glucose is fermented to fatty acids rather than oxidized directly to carbon dioxide (80). Thermodynamic considerations support this idea, suggesting that microorganisms attempting to completely oxidize glucose to carbon dioxide with Fe(III) would be at a competitive disadvantage with microorganisms that convert glucose to fermentation products (80). These findings have led to the hypothesis that, in Fe(III)-reducing environments, fermentative microorganisms metabolize sugars and amino acids with relatively little Fe(III) reduction during this initial step.

Most of the electron transfer to Fe(III) during the metabolism of sugars and amino acids results from the oxidation of the fermentation products (71). Acetate is considered to be the most important fermentation product in Fe(III)-reducing sedimentary environments (80). The freshwater isolate, *Geobacter metallireducens* (formerly strain GS-15) was the first known acetate-oxidizing Fe(III) reducer (76, 79, 86). *G. metallireducens* conserves energy to support growth by the reaction:

$$\text{acetate}^- + 8\ \text{Fe(III)} + 4\ H_2O \rightarrow 2\ HCO_3^- + 8\ \text{Fe(II)} + 9\ H^+.$$

G. metallireducens oxidizes various other volatile fatty acids and simple alcohols.

G. metallireducens is in the delta proteobacteria (76). Its closest known relative is *Desulfuromonas acetoxidans*. This organism was previously known primarily for its unique ability to couple the oxidation of acetate to the reduction of S^0. However, *D. acetoxidans* can also oxidize acetate with Fe(III) as the electron acceptor (116). This finding demonstrates that some marine organisms can also effectively couple the oxidation of organic compounds to Fe(III) reduction.

Another acetate-oxidizing Fe(III) reducer, strain 172, was recovered from deep subsurface sediments of an Atlantic Coastal Plain aquifer (75). Although a detailed characterization of 172 is not yet available, the preliminary evidence suggests that it is not a *Geobacter* or *Desulfuromonas* species.

Shewanella putrefaciens (formerly *Alteromonas putrefaciens*) can also conserve energy to support growth by coupling the oxidation of organic compounds to the reduction of Fe(III) (74, 84, 96). Formate is oxidized to carbon dioxide whereas lactate and pyruvate are incompletely oxidized to carbon dioxide and acetate:

$$\text{formate}^- + 2\ \text{Fe(III)} + H_2O \rightarrow HCO_3^- + 2\ \text{Fe(II)} + 2\ H^+;$$

$$\text{lactate}^- + 4\ \text{Fe(III)} + 2\ H_2O \rightarrow \text{acetate}^- + HCO_3^- + 4\ \text{Fe(II)} + 5\ H^+;$$

$$\text{pyruvate}^- + 2\ \text{Fe(III)} + 2\ H_2O \rightarrow \text{acetate}^- + HCO_3^- + 2\ \text{Fe(II)} + 3\ H^+.$$

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Formate oxidation coupled to Fe(III) reduction is a potentially important process in sediments if formate replaces H_2 as an important fermentation product in Fe(III)-reducing environments (84). However, the metabolism of lactate and pyruvate by *S. putrefaciens* is expected to be a minor pathway for carbon and electron flow in Fe(III)-reducing environments (71, 84).

The estuarine microorganism, strain BrY (22), and several *Desulfovibrio* species (D. R. Lovley, E. J. P. Phillips, J. C. Woodward & E. E. Roden, submitted) also incompletely oxidize lactate to acetate and carbon dioxide with Fe(III) as the electron acceptor. Like *S. putrefaciens,* BrY conserves energy to support growth from Fe(III) reduction. In contrast, there is no net growth during Fe(III) reduction by the *Desulfovibrio* species, but this metabolism may provide some energy.

Aquaspirillum magnetotacticum grows slowly and reduces Fe(III) in anaerobic medium that contains succinate as the sole electron donor (42). The authors of this study concluded that it is not clear whether *A. magnetotacticum* conserves energy from electron transport to Fe(III), and no data on succinate metabolism were provided.

Although the heterotrophic Fe(III)-reducing microorganisms discussed above grow best at circumneutral pH, numerous acidophilic heterotrophic bacteria reduce Fe(III) while growing in complex medium containing glucose or glycerol as well as tryptone (54). One isolate studied in detail reduces Fe(III) under both aerobic and anaerobic conditions. Evidence that energy is conserved from Fe(III) reduction is the finding that there is more cell growth as more Fe(III) is provided.

Several microorganisms can couple the oxidation of H_2 to the reduction of Fe(III). A *Pseudomonas* sp. (3), *S. putrefaciens* (84), and BrY (22) conserve energy to support growth via the reaction:

$$H_2 + 2\ \text{Fe(III)} \rightarrow 2\ H^+ + 2\ \text{Fe(II)}.$$

Data (71) did not substantiate claims that another organism could grow by coupling the oxidation of H_2 to the reduction of Fe(III) (55).

Several *Desulfovibrio* species also oxidize H_2 with the reduction of Fe(III) at rates comparable to those observed with other Fe(III) reducers, but no net cell growth occurs (D. R. Lovley, E. J. P. Phillips, J. C. Woodward & E. E. Roden, submitted). The minimum threshold for H_2 uptake in *D. desulfuricans* is lower with Fe(III) serving as the electron acceptor than with sulfate, suggesting that under conditions of limiting electron donor availability, *Desulfovibrio* species will preferentially reduce Fe(III).

A wide variety of monoaromatic compounds can be completely oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor (68, 85). *G. metallireducens* is the only aromatic-oxidizing, Fe(III)-reducing microor-

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ganism as yet available in pure culture (73, 77). Aromatics oxidized by *G. metallireducens* include prevalent contaminants such as toluene, *p*-cresol, and phenol. The metabolism of *G. metallireducens* serves as a model for the oxidation of aromatic contaminants coupled to Fe(III) reduction frequently observed in polluted aquifers (73, 77).

Long-chain fatty acids are likely another important component of organic matter that is metabolized in sediments. Enrichment cultures that can oxidize long-chain fatty acids have previously been established (71), but no isolates have been purified and the pathways for oxidation of long-chain fatty acids in Fe(III)-reducing environments have not been elucidated.

In acidic environments, elemental sulfur can serve as an electron donor for Fe(III) reduction. *Thiobacillus ferrooxidans, Thiobacillus thiooxidans,* and the thermophile, *Sulfolobus acidocaldarius,* reduce Fe(III) by the following reaction (19):

$$S^0 + 6\ Fe(III) + 4\ H_2O \rightarrow HSO^-_4 + 6\ Fe(II) + 7\ H^+.$$

Initial studies indicated that neither *T. thiooxidans* nor *T. ferrooxidans* (58, 118, 130) could conserve energy to support growth from this reaction. However, subsequent studies have demonstrated that Fe(III) reduction provides energy to support amino acid transport (111) and growth (110) in *T. ferrooxidans*. Microorganisms that oxidize sulfur with the reduction of Fe(III) at circumneutral pH have not been reported.

In addition, *S. acidocaldarius* provides evidence for the existence of thermophilic Fe(III) reducers. The recovery of large quantities of ultrafine-grained magnetite from depths as great as 6.7 km below the land surface presumably indicates the presence of Fe(III)-reducing life in such environments (37).

Electron Transport to Fe(III)

Investigations into electron transport to Fe(III) in dissimilatory Fe(III) reducers are important not only for a better understanding of the mechanisms for Fe(III) reduction but also because of the potential evolutionary significance of Fe(III) reduction. The geological evidence suggests that microbial Fe(III) reduction may have evolved before other respiratory processes, such as sulfate reduction, nitrate reduction, and oxygen reduction, which can also completely oxidize multicarbon organic compounds back to carbon dioxide (71).

Much of the early work on electron transport to Fe(III) conducted with organisms grown under aerobic conditions was recently reviewed (71). The relevance of these studies to anaerobic dissimilatory Fe(III) reduction has been questioned because aerobically grown cells produce Fe(III) reductases that are not linked to energy conservation (71).

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The three strains of *S. putrefaciens* that grow on nonfermentable substrates under anaerobic conditions with Fe(III) as the sole electron acceptor (28, 84, 96) must produce Fe(III) reductases that are linked to energy conservation. When cell suspensions of anaerobically grown *S. putrefaciens* are provided with Fe(III), the pH of the external medium drops, suggesting that electron transport to Fe(III) is associated with proton translocation (98). Growth under anaerobic conditions stimulates the production of *c*-type cytochromes in the outer membrane of *S. putrefaciens* (95). If these cytochromes can reduce Fe(III), this suggests a mechanism by which *S. putrefaciens* might transfer electrons to insoluble Fe(III) oxides (95). Although the Fe(III) reductase in *S. putrefaciens* has yet to be identified, it appears to be a different enzyme than the nitrate reductase (28, 98).

G. metallireducens contains a membrane-bound Fe(III) reductase also distinct from the nitrate reductase (39), as well as several membrane-bound and soluble *c*-type cytochromes (76, 99; J. E. Champine & S. Goodwin, submitted), some of which appear to be involved in electron transport to Fe(III) and other metals (76). *c*-Type cytochromes may also serve as electron carriers during Fe(III) reduction in the closely related *D. acetoxidans* (116). Menaquinone and ferredoxin are other likely components of the electron transport chain to Fe(III) in *G. metallireducens* (76; J. E. Champine & S. Goodwin, submitted). *G. metallireducens* derives electrons for Fe(III) reduction by oxidizing acetate via the citric acid cycle (24).

Thiobacillus ferrooxidans contains several enzymes that can reduce soluble Fe(III) under acidic conditions and could be involved in dissimilatory Fe(III) reduction with reduced sulfur compounds as the electron donor. A periplasmic enzyme purified to an electrophoretically homogeneous state (127) catalyzes the oxidation of sulfide to sulfite with Fe(III) as the electron acceptor (125). Another Fe(III) reductase activity located in the plasma membrane oxidizes sulfite to sulfate (126).

The first enzyme capable of reducing insoluble Fe(III) oxide at circumneutral pH was purified from the dissimilatory Fe(III) reducer, *Desulfovibrio vulgaris* (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted). Attempts to find the U(VI) reductase in this organism (see below) indicated that the c_3 cytochrome was an active metal reductase. When a combination of hydrogenase and H_2 was used as a source for electrons for c_3, c_3 reduced a poorly crystalline Fe(III) oxide-producing soluble Fe(II) and the magnetic mineral magnetite. Electron transport via cytochrome c_3 may account for the ability of *D. vulgaris* and other *Desulfovibrio* species to reduce Fe(III) oxides.

Environmental Significance and Ecology of Fe(III) Reduction

Dissimilatory Fe(III) reduction has a greater overall environmental impact than microbial reduction of any other metal. Microbial Fe(III) reduction has

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been directly shown or implicated as an important process in the following phenomena: organic matter decomposition in a variety of freshwater, estuarine, and marine sediments; the decomposition of aromatic hydrocarbons in contaminated aquifers; the generation of undesirably high concentrations of dissolved iron in deep pristine aquifers; the oxidation of organic matter coupled to Fe(III) that resulted in the accumulation of magnetite in the Banded Iron Formations; the accumulation of magnetite around hydrocarbon seeps and formation of ultrafine-grained magnetite in aquatic sediments; the formation of other ferrous iron minerals such as siderite and vivianite; the control of the extent of methane formation in shallow freshwater environments; the release of phosphate and trace metals into water supplies; soil gleying; and corrosion of steel. A recent review examined the literature on this subject (71).

Although several microorganisms can now serve as models for the enzymatically catalyzed reduction of Fe(III), it is not clear that any of the organisms currently available in pure culture are the dominant Fe(III) reducers in sedimentary environments. For example, a study on the mechanisms for siderite ($FeCO_3$) concretion formation in salt marsh sediments found that organisms related to *G. metallireducens* or *S. putrefaciens* were not abundant in the zone of Fe(III) reduction (25a). The concretions were enriched with *Desulfovibrio*-type organisms that are now known to be active dissimilatory Fe(III) reducers (see above), though at the time they were not generally regarded as such. Further circumstantial evidence for the potential role of sulfate-reducing organisms in dissimilatory Fe(III) reduction is that sulfate reducers are abundant in the zones of deep aquifers of the Atlantic Coastal Plain in which Fe(III) reduction is the terminal electron-accepting process (25, 94). Sulfate reducers living for such long periods in these subsurface environments, in which no detectable sulfate reduction occurs, probably gain energy for survival from electron transport to Fe(III). Such findings emphasize the need for more intensive study of community structure of Fe(III)-reducing environments.

Mn(IV) REDUCTION

Mn(IV)-Reducing Microorganisms

Microbial reduction of Mn(IV) to Mn(II) greatly parallels dissimilatory Fe(III) reduction. Most of the microorganisms that reduce Mn(IV) reduce Fe(III) and vice versa. Furthermore, the fact that Fe(II) rapidly reduces Mn(IV) means that, in most environments, any microorganism that can enzymatically reduce Fe(III) will also indirectly reduce Mn(IV) (78, 97). As with Fe(III), much of the early work on dissimilatory Mn(IV) reduction was primarily conducted with microorganisms that only used Mn(IV) as a minor electron acceptor

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during metabolism. This early literature was reviewed previously (31, 32, 36).

In general, Fe(III)/Mn(IV) reducers oxidize the same electron donors with Mn(IV) as they do with Fe(III). The exception is the H_2-oxidizing, Fe(III)-reducing *Pseudomonas* sp., which cannot oxidize H_2 with Mn(IV) (3). All of the other well-studied microorganisms that can conserve energy from dissimilatory Fe(III) reduction such as *G. metallireducens* (79), *S. putrefaciens* (84, 96), strain BrY (22), and *D. acetoxidans* (116) can also grow with Mn(IV) as the sole electron acceptor. However, in most instances, studies on electron donor metabolism with Mn(IV) as the electron acceptor have not been as extensive as studies with Fe(III). For example, although *G. metallireducens* can reduce Mn(IV) when benzoate is provided as an electron donor (D. R. Lovley, unpublished data) the oxidation of aromatic compounds coupled to Mn(IV) reduction in this or other organisms has not been studied in detail. It seems likely, however, that organic matter could be oxidized to carbon dioxide with the reduction of Mn(IV) in a manner similar to that proposed in Figure 1 for Fe(III) reduction.

Electron Transport to Mn(IV)

Electron transport systems to Mn(IV) have been investigated in several *Bacillus* species that reduce Mn(IV) (27, 31, 36). However, the available evidence suggests that this Mn(IV) reduction is a minor side reaction that does not conserve energy to support growth for these organisms, and thus this metabolism may have little relevance to the bulk of dissimilatory Mn(IV) reduction in sedimentary environments (71).

Almost nothing is known about electron transport to Mn(IV) in organisms that conserve energy to support growth from Mn(IV) reduction. Growth of *G. metallireducens* (79), *S. putrefaciens* (84, 96), strain BrY (22), and *D. acetoxidans* (116) on nonfermentable substrates with Mn(IV) as the electron acceptor implies energy conservation through electron transport to a Mn(IV) reductase. Evidence of the potential for energy conservation as the result of electron transport to Mn(IV) was the finding that cell suspensions of *S. putrefaciens* translocated protons to the external medium when provided with lactate and Mn(IV) (98). The *c*-type cytochrome(s) in *D. acetoxidans* are oxidized by Mn(IV), suggesting that they are involved in electron transport to Mn(IV) (116).

Environmental Significance of Mn(IV) Reduction

The environmental significance of enzymatic mechanisms for Mn(IV) reduction is less clear than for Fe(III) reduction. This is because Mn(IV) is more easily reduced through nonenzymatic mechanisms than is Fe(III) (71). Most notably, no practical method has been devised for differentiating between

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enzymatic reduction of Mn(IV) and nonenzymatic reduction of Mn(IV) by Fe(II). However, the distinction in this case may not be meaningful because the enzymatic reduction of Fe(III) oxide followed by nonenzymatic reduction of Mn(IV) by Fe(II) results in the same end products, Mn(II) and Fe(III) oxide, as does direct enzymatic reduction of Mn(IV) (78).

In analogy to Fe(III) reduction, microbial Mn(IV) reduction has the potential to be important in the formation of reduced manganese minerals, the release of dissolved manganese into sediment pore and ground waters, and the release of trace metals bound to Mn(IV) oxides (71). Mn(IV) reduction may also serve to oxidize organic matter in aquatic sediments, deep pristine aquifers, and shallow aquifers contaminated with organics, but quantitative data are scarce (71).

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U(VI) REDUCTION

Microbial reduction of soluble U(VI) to insoluble U(IV) is an important process in the global uranium cycle and may also be a useful technique for removing uranium from contaminated environments (40, 81–83). Early evidence that microorganisms might reduce U(VI) to U(IV) was the finding that cell-free extracts of *Vellionella atypica* (formerly *Micrococcus lactilyticus*) reduce U(VI) along with a variety of other metals (141). However, there is no evidence that this metal reduction is an enzymatic reaction or has any physiological significance. Subsequent studies have demonstrated that whole cells of *V. atypica* do not reduce U(VI) (D. R. Lovley, unpublished data).

U(VI)-Reducing Microorganisms

G. metallireducens was the first organism found to use U(VI) as a terminal electron acceptor. *G. metallireducens* grows by carrying out the reaction:

$$\text{acetate}^- + 4\ \text{U(VI)} + 4\ H_2O \rightarrow 2HCO_3^- + 4\ \text{U(IV)} + 9\ H^+.$$

S. putrefaciens can also grow with U(VI) as the sole electron acceptor and H_2 as the electron donor:

$$H_2 + \text{U(VI)} \rightarrow 2\ H^+ + \text{U(IV)}.$$

Both organisms will grow in high-concentration (8 mM) dissolved uranium.

Several *Desulfovibrio* species can also enzymatically reduce U(VI) with either H_2 or lactate (82) (D. R. Lovley, unpublished data). However, attempts to grow *D. desulfuricans* with U(VI) as the sole electron acceptor have been unsuccessful (82).

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Electron Transport to U(VI)

The enzymatic mechanisms for U(VI) reduction by *G. metallireducens* and *S. putrefaciens* are ill defined. The fact that these organisms conserve energy to support growth by oxidizing nonfermentable substrates with U(VI) as the sole electron acceptor suggests that electron transport-linked phosphorylation must be involved. Electron transport to or through *c*-type cytochrome(s) is likely based on the observation that U(VI) oxidizes the *c*-type cytochromes in whole-cell suspensions of *G. metallireducens* (76).

A U(VI) reductase has been isolated from the U(VI) reducer, *D. vulgaris* (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted). The soluble fraction of *D. vulgaris* rapidly reduces U(VI) with H_2 as the electron donor. If cytochrome c_3 is removed from the soluble fraction of *D. vulgaris,* then all capacity for U(VI) reduction is lost. If cytochrome c_3 is added back, then the capacity for U(VI) reduction is restored. U(VI) rapidly oxidizes previously reduced cytochrome c_3. U(VI) is rapidly reduced when c_3 is combined with hydrogenase, the physiological electron donor for c_3, and H_2.

Environmental Significance of U(VI) Reduction

The environmental significance of microbial U(VI) reduction is that U(VI) is highly soluble in most natural waters, whereas U(IV) is highly insoluble (65). The reduction of U(VI) to U(IV) in anaerobic marine sediments is the most globally significant sink for dissolved uranium (1, 59, 136). The reductive precipitation of uranium from ground water is considered to be the mechanism for the formation of some sandstone or roll-type uranium ores (50, 53, 65). The earlier geochemical literature (50, 53, 65) suggested that U(VI) reduction in anaerobic environments results from nonenzymatic reduction of U(VI) reduction by sulfide or H_2. However, neither are effective U(VI) reductants at the temperatures and pH typical of most aquatic sediments or ground waters, and sterilization of anaerobic sediments inhibits U(VI) reduction (83). These findings suggest that U(VI)-reducing enzymes are responsible for U(VI) reduction in these environments.

Microbial U(VI) reduction may be used to remove uranium from contaminated waters and soils. In most natural surface and ground waters, U(VI) is in the form of uranyl-carbonate complexes (65). Furthermore, uranyl-carbonate complexes are the typical dissolved uranium form that results from various human activities utilizing uranium (81). Studies with *G. metallireducens* (40) and *D. desulfuricans* (82) demonstrated that U(VI)-reducing microorganisms can reduce the U(VI) in U(VI)-carbonate complexes. The U(IV) precipitates as uraninite (UO_2), all of which is extracellular. Thus, microbial uranium reduction can potentially take uranium that is dispersed in a large volume of liquid and concentrate it into a very pure, compact solid.

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D. desulfuricans was chosen for detailed studies of microbial removal of U(VI) from contaminated environments because of the ease in mass culturing this organism and because its U(VI)-reducing capacity is extremely stable. For example, freeze-dried cells kept under air at room temperature lose none of their potential for U(VI) reduction even after six months of storage (81). *D. desulfuricans* effectively reduces U(VI) both at very high (24 mM) and at relatively low (< 50 nM) concentrations (81). Of the wide variety of potentially inhibiting anions and cations evaluated, only exceptionally high concentrations (> 20 μM) of copper inhibited U(VI) reduction. *D. desulfuricans* readily removed soluble U(VI) from several mine drainage waters and contaminated ground waters from a Department of Energy site.

In addition to treating uranium-contaminated waters, microbial U(VI) reduction can be used as part of a technique to concentrate uranium from contaminated soils (D. R. Lovley, E. J. P. Phillips & E. R. Landa, in preparation). In this process, uranium is leached from the soils with a bicarbonate solution and then microbial U(VI) reduction precipitates the uranium from the extract.

Microbial U(VI) reduction has several advantages over other previously proposed treatment techniques (81). These include: the ability to precipitate uranium from U(VI)-carbonate complexes; the recovery of uranium in a highly concentrated and pure form; high uranium removal per amount of biomass; the potential to simultaneously treat organic contaminants and uranium by using the organic as an electron donor for U(VI) reduction; and the potential for in situ remediation of both ground and surface waters.

Se(VI), Se(IV), AND Se(0) REDUCTION

Although selenium is classified a metalloid rather than a true metal, it is included here because of the obvious parallels between dissimilatory selenium reduction and dissimilatory metal reduction and because of its environmental significance. The discovery that toxic concentrations of selenium were adversely affecting bird populations at the Kesterson National Wildlife Refuge in California led to a surge in research on microbial metabolism of selenium. The predominant redox states of selenium in natural environments are Se(VI) (selenate, SeO_4^{-2}), Se(IV) (selenite, SeO_3^{-2}), Se(0) (elemental selenium), and Se(−II) (selenide) (29). The first three of these can potentially serve as electron acceptors for microbial metabolism.

Se(VI)-, Se(IV)-, and Se(0)-Reducing Microorganisms

A high percentage of the heterotrophic microorganisms that can be isolated from soil can reduce selenate to elemental selenium (13). *Clostridium* (56), *Citrobacter, Flavobacterium,* and *Pseudomonas* species (21) as well as an

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unidentified gram-negative rod (89) all reduce selenate. However, the mechanisms for selenate reduction in these organisms were not studied in detail and no evidence of selenate-dependent growth was provided.

Several organisms that can conserve energy to support growth via selenate reduction have been described. Strain SeS was purified from intertidal sediments of San Francisco Bay (105). SeS obtains energy to support growth by the reaction:

$$4\ CH_3COO^- + 3\ SeO_4^{-2} \rightarrow 3\ Se^0 + 8\ CO_2 + 4\ H_2O + 4\ H^+.$$

Pseudomonas sp. AX, which was isolated from biological reactors used for selenium removal (88) has a different mode of selenate reduction, reducing selenate to selenite:

$$CH_3COO^- + H^+ + 4\ SeO_4^{-2} \rightarrow 2\ CO_2 + 4\ SeO_3^{-2} + 2\ H_2O.$$

When *Pseudomonas* sp. AX is cocultured with a selenite-reducing microorganism, selenate is rapidly reduced to elemental selenium (88). Another selenate-reducing microorganism, designated SeS-3, grows in defined medium with lactate as the electron donor and selenate as the electron acceptor (104, 122). Selenate is reduced to selenite and elemental selenium.

The ability of microorganisms to reduce selenite to elemental selenium has been known since the turn of the century (67 and references therein) and a wide range of microorganisms are known to catalyze this reaction (29, 104). This metabolism has never been found to be associated with energy conservation and may be a detoxification mechanism (29, 104). Cell suspensions of *T. ferrooxidans* reduce red elemental selenium to selenide (2).

Environmental Significance and Ecology of Selenium Reduction

Selenate and selenite reduction to insoluble elemental selenium has been documented in a variety of soils and aquatic sediments, and the evidence suggests that this reduction is enzymatically catalyzed (30, 89, 105, 123). Because of the low concentrations of selenate and selenite in the environment, they are not important electron acceptors for organic matter oxidation (105). However, microbial selenate and selenite reduction play a central role in affecting the fate of selenium in aquatic environments and may be the principle mechanism for the removal of selenate and selenite from agricultural wastewater evaporation ponds and high-selenium ground waters (104, 106, 107).

Various lines of evidence indicate that sulfate-reducing microorganisms are not responsible for selenate reduction in sediments. Selenate and selenite are

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reduced at shallower depths than the zone of sulfate reduction (105, 106). At the depths where selenate is reduced, the sulfate concentrations are at levels that inhibit selenate reduction by sulfate reducers (143). Furthermore, molybdate, a specific inhibitor of sulfate reduction, did not inhibit selenate reduction (105).

Nitrate and Mn(IV) are preferred electron acceptors for selenate reducers (105, 122). Selenate reduction might be catalyzed by enzyme(s) that are also involved in dissimilatory nitrate reduction (104). The distribution of the maximum potential for denitrification and selenate reduction potentials in sediments are similar (106, 123), and the inhibition of selenate reduction in some sediments by tungstate but not molybdate might indicate the involvement of a molybdenum-containing nitrate reductase (105, 123). However, nitrate does not always inhibit selenate reduction at the low concentrations found in pore waters (106).

The immobilization of selenium that results when selenate is reduced to elemental selenium may be exploited to enhance removal of selenate from contaminated waters (89). For example, at Kesterson, flooding of previously exposed pond sediments with selenium-free water immobilized 66–110% of the dissolved selenium that had been present in the upper 1.22 m of soil (69). Immobilization is thought to result from the development of anaerobic conditions, which should stimulate selenate reduction. However, in situ stimulation of selenate reduction may not be a suitable long-term solution to the selenium-contamination problem as elemental selenium may enter the food chain through the bottom-feeding organisms (87).

Ex situ treatment processes for contaminated waters are also possible. Selenate is microbially reduced to insoluble elemental selenium when uranium-mine discharge water is passed through a soil column (56). Several investigators have suggested using combined algal-bacterial systems to remove selenium from contaminated waters (35, 106). Nitrate, which inhibits selenate reduction, is removed via the growth of algae. The water thus treated is then fed into an anaerobic digestor where a portion of the algae are oxidized and thereby become electron donors for removal of any remaining nitrate (through denitrification), and then selenate is reduced and precipitated as elemental selenium.

Cr(VI) REDUCTION

Chromium contamination of the environment is extensive (12). The reduction of highly toxic and mobile Cr(VI) to the less toxic, less mobile Cr(III) is likely to be a useful process for the remediation of contaminated waters and soils (108). This problem has stimulated interest in microorganisms that can use Cr(VI) as an electron acceptor.

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Cr(VI)-Reducing Microorganisms

Early investigations demonstrated that facultative anaerobes such as *Pseudomonas dechromaticans* (117), *Pseudomonas chromatophila* (66), and *Aeromonas dechromatica* (64) remove Cr(VI) from solution by the formation of a Cr(III) precipitate, presumably $Cr(OH)_3$. Subsequent studies have demonstrated that the capacity for Cr(VI) reduction is widespread and found in such organisms as *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter eurydice, Micrococcus roseus,* and *Escherichia coli* (43), as well as *Pseudomonas ambigua* (48), *Pseudomonas fluorescens* (17), *Enterobacter cloacae* (137), *Streptomyces* spp. (26), *Pseudomonas putida* (52), *D. desulfuricans,* and *D. vulgaris* (D. R. Lovley & E. J. P. Phillips, in preparation).

Many of these organisms reduce Cr(VI) better under aerobic conditions than under anaerobic conditions and the physiological role of this aerobic Cr(VI) reduction has not been well defined. Cr(VI) reduction is not a Cr(VI)-resistance mechanism (17), and Cr(VI) reduction in *P. putida* and possibly other Cr(VI) reducers is a side activity for enzymes that have other, as yet unidentified, natural substrates (23, 52).

Although some microorganisms reduce Cr(VI) during anaerobic growth in media in which Cr(VI) is provided as the sole electron acceptor, in no instance has Cr(VI) reduction definitely been shown to yield energy to support anaerobic growth. For example, *Pseudomonas chromatophila* purportedly uses Cr(VI) as an electron acceptor to support growth under anaerobic conditions with a variety of electron acceptors, including the nonfermentable substrate, acetate (66). However, in the only data shown, most of the Cr(VI) reduction took place after growth stopped. No studies demonstrating that anaerobic growth depended upon the presence of Cr(VI) were presented.

Pseudomonas fluorescens LB300, which reduces Cr(VI) while growing aerobically in a glucose medium, also grows in an anaerobic chamber containing oxygen-free N_2 on agar plates containing acetate as a potential electron donor and Cr(VI) as the potential electron acceptor (17). However, no Cr(VI) reduction occurs in anaerobic liquid cultures. Neither acetate oxidation nor Cr(VI) reduction under anaerobic conditions was documented. Further studies on whether electron transport to Cr(VI) can yield energy to support growth of this organism seem warranted given the difficulties in maintaining oxygen-free conditions in anaerobic chambers under a N_2 atmosphere.

Enterobacter cloacae strain HO1 reduces Cr(VI) while growing anaerobically in a medium that contains acetate and Casamino acids as potential electron donors (137). O_2 rapidly inhibits Cr(VI) reduction, but reduction

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resumes when O_2 is removed (62). Amino acid mixtures are the best electron donors for Cr(VI) reduction (102). The fate of the electron donors (i.e. whether they are oxidized to carbon dioxide or whether they are fermented) has not been reported. *E. cloacae* can grow anaerobically in the absence of added Cr(VI) and no evidence for Cr(VI)-dependent growth has been presented (137). In fact, in one study, the number of viable cells decreased in the initial stages after Cr(VI) was added, and ~50% of the added Cr(VI) was reduced before viable cell numbers increased over what was present prior to Cr(VI) addition (100).

Enzymatic Mechanisms for Cr(VI) Reduction

Cr(VI) reduction is an enzymatically catalyzed reaction in the Cr(VI) reducers that have been studied in detail. For example, spent medium from cultures of *P. fluorescens* LB300 does not reduce Cr(VI), and washed cell suspensions reduce Cr(VI) only if glucose or another suitable electron donor is provided (17). Cyanide (10^{-2} M) or azide (10^{-3} M) inhibit Cr(VI) reduction in crude cell extracts, and the Cr(VI)-reducing capacity is lost when the membrane fraction is removed.

Evidence for an enzymatic role in Cr(VI) reduction in *E. cloacae* is that: Cr(VI) is reduced faster with higher cell densities; no Cr(VI) reduction occurs in cell-free controls; and inhibition of growth with antibiotics inhibits Cr(VI) reduction (137). Cell-free filtrates of cultures do not reduce Cr(VI) (138). Several metabolic poisons as well as molybdate, vanadate, and tellurate inhibit Cr(VI) reduction. Cr(VI) also inhibits Cr(VI) reduction—the rate of reduction declines as the concentration rises above 1 mM (100). Temperature and pH optima for Cr(VI) reduction are characteristic of an enzymatically catalyzed reaction (63).

The Cr(VI) reductase activity in *E. cloacae* is located in the membrane fraction (138). When membrane vesicles are reduced with NADH and then exposed to Cr(VI), the *c*- and *b*-type cytochromes are oxidized (139). Further analyses have suggested that of the identifiable cytochromes in the membrane vesicles (c_{548}, c_{549}, c_{550}, b_{555}, b_{556}, and b_{558}), c_{548} might be specifically involved in Cr(VI) reduction, serving as a branch point between Cr(VI) and O_2 reduction.

In contrast to *P. fluorescens* and *E. cloacae*, the Cr(VI)-reducing activity in *P. ambigua* and *P. putida* is in the soluble fraction of the cell (48, 52). A 65-kDa protein has been purified from *P. ambigua* that can reduce Cr(VI) with NADH or NADPH serving as the electron donor (134). The enzyme initially reduces Cr(VI) to Cr(V), which is subsequently reduced to Cr(III). NADH also reduces Cr(VI) to Cr(V) in the absence of the enzyme, but at slower rates than with the enzyme.

Washed cell suspensions of *D. desulfuricans* and *D. vulgaris* rapidly reduce

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Cr(VI) to Cr(III) under anaerobic conditions with H_2 as the electron donor (D. R. Lovley & E. J. P. Phillips, in preparation). H_2-dependent, Cr(VI) reductase activity in the soluble, cell-free fraction of *D. vulgaris* is lost when the soluble fraction is passed over a cation-exchange column that removes cytochrome c_3, a periplasmic protein. The capacity for Cr(VI) reduction is restored when cytochrome c_3 is added back. In the presence of H_2 and an excess of hydrogenase, cytochrome c_3 reduces Cr(VI) at a rate 50-fold faster than the maximum rate for Cr(VI) reduction by the Cr(VI) reductase purified from *P. ambigua*.

Environmental Significance of Cr(VI) Reduction

Most of the earth's chromium is tied up as insoluble Cr(III) (12). In unpolluted fresh and sea waters the concentrations of chromium are generally less than 50 nmol/liter (113). Therefore, microbial Cr(VI) reduction is of primary concern in contaminated environments or for the treatment of Cr(VI)-containing wastes. Cr(VI) is readily reduced to Cr(III) in anaerobic aquatic sediments, but nonenzymatic processes such as Cr(VI) reduction by Fe(II) are likely to be at least as important as enzymatic Cr(VI) reduction (91).

The most extensive studies on the potential use of Cr(VI)-reducing microorganisms for the removal of Cr(VI) from waste streams have been conducted with *E. cloacae* strain HO1. Early studies with this organism revealed that during Cr(VI) reduction the yellow medium turns white and turbid, presumably as the result of the formation of insoluble Cr(III) hydroxide (137). However, the Cr(III) does not readily precipitate and cannot be effectively removed with centrifugation (61). Cr(III) is removed more effectively if *E. cloacae* is placed on one side of a semipermeable membrane and the Cr(VI)-containing medium is on the other (61). Cr(VI) diffuses into culture and *E. cloacae* reduces the Cr(VI) to Cr(III), which is retained on the culture side, especially when an anion-exchange membrane that does not permit passage of Cr(III) is used (60). Despite successful removal of Cr(VI) from culture medium with *E. cloacae*, removal from industrial effluents has been problematic as heavy metals and sulfate in the effluents can inhibit Cr(VI) reduction (45, 101). Whether microbial Cr(VI) reduction would have advantages over simple chemical Cr(VI) reduction in the removal of Cr(VI) does not appear to have been evaluated.

Hg(II) REDUCTION

Many aerobic and facultative microorganisms reduce soluble ionic Hg(II) to volatile Hg(0) as a detoxification mechanism (20, 114, 132). The Hg(II) is reduced during aerobic growth and Hg(II) reduction is not linked to energy-conserving electron transport. In contrast to most types of dissimilatory metal

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reduction, about which very little is known, many of the mechanisms for Hg(II) reduction have been beautifully elucidated for both gram-negative and gram-positive bacteria. This includes understanding of the mechanisms for Hg(II) transport into the cell and the intracellular reduction of Hg(II), as well as mapping of the organization of the mercury-resistance (*mer*) operons and determination of the structure of the Hg(II) reductase. These topics have been reviewed frequently (for recent updates see 119, 121, 131 and references therein) and thus, because of space limitations, are not discussed further here. As recently suggested, the respiratory metal-reducing microorganism, *G. metallireducens,* may be able to reduce Hg(II) through its dissimilatory metal-reducing pathway (76).

Environmental Significance and Ecology of Microbial Hg(II) Reduction

Microbial reduction of Hg(II) may play an important role in the fate of mercury in both aquatic and terrestrial environments (11, 38). For example, microbial Hg(II) reduction in ocean waters has been proposed to be a significant source of atmospheric mercury (57). However, the study of Hg(II) reduction in the environment is complicated by the fact that the mercury cycle is much more complex than the geochemical cycles of other redox sensitive metals such as iron (51). In addition to microbial Hg(II) reduction, microbially catalyzed methylation and demethylation reactions as well as a myriad of abiotic reactions also influence mercury cycling (11, 38). Furthermore, although some of the Hg(II) reduction in natural waters clearly depends upon the presence of living microorganisms, in some instances Hg(II) reduction is primarily a nonbiological process (6, 8, 11, 112).

Mercury contamination of the environment generally enhances the capacity for microbial Hg(II) reduction. In uncontaminated waters the rate of biological Hg(II) reduction typically lags for 6–24 h until the microbial population can adapt (6, 11). This lag is in contrast to the immediate microbial Hg(II) reduction in water samples from contaminated sites or pristine waters that have been artificially preexposed to Hg(II). Mercury-contaminated environments have increased abundance of Hg(II)-resistant bacteria and/or *mer* genes as well as increased rates of Hg(II) reduction (4–6, 8–11, 103, 115). Although many of the Hg(II)-reducing microorganisms in mercury-contaminated environments do not have genes that hybridize to *mer* gene probes under highly stringent hybridization conditions (7, 8, 103, 115), current evidence indicates that these organisms also volatilize Hg(II) with a NADPH-dependent Hg(II) reductase (7).

One proposed bioremediation strategy for removing mercury from contaminated environments would be to stimulate *mer*-mediated reduction of Hg(II)

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(11, 38). This could be accomplished by increasing the density of working *mer* operons, either by adding Hg(II) reducers, stimulating the growth of indigenous populations, amplifying the number of *mer* operons in indigenous microorganisms, or increasing the percentage of indigenous *mer* operons that are expressed (11, 38). However, these approaches assume that Hg(II) reduction is enzyme limited. A greater limitation in the use of microbial Hg(II) reduction to remove Hg(II) from some contaminated environments is that not all of the Hg(II) may be available for microbial reduction. For example, typically less than half and sometimes less than 10% of the Hg(II) in contaminated waters is volatilized by microbial reduction (11, 112). Much of the mercury may be bound in nonreducible forms to suspended particulate matter in the water (11). Mercury in aquatic sediments may also be unavailable for microbial reduction because sterile sediment added to water inhibits Hg(II) reduction (11) and sediment-water incubation systems volatilize less mercury than systems with water alone (112). Further evidence that mercury was in nonreducible forms in aquatic sediments was the finding that the abundance of *mer* genes in contaminated bottom sediments was two orders of magnitude lower than in the water column even though the sediment had very high mercury concentrations (11).

Preliminary experiments demonstrated that microbial Hg(II) reduction has the potential to volatilize Hg(0) from Hg(II)-containing mine- and industrial-waste waters (133) as well as residential sewage (44). However, I have been unable to find documentation of any instance in which microbial Hg(II) reduction is currently being applied for the removal of mercury from contaminated waters or waste streams.

Tc(VII) REDUCTION

Technetium is a long-lived (half-life, 2.15×10^5 years) radioactive contaminant in the environment that is a by-product of fission reactions occurring in atomic explosions and in nuclear power stations (135). Under aerobic conditions, technetium is primarily in the form of pertechnetate [Tc(VII); TcO_4^-), which is highly soluble (135). The reduced form of technetium, Tc(IV) is highly insoluble (135). Several studies have demonstrated that the development of reducing conditions in soils greatly decreases the solubility and mobility of technetium, presumably as the result of Tc(VII) reduction to Tc(IV) (120 and references therein).

One of the few studies that have directly examined the potential for microorganisms to reduce Tc(VII) found that a mixed culture of anaerobic bacteria produced more insoluble and/or adsorbed technetium from pertechnetate than mixed or pure cultures of aerobic bacteria (46). Forms of technetium

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other than pertechnetate were also associated with dissolved organics. However, if pertechnetate was added to bacteria-free filtrates of the mixed anaerobic cultures, no organic-associated technetium was formed. These results suggested that Tc(VII) might have served as an electron acceptor in anaerobic respiration.

Tc(VII) was reduced during the stationary growth phase of *Moraxella* and *Planococcus* spp. as oxygen was depleted from the medium (109). Studies with heat-killed cells and incubations with live cells at different temperatures suggested that Tc(VII) reduction resulted from an enzymatically catalyzed reaction.

Sulfate-reducing bacteria may also be involved in Tc(VII) reduction. The sulfate-reducing microorganisms, *Desulfovibrio gigas* and *D. vulgaris*, were even more effective than a mixed anaerobic microbial culture in converting Tc(VII) to insoluble and/or adsorbed forms (46). Molybdate, a specific inhibitor of sulfate reducers, inhibited Tc(VII) reduction in a mixed culture of anaerobic marine microorganisms (109). Both studies suggested that the ability of sulfate-reducers to remove technetium from solution was due, at least in part, to the formation of insoluble technetium sulfides and/or the release of other reducing agents. However, the only evidence for nonenzymatic reduction of Tc(VII) was the association of some technetium with dissolved organics when pertechnetate was added to bacterial-free filtrates of the sulfate-reducing cultures (46). Given the recent finding that sulfate reducers can enzymatically reduce other metals [see sections on Fe(III), U(VI), and Cr(VI) reduction, above], sulfate reducers might also be able to enzymatically reduce Tc(VII).

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V(V) REDUCTION

Although microbial V(V) reduction has not been studied intensively, this metabolic capability may be widespread. Almost all of the heterotrophic bacteria and fungi isolated from a silty clay loam soil could reduce V(V) (13). However, the mechanisms for V(V) reduction were not determined.

Two V(V)-reducing microorganisms have subsequently been studied in greater detail (142). *Pseudomonas vanadium reductans* was isolated from the effluent of a metallurgical factory, and *Pseudomonas isachenkovii* was isolated from sea water. Evidence for V(V) reduction is primarily qualitative. During the anaerobic growth of these microorganisms, the V(V)-containing medium changes color from the pale yellow characteristic of dissolved V(V) to blue, which is characteristic of V(IV). With further incubation the medium turns black and opaque, presumably from the accumulation of colloidal V(III) particles. All of the V(V) is reduced within 10 days of incubation; there is

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no loss of V(V) in sterile controls. Several sugars, amino acids, lactate, glycerol, H_2, and CO can serve as electron donors for V(V) reduction.

Given the similarities between the geochemistry of uranium and vanadium (50), microbial vanadium reduction could be responsible for the commonly observed (47, 50) precipitation of vanadium in anaerobic environments, as seen with uranium reduction (see above), and this metabolism could potentially be used to remove vanadium from ore-processing waste streams.

Mo(VI) REDUCTION

Pseudomonas guillermondii and a *Micrococcus* sp. reduce Mo(VI) to blue reduced molybdenum [presumably Mo(V)] during growth on aerobic heterotrophic medium or in cell suspensions (13). The mechanisms for Mo(VI) reduction were not determined.

At high temperature (60°C), low pH (< 2), and with S^0 as the potential electron donor, *Sulfolobus brierleyi* and *S. acidocaldarius* reduce Mo(VI) to Mo(V) under aerobic or anaerobic conditions (18). Mo(VI) is not reduced when the organisms are grown on yeast extract instead of S^0.

Cell suspensions of the mesophile *T. ferrooxidans* also reduce Mo(VI) with S^0 (129). The rate of Mo(VI) reduction is proportional to the amount of cell protein added and the initial S^0 and Mo(VI) concentrations. The hydrogen sulfide:ferric ion oxidoreductase [see section on Fe(III) reduction] purified from *T. ferrooxidans* catalyzes S^0 oxidation to sulfite coupled to Mo(VI) reduction (129). However, Fe(III) is considered to be the physiological electron acceptor for this enzyme because the rate of Mo(VI) reduction is 20-fold lower than the rate of Fe(III) reduction.

Apparently no ecological investigations have examined the role of microbial Mo(VI) reduction in the environment. The reduction of Mo(VI) under acidic conditions could potentially influence molybdenum cycling during ore leaching. Microbial Mo(VI) reduction at circumneutral pH might be involved in the concentration of insoluble molybdenum in anaerobic marine sediments (15) and the reduction spots found in rocks (47).

Cu(II) REDUCTION

Washed cell suspensions of *T. ferrooxidans* reduce Cu(II) to Cu(I) in the presence of S^0 as a potential electron donor (128). Cu(II) is reduced under both aerobic and anaerobic conditions. However, only net reduction occurs under aerobic conditions when azide or cyanide are added to prevent the iron oxidase from oxidizing Cu(I). The hydrogen sulfide:ferric ion oxidoreductase that reduces Fe(III) and Mo(VI) (see above) also reduces Cu(II) (128). Cu(II) reduction by *T. ferrooxidans* may play a role in copper leaching (124).

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Au(III), Au(I), AND Ag(I) REDUCTION

Bacillus subtilis, Aspergillus niger, Cholorella vulgaris, and *Spirulina platentis* reduce Au(III) to Au(0) (16, 34, 41). Within 5 min of exposure of isolated walls of *B. subtilis* to Au(III) chloride, granules of elemental gold form within the walls (16). Au(III) and Au(I) adsorb onto cells of *C. vulgaris* with subsequent reduction to Au(0) (41). Reduction of Au(III) to Au(I) is much faster than the reduction of Au(I) to A(0), which takes place over several days. Gold crystals form on the cell surfaces of *C. vulgaris* and within the cells (49). In some instances the gold crystals are larger than the organisms themselves (34). The mechanisms for Au(III) reduction in these organisms have not been elucidated.

In a similar manner to Au(III) and Au(I), Ag(I) adsorbs onto the cell walls of bacteria and fungi with the subsequent reduction of Ag(I) to colloidal Ag(0) (92, 93). Reduction of Ag(I) to Ag(0) through the production of unknown reducing compounds may account for silver resistance in some bacteria (14).

Dissimilatory Fe(III)-reducing microorganisms can probably also reduce Au(III) and Ag(I). Addition of Au(III) or Ag(I) resulted in the oxidation of the *c*-type cytochrome(s) in *G. metallireducens,* suggesting that the *c*-type cytochromes or a terminal reductase can transfer electrons to Au(III) (76). Washed cell suspensions of *D. vulgaris* rapidly reduce Au(III) to Au(0), and the c_3 cytochrome from *D. vulgaris* reduces Au(III) with the accumulation of Au(0) (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted).

Microbial Au(III) reduction may lead to the formation of gold deposits (140) and could potentially be used for the removal of gold from waters and waste streams (34, 41, 90).

CONCLUSIONS

The intrinsic activity of dissimilatory metal-reducing microorganisms that use Fe(III), Mn(IV), U(VI), or Se(VI) as terminal electron acceptors can greatly influence the fate of these metals in aquatic sediments and groundwater. Fe(III) [and possibly Mn(IV)] reduction can also be an important mechanism for the oxidation of naturally occurring organic matter and organic contaminants in these environments. Dissimilatory reduction of U(VI), Se(VI), Cr(VI), Hg(II), Tc(VII), V(V), Au(III), and Ag(I) is a potential mechanism for removing these metals from contaminated environments or waste streams. Although microorganisms are available in pure culture that can serve as models for the reduction of each of these metals, there is no substantive information about which microorganisms are important in catalyzing metal reduction in natural environments. Furthermore, with the exception of Hg(II) reduction, very little is known about the physiology and biochemistry of dissimilatory metal reduction.

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BIOTECHNOLOGIES FOR METAL AND TOXIC INORGANIC REMOVAL FROM MINING PROCESS AND WASTE SOLUTIONS

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ABSTRACT

Removal of metals, such as selenium, from solutions and wastewaters are some of the most complex environmental problems facing the nation today. Numerous bacterial isolates, obtained from agricultural and mining wastes, have been screened to isolate and enhance the best metal and toxic inorganic reducers. *Pseudomonas stuzeri* isolates have been identified that are able to grow and reduce selenium from solutions containing up to 1000 mg/L selenate, 285 mg/L sulfate, over a broad pH range and under aerobic conditions. Selenium removal has been demonstrated at bench-scale for periods of about nine months. Selenium was removed from mining wastewaters from 0.62 mg/L to below drinking water standards using an 18 hr retention time. Retention times as short as 6 hr have been demonstrated from process solutions containing 33 mg/L selenium. Enzymatic bioremediation processes have been used to treat mining solutions containing 0.6 to 30 mg/L selenium. Effluents containing <0.01 mg/L selenium were obtained for over four months. Tests combining immobilized enzymes preparations for cyanide-oxidation and selenium-reduction simultaneously removed both contaminants from a wastewater containing 102 mg/L cyanide and 31.1 mg/L selenium. Tests using live microorganisms and immobilized enzyme preparations removed over 53 mg/L nitrate from a spiked mining process solution using an 18 hr retention time.

INTRODUCTION

Management of mine and mineral drainage, mineral processing streams, and mine wastewaters to ensure safe and cost-effective removal of metal contaminants is a serious responsibility. This management requirement is driven by regulatory guidelines for removal of site specific pollutants. Techniques for the removal of dissolved metals from waters are needed for a variety of mining and environmental related applications. Biotechnology has the capability to meet or exceed the stringent target treatment levels set forth under the Best Demonstrated Available Technology (BDAT) concentrations at lower costs than conventional technologies.

Various metals can be used as electron acceptors by microorganisms, and are subsequently reduced in the process. In general, microbial metal reduction may represent either a detoxification mechanism or anaerobic respiration. The reduction of some metals, such as arsenic, chromium, uranium, and selenium, produces less soluble metal forms that may subsequently be removed from solution. Toxic inorganics, such as cyanide and nitrate can be used as nutrients for microbial growth.

Selenium contamination is common in the United States, especially in the Western US. Contaminated waters include agricultural drainage, mineral process solutions and recreational waters. Selenium is most often present as selenate $(SeO_4)^{6+}$ and/or selenite $(SeO_3)^{4+}$, depending on the oxidation potential and pH of the water. Cost-effective, off-the-shelf technology is not available for selenium removal from large-volume wastewaters. In this paper, selenium reduction is presented as an example of microbial metal reduction that shows potential for application to selenium removal from industrial wastewaters. Microbial reduction of selenium can occur under both aerobic and anaerobic conditions. The experiments discussed herein concentrate on the removal of selenate and selenite from various process and waste waters and nitrate from a spiked wastewater. A biopolymer, alginate, with and without bacteria was used to remove metals from various mining waters.

METHODS

Bacterial Culture, Isolation and Testing

Bacteria were isolated from mine drainage and agricultural waters and soils and adapted to high metal and selenium concentrations. Samples were collected in sterile tubes and grown on nutrient agar and trypticase soy agar at room temperature for 72 hours. Individual colonies were isolated further on trypticase soy agar plates containing 100 ppm selenate. Over one-hundred fifty bacterial isolates were obtained and screened through adaptation and enhancement procedures for selenium, arsenic and uranium reduction and nitrate and cyanide destruction. One uranium reducing isolate was obtained from Battelle Pacific Northwest Labs.

Batch and reactor metal removal and toxic inorganic destruction tests were conducted with the adapted-stimulated cultures. All tests were conducted at 24 C using actual mining process and wastewaters. Metal-spiked tests also used actual mining wastewaters.

Chemicals and Labware

Reagent grade chemicals were used for all experiments. Procedures to remove metal contaminants were used according to Standard Methods (APHA, 1992, Section 3010 C). Procedures detailed in the APHA Standard Methods (APHA 92) were followed to determine metal concentrations. Stock solutions were prepared in stoppered 50 ml glass volumetric flasks. Working standards were diluted 1000 times and prepared fresh on the day of the test or assay.

RESULTS AND DISCUSSION

Selenium-Reducing Isolates

Selenium-reducing capabilities of microbes can vary greatly from species to species and from isolate to isolate within species. The selenium-reducing capabilities of numerous bacteria were compared in tube and flask tests, conducted at 24 C with initial selenium concentrations ranging from 1,000 to 50 mg/L. Different microbes demonstrated a varing capability to reduce selenate and selenite ions to elemental selenium. For example, *E. coli* and *P. stutzeri* were both able to reduce selenite. *E. coli* reduced only selenite and at a much slower rate than *P. stutzeri*. *P. stutzeri* reduces both selenate and selenite, however, considerable differences in the selenium-reducing abilities of several different *P. stutzeri* isolates was observed. A *P. stutzeri* isolate has been isolated and acclimated to mining process waters that is capable of removing 33 mg/L selenate from mining process solutions, containing 285 mg/L sulfate, using bioreactor retention times as short as 6 hr. Numerous selenium-reducing microorganisms have been isolated from contaminated agricultural drainage and various mining process waters and soils. The best selenium reducers screened are all aerobic and heterotrophic.

Live Microorganism and Enzymes for Selenium Removal

Laboratory testing of many selenium-containing process solutions and wastewaters in specially-developed bioreactors produced effluents that meet or exceed drinking water criteria. This biotechnology uses simple bioreactors consisting of naturally-occurring, selenium-reducing *Pseudomonas* bacteria and/or selenium-reducing enzymes extracted from these bacteria and immobilized on solid surfaces, such as carbon or in gel beads. The microbial biofilm reactor was developed by pretreating carbon surfaces with nutrients and biopolymers to stabilize *Pseudomonas* bacteria on the carbon surface. This biofilm performed for about nine months removing 0.62 mg/L selenium from mine wastewaters to produce effluents containing <0.01 mg/L selenium.

In the test shown in Figure 1, a mine wastewater containing 0.62 mg/L selenium was treated with live bacteria and an immobilized enzyme preparation. The enzyme preparations produced effluents of <0.01 mg/L for approximately four months before breakthrough occurred. Live microbial biofilms produced effluents <0.01 mg/L for about nine months without supplemental nutrients. This was achieved in a single-pass reactor operated at room temperature and a retention time of 18 hr.

Simultaneous Removal of Cyanide and Selenium

Bioreactors containing immobilized enzymes simultaneously removed both selenium and cyanide from a mining process solution. Immobilized enzyme preparations in alginate gel beads were tested to determine if cyanide and selenium could be simultaneously removed from a mining process solution. Simultaneous removal of these contaminants, using live microbes, has not been demonstrated because cyanide either kills the selenium-reducing bacteria or inhibits selenium reduction. However, enzyme preparations from cyanide-oxidizing bacteria and selenium-reducing bacteria, immobilized in alginate beads, were effective in simultaneous removal of cyanide and selenium from process solutions containing 102 mg/L cyanide and 31.1 mg/L selenium, Figure 2.

Nitrate Removal from Spiked Mine Waters

In initial comparison tests of *Pseudomonas* bacteria as live cells and as an enzyme preparation, containing no live cells, both produced effluents containing <0.1 mg/L nitrate using mine wastewaters spiked with 53 mg/L nitrate. Bioreactor tests using a nitrate-spiked mine wastewater at room temperature with a retention time of 18 hr demonstrated nitrate removal rates >3.3 mg/L/hr.

Biopolymers for Metal Removal

Alginate/Bacteria beads removed uranium from Midnite Mine waters and produced effluents with less than 0.1 mg/L uranium. Research at SLRC have shown that certain bacteria including *Pseudomonas sp.* incorporated into the alginate beads can more than double the removal of certain metals, such as uranium. Using a 24 hr treatment time, the bacteria-bead system removed uranium from Midnite Mine waters containing 24.5 mg/L and produced effluents with less than 0.1 mg/L uranium. Bead loading levels of up to 15 percent uranium, and 7 percent selenium have been observed.

A new technique sprays an alginate-bacterial mix directly into mine water through an air spray nozzle, similar to a paint sprayer. The alginate and bacteria interact with the metals present in the mine waters and form self-separating beads that remove ninety-nine percent of uranium and lead. Tests have been completed using Berkley Pit, Midnite Mine, and spiked Midnite waters with good metal removals for As, Hg, Se, U, and Pb. Alginate (8 g/L) sprayed into Midnite Mine water, spiked with 50 mg/L of various metals, demonstrated significant metal removal using a <15 min contact time, Table 1.

Table 1. Alginate spray metal removal efficiency using spiked Midnite Mine waters*.

Metal	Single Metal Spike (%)	Multiple Metal Spike (Hg, U, Pb, Se, As) (%)
Hg	33	26
U	99	41
Pb	99	97
Se	31	26
As	38	21

*Midnite Minewater naturally contains uranium at 23 mg/L

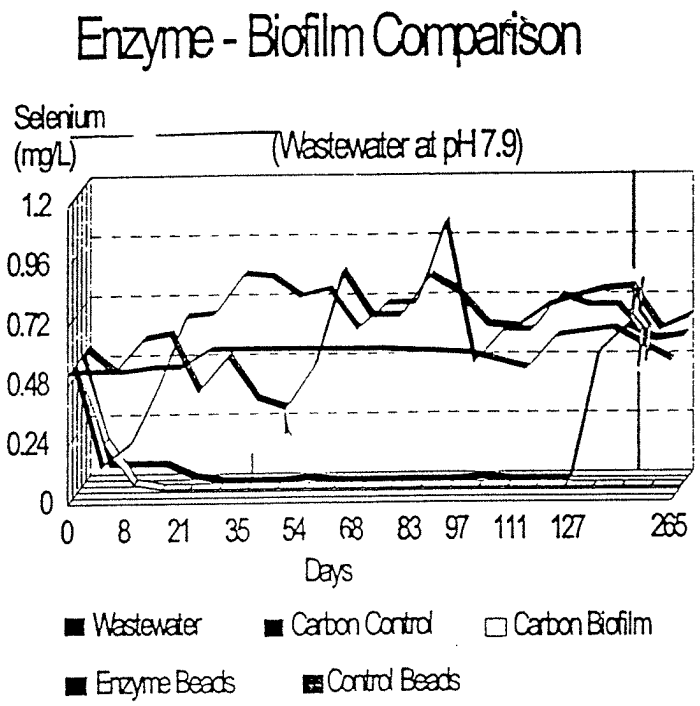


Figure 1. Single-pass bioreactors containing live microorganisms and immobilized enzyme preparations were tested for their ability to remove selenium at 0.62 mg/L from a mining wastewater using an 18 hr retention time.

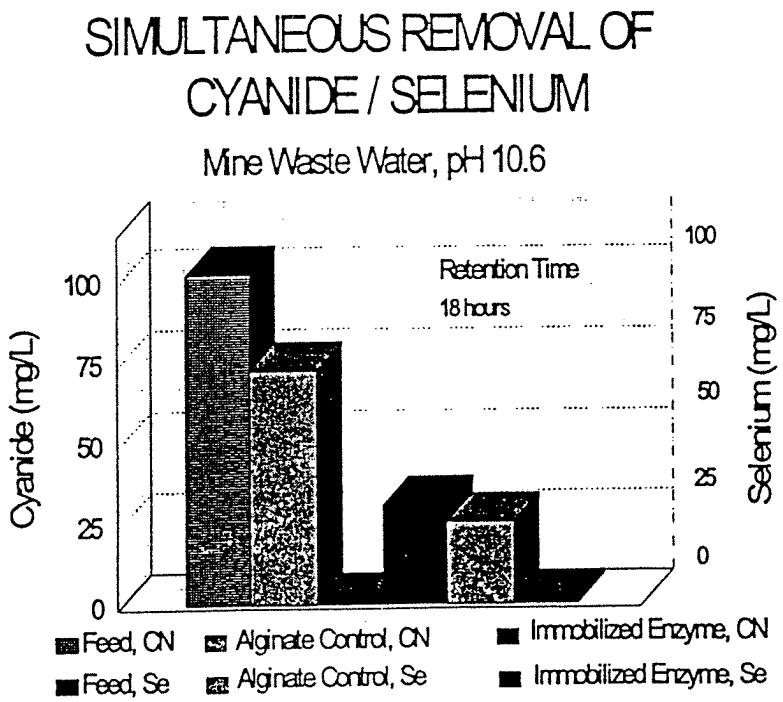


Figure 2. Simultaneous removal of cyanide and selenium from a mining process solution at pH 10.6 containing 102 mg/L cyanide and 31.1 mg/L selenium.

SUMMARY

Microbial isolates varry greatly in their ability to reduce selenate and selenite to elemental selenium. Overall, the use of live microbial biofilms and/or immobilized enzymes shows considerable promise for selenium removal from various waters. The live microbial biofilm, on pretreated carbon, produced effluents containing <0.01 mg/L selenium for nine months without supplemental nutrients.

The advantages of enzymes over live systems include (1) potential for greatly increased kinetics and (2) the effect of a toxic process solution was eliminated. The cyanide did not have to be removed prior to treatment for selenium reduction. This suggests the possibility of simultaneous multiple metal removal from wastewaters.

Bacteria and enzyme systems both removed nitrates from a mining wastewater spiked with 53 mg/L nitrate. Biopolymers were quite effective in removing various metals from spiked and unspiked mining wastewaters. Uranium and lead were most effectively removed.

The Weber State University Bioremediation Program, Odgen, UT is a newly formed, state-of-the-art bioprocess research, development and testing facility. We are seeking cooperators interested in pilot-scale evaluation of various bioprocesses for metal removal and recovery. The Program scientists have expertise in water treatment, soils and sediments remediation, microbiology, biotechnology, bench- and pilot-scale bioprocess development, chemistry, engineering and geophysics. Additional technologies under development and evaluation include:

- ARSENIC REMOVAL - PILOT-SCALE
- URANIUM/LEAD REMOVAL - BENCH-SCALE
- AMD CONTROL - R & D
- BIOMOLECULES FOR METAL REMOVAL/RECOVERY - R & D / BENCH-SCALE

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BIOREDUCTION OF SELENATE AND SELENITE

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Abstract

The U.S. Bureau of Mines, Salt Lake City Research Center and CANMET, Department of Energy, Mines, and Resources, Ottawa, Ontario, Canada, are cooperating on research investigating the biological removal of selenate and selenite from process wastewaters. Bacterial isolates, obtained from agricultural (Alcaligenes xylosoxydans) and mining wastes (Hydrogenophaga pseudoflava), reduce both selenate and selenite ions in mining wastewaters. Selenium reduction has been observed under both aerobic and microaerophilic conditions. Eighty-three to ninety-seven percent selenium removal from wastewaters, containing 0.6 to 30 ppm selenium, has been attained using these bacteria in trickling-column and stirred-tank bioreactors. In laboratory tests, rapid selenate and selenite reductions were obtained with Pseudomonas stutzeri and Escherichia coli. In other tests with P. stutzeri, both selenate and selenite reduction were unaffected by a number of other anions present. An exception was the inhibition of both growth and selenite reduction by the sulfite ion.

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Introduction

Management of mine and mineral drainage, mineral processing streams, and mine wastewaters to ensure safe and cost-effective removal of metal contaminants is a serious responsibility. This management requirement is driven by regulatory guidelines for removal of site specific pollutants. Techniques for the removal of dissolved metals from waters are needed for a variety of mining and environmental related applications.

The U.S. Bureau of Mines (Bureau) is developing bioremediation technologies to reduce or eliminate inorganic contamination from mining and milling wastes. The objective of Bureau research is to reduce concentrations of toxic inorganics and heavy metals to levels that meet site specific discharge constraints, with an overall goal of meeting National Drinking Water Standards.

Laboratory tests have demonstrated that microbial reduction of metals holds promise for the removal of metal contaminants from various mining process waters. Various metals can be used as electron acceptors, by microorganisms, and are subsequently reduced in the process, Table 1 (1-45). In general, microbial metal reduction may represent either a detoxification mechanism or anaerobic respiration. The reduction of some metals, such as arsenic, chromium, uranium, and selenium, produces less soluble metal forms that may subsequently be removed from solution. In this paper, selenium reduction is presented as an example of microbial metal reduction that shows potential for application to selenium removal from industrial wastestreams. Microbial reduction of selenium can occur under both aerobic and anaerobic conditions. The experiments discussed herein concentrate on the removal of selenate and selenite from various process waters. This research was conducted as a joint effort between the Bureau's Salt Lake City Research Center (SLRC) and CANMET, Department of Energy, Mines, and Resources, Ottawa, Ontario, Canada, under a cooperative agreement.

Background

Inorganic selenium is most commonly found in four oxidation states (Se^{6+}, Se^{4+}, Se^{0}, and Se^{2-}). Selenate $(SeO_4)^{=}$ and selenite $(SeO_3)^{=}$ are highly water soluble, elemental selenium $(Se)^{0}$ is much less soluble in water. The most reduced form, hydrogen selenide (H_2Se) occurs as a toxic gas, but is readily oxidized to elemental selenium in the presence of air. A typical selenium contaminated wastewater contains a mixture of selenate, selenite, and elemental selenium; the relative proportions depend on the oxidation potential of the water as well as the pH. Elemental selenium can be oxidized to selenite in alkaline or mildly acidic conditions; further oxidation results in the conversion to selenate. Well aerated surface waters, especially those with alkaline conditions, represent a highly oxidized condition that would contain the majority of selenium as selenate. Selenite is reduced to elemental selenium under mildly reducing conditions while selenate reduction would only occur under stronger reducing conditions (1, 3, 31).

Selenium contamination has been found in at least six western states and has been identified in areas affected by agricultural runoff and in mine drainage and mineral-processing waste systems. An example of agricultural contamination is the Kesterson Wildlife Refuge, which received agricultural drainage waters from farms in the San Joaquin Valley of California where selenium occurs naturally in the rock and soils.

Selenium has also been detected in precious metals process solutions at concentrations up to 30 ppm. While most of those operations are currently

zero-discharge facilities, eventual treatment prior to discharge and closure will necessitate removing the selenium to meet discharge permit requirements. Under new regulations that are to be implemented by the U.S. Environmental Protection Agency (EPA), discharge standards for selenium and its compounds are based on "Best Demonstrated Available Technology" (BDAT). For wastewaters containing selenium, precipitation was determined to be the BDAT. Using this criterion, EPA met the standard for discharge of selenium in wastewater at 1.0 ppm Se (46, 47).

Cost-effective, off-the-shelf technology is not available for selenium removal from large-volume wastewaters. Most of the reported selenium remediation methods, including neutralization/precipitation, reverse osmosis, ion exchange, and reduction/precipitation will decrease selenium concentrations to acceptable levels; however, the treatment costs are prohibitive for large volumes. Because of these high costs, the Bureau and CANMET are examining the potential of selenium remediation using biological reduction

Table 1 Examples of metal ions which can serve as electron acceptors

Metal Ion	Microorganisms Involved	References
Arsenic As^{5+} & As^{3+}	Bacteria - Micrococcus, Pseudomonas, Alcaligenes Algae - Chorella Fungi - Aspergillus, Fusarium, Candida	1-11
Chromium Cr^{6+} & Cr^{3+}	Bacteria - Pseudomonas, Aeromonas, Bacillus, Arthrobacter, Micrococcus	1, 3, 12
Iron Fe^{3+} & Fe^{2+}	Bacteria - Pseudomonas, Enterobacter, Bacillus, Thiobacillus, Micrococcus, Staphylococcus Fungi - Alternaria, Fusarium	1, 3, 13-15
Mercury Hg^{2+}	Bacteria - Pseudomonas, Alcaligenes, Vibrio, Thiobacillus, Flavobacterium, Bacillus	1, 3, 16-18
Manganese Mn^{3+} & Mn^{4+}	Bacteria - Pseudomonas, Alteromonas, Arthrobacter, Micrococcus, Bacillus	3, 3, 19-21
Molybdenum Mo^{6+}	Bacteria - Sulfolobus	3, 22, 23
Selenium Se^{6+}, Se^{4+}, & Se^{0}	Bacteria - Pseudomonas, Escherichia, Bacillus, Micrococcus, Salmonella, Desulfovibrio, Clostridium Fungi - Candida Yeasts - Saccharomyces, Actinomycetes	1, 3, 10, 24-40
Tellurium Te^{6+} & Te^{4+}	Bacteria - Streptococcus, Corynebacteria, Micrococcus	1, 3, 10, 29
Uranium U^{6+}	Bacteria - Micrococcus, Alteromonas	3, 10, 41-45
Vanadium V^{5+}	Bacteria - Micrococcus, Desulfovibrio	3, 10, 26

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Selenium-Reducing Microorganisms and Growth Media

The selenium-reducing microorganisms used in experiments conducted at the SLRC were isolated from a selenium-contaminated agricultural drainage and uranium-mining process waters. Indigenous selenium-reducing bacteria were isolated and characterized using standard biochemical tests and the MIDI Microbial Identification System based on gas chromatograph fatty acid analysis. Alcaligenes xylosoxydans was isolated from the agricultural drainage water and Hydrogenophaga pseudoflava was isolated from the uranium process water; both microorganisms are aerobes and heterotrophic. In addition, the entire bacterial population indigenous to a uranium process water, including H. pseudoflava was used in several experiments.

The media used in SLRC research included (1) PGY (5 g/L peptone, 2.5 g/L glycerol, and 0.5 g/L yeast), (2) peptone (5 g/L), and (3) ammonium sulfate (1 g/L). Sodium selenate (F.W. 188.94) was used at the concentrations indicated for some tests. E-pure water was used to prepare all reagents and media. Stock cultures were maintained and assayed on PGY or nutrient agar. Bacteria were maintained on PGY agar and were transferred to PGY broth for 24 h at room temperature to obtain a 10-pct inoculum to begin each SLRC experiment.

The CANMET study used selenium-reducing Escherichia coli (Strain R-2 - National Research Council Laboratory in Saskatoon, Canada) and Pseudomonas stutzeri (ATCC # 51152, isolated at SLRC). Bacteria were maintained on Tryptic Soy Agar (TSA) and were transferred to Tryptic Soy Broth (TSB) for 24 h at 30° C to obtain a 5-pct inoculum to begin each CANMET experiment.

Selenium Analysis

Selenium analysis at SLRC used approximately 25 mL of filter sterilized (Nalgene 0.45 μm) culture supernatant; samples were stored at 4° C until analyzed. Total selenium and selenite were determined using a hydride generation atomic absorption spectrophotometry (AA) procedure. Selenite was determined directly by hydride generation. Total selenium was then determined by oxidizing all selenium in the sample to selenate in a potassium persulfate-nitric acid digestion followed by reduction to selenite with HCl. Selenate was calculated as the difference between total selenium and selenite.

At CANMET selenium analysis used 10 mL of acidified (HCl), filter sterilized (Nalgene 0.45 μm) culture supernatant. Samples were stored at 4° C until analyzed by AA.

Test Procedures and Results

The potential of using selenium-reducing bacteria to remediate selenium-contaminated process solutions was investigated in two separate series of laboratory tests. Tests at SLRC were conducted in flasks, a 3-L stirred-tank bioreactor, and trickling column reactors. CANMET experiments included tests in flasks and a rotating biological contractor (RBC).

SLRC Studies

Flask Tests. A series of flask experiments, using A. xylosoxydans, H. pseudoflava, and uranium mine wastewater U-1, were conducted to investigate the effect of aeration and nutrient supplement on selenium reduction. Nutrient supplements of PGY, peptone, or ammonium sulfate in combination with dextrose, fructose, maltose, or molasses were used under static and shaken conditions.

Past laboratory tests with A. xylosoxydans demonstrated a PGY supplement to be effective for selenium reduction in various process waters (26, 27). However, for large scale application, a more cost-effective supplement is required. Experiments were conducted for 10 days at room temperature in 250 mL Erylenmyer flasks containing 100 mL of filter sterilized U-1 wastewater, supplemented with a 10 pct inoculum (10 mL at 10^8 cells/mL) of A. xylosoxydans or H. pseudoflava. Figures 1a and 1b show that selenium reduction by A. xylosoxydans had distinct optimum aeration levels with different nutrient supplements. Under conditions of aeration (shaker flasks - 100 rpm) use of PGY and peptone supplements resulted in significantly lower selenium levels than the ammonium sulfate and dextrose supplements. However, when oxygen levels were low (static flasks), the ammonium sulfate and sugar supplements performed best. Selenium reductions to 0.00 ppm were achieved with A. xylosoxydans using ammonium sulfate based media. Tests with H. pseudoflava, the uranium mine isolate, produced similar results.

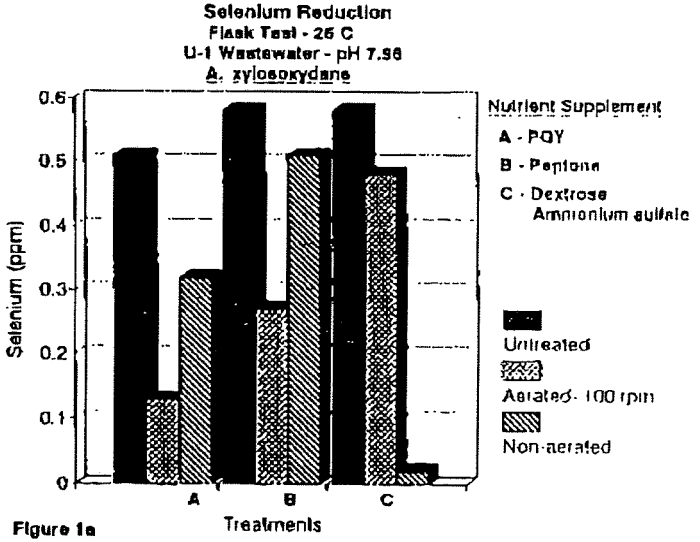


Figure 1a

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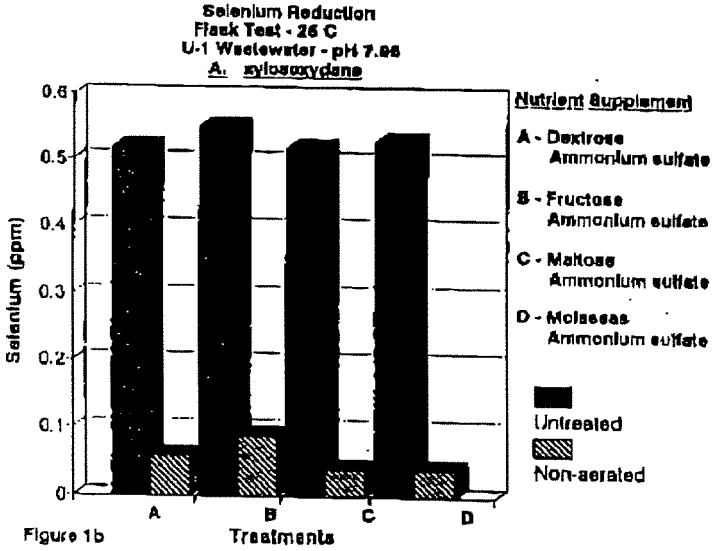


Figure 1b

Figures 1a and 1b - Aeration and media determined selenium reduction by Alcaligenes xylosoxydans in uranium mine wastewater.

The ammonium sulfate and sugar supplements did not induce the rapid microbial growth observed with the PGY or peptone supplements, however, microbial cell numbers did increase slowly and both selenium anions were reduced. This suggests that (1) sugar and ammonium sulfate may be used to maintain an established selenium-reducing bacterial population, (2) ammonium sulfate plus dextrose may be an effective alternative to PGY as a nutrient, and (3) test procedures, such as air addition, can be simplified. An additional benefit of using ammonium sulfate is the reduction of odor associated with microbial growth in organic media.

3-L Bioreactor Tests. A 3-L, temperature-controlled fermentation vessel with a shaft-driven impeller and a bottom air-sparging ring was operated in batch mode. The bioreactor was sterilized immediately preceding the start of each test; 2.1 L of selenium-contaminated process solution, supplemented with appropriate nutrients, was filter sterilized and then added to the vessel.

In the initial test series, A. xylosoxydans was used to treat process solutions, including tailings pond water from an operating silver mine (S-1), leak collection system water from a gold dump leach operation (G-1), barren solution from a gold heap leach operation (G-2), pregnant solution from a second gold heap leach operation (G-3), and wastewater solutions from two uranium operations (U-1 and U-2). The silver and gold process solutions were chemically treated to oxidize residual cyanide prior to bacterial selenium reduction (Table II).

Table II Bacterial selenium removal from contaminated process solutions (26, 27)

Process Solution	S-1[1,2]	G-1[1,3]	G-2[1,3]	G-3[1,3]	U-1[4]	U-2[4]
Selenium Concentration Feed (ppm)	4.2	0.6	30	1.7	0.58	1.6
Time (hours)	48	160	48	110	48	48
Selenium Concentration Effluent (ppm)	0.16	0.5	22	0.25	0.03	0.14
Process Solution pH	8.5	8.5	8.5	8.5	7.96	7.5

[1]The process solutions were treated with peroxide and bleach to destroy the cyanide prior to bacterial selenium reduction.
[2]Silver process solution - PGY supplement.
[3]Gold process solution - PGY supplement.
[4]Uranium process solution - Dextrose and ammonium sulfate supplement

Operating parameters for treating S-1, G-1, G-2, and G-3 included adding a 135 mL bacterial inoculum (containing 10^9 cells/mL) to 2.1 L of solution at 25 C, stirring at 350 rpm, using PGY as the nutrient, and sparging with 60 cc/min H_2 and 10 cc/min air (these operating conditions were established in previously reported research (26, 27)). Sparging with this gas mixture produced about 2 ppm dissolved oxygen. During the bioreactor batch tests, the viable bacterial cells increased to approximately 10^9 cells/mL and remained at this level for the duration of the test. In batch testing, the selenium in these solutions was reduced from 4.2, 0.6, 1.7, and 30 ppm to 0.16, 0.5, 0.25, and 22 ppm, respectively.

Operating parameters for treating U-1 and U-2 included adding a 210-mL bacterial inoculum (containing approximately 10^8 cells/mL) to 2.1 L of solution at 25°C, stirring at 150 rpm, and using ammonium sulfate and dextrose, each at 1 g/L, as the nutrient. The solutions were not sparged; filtered room air was allowed to freely diffuse into the bioreactor. During the tests, the viable cell counts remained fairly stable and in some cases increased slightly. Batch testing of uranium process waters resulted in selenium reductions from 0.58 and 1.6 ppm selenium to 0.03 and 0.16 ppm, respectively.

A subsequent test series, Figures 2a-c, was conducted in which uranium process solution, U-2, was treated with (1) A. xylosoxydans, (2) H. pseudoflava, and (3) the entire bacterial population indigenous to uranium waters U-2, including H. pseudoflava. Test conditions were identical to those previously used in the treatment of U-1 and U-2. The selenium feed concentration was reduced from 1.6 ppm to 0.04 ppm using A. xylosoxydans to 0.08 ppm using H. pseudoflava, and to 0.14 ppm using the indigenous bacterial population. Continued testing with the U-2 indigenous population revealed that the selenium-reducing bacterium, H. pseudoflava, was gradually displaced by non-selenium reducing strains. As a result, selenium reduction efficiency gradually decreased.

These test series demonstrated the feasibility of using biological selenium reduction as a means of remediating selenium-contaminated process solutions. In addition, when comparing treatment of the precious metals solutions to the

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uranium solutions, the test data support results obtained in flask tests indicating that (1) ammonium sulfate plus dextrose may be an effective alternative to FCV as a nutrient and (2) test procedures, such as air addition, can be simplified. Additional wastewater solutions will be tested to confirm this observation.

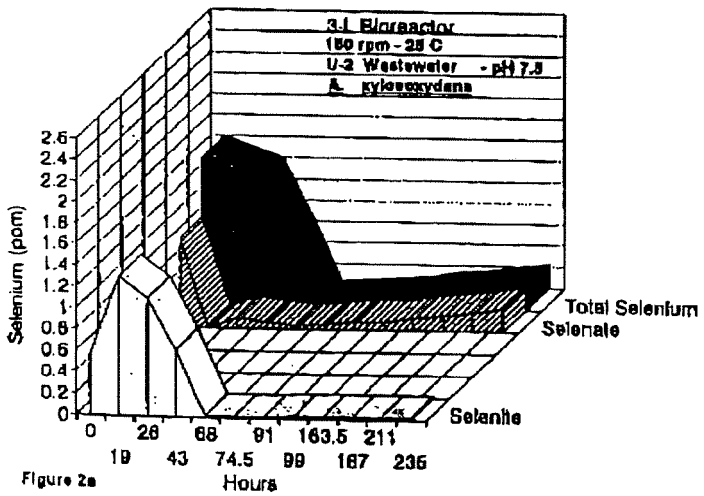


Figure 2a

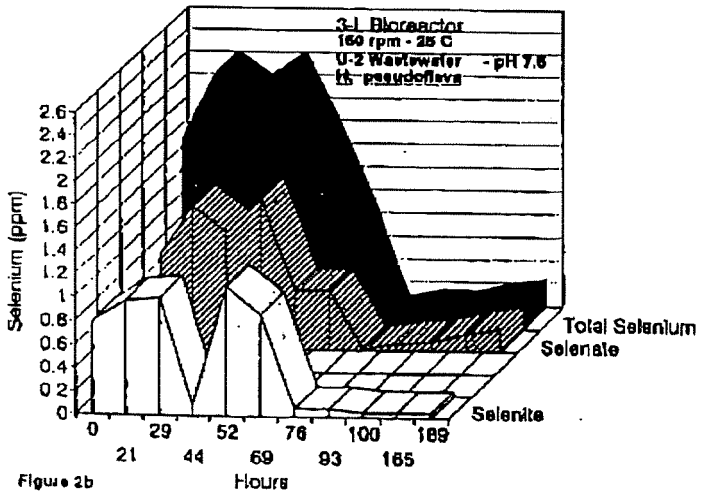


Figure 2b

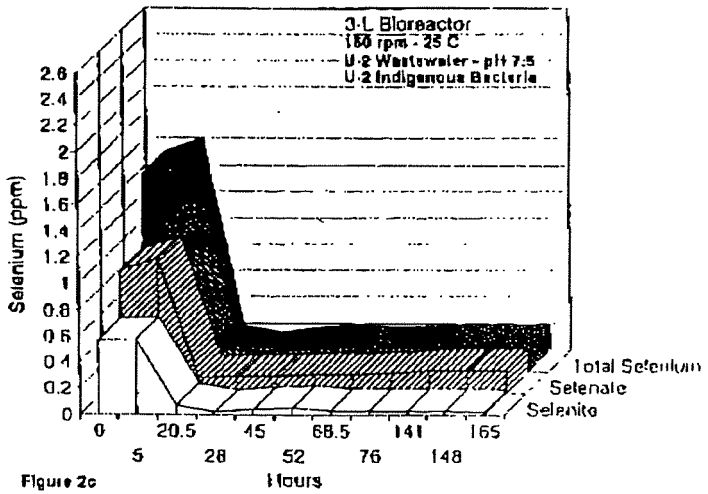


Figure 2c

Figures 2a, 2b, and 2c - Selenium reduction in uranium mine wastewater using the 3-L bioreactor.

Trickling Column Reactor Tests. The column bioreactors were 1-in ID glass columns containing 1-in gauze on top of 3-in packed polypropylene beads; bed volumes were 40 mL. Three columns were operated as down-flow, single-pass, trickling reactors. Column A was the control column, with no bacteria. Column B was inoculated with A. xylosoxydans and column C was inoculated with Th. pseudoflava. The inoculation procedure involved circulating 40 mL of 10^9 cells/mL inoculum through columns B and C for 6 days. Column feed solutions (U-1 and U-2) were supplemented with 1 g/L ammonium sulfate and 1 g/L dextrose and filter sterilized. The solution flow rate through the columns was 4.7 mL/h.

The U-1 solution, naturally containing 0.56 ppm Se, was spiked with sodium selenate to produce a feed solution containing 4 ppm total selenium. After 1 days of treatment, a red precipitate (elemental selenium) had formed in columns B and C indicating that the bacteria were removing selenium from solution. During the 30 day test with the U-1 solution, the total selenium in the effluent solutions from columns B and C ranged from 0.72 to 0.4 and 0.3 to 0.22 ppm respectively; selenium removal gradually decreased in both columns (Figures 3a and 3b).

After 30 days, the feed solution was switched to U-2, which naturally contains 1.6 ppm Se; this solution was also spiked with sodium selenate to yield a feed solution containing 4 ppm total selenium. The change in feed solution adversely affected the selenium reduction efficiency in column B (A. xylosoxydans) (Figure 3a). However, over an 18-day test period, the selenium removal efficiency gradually improved and the effluent selenium concentration was reduced to <0.5 ppm. Figure 3b shows that the selenium removal efficiency

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in column C (H. pseudoflava) increased throughout the same 18-day test period and the effluent selenium concentration was reduced to <0.25 ppm. The data indicate that A. xylosoxydans, a bacterial strain not indigenous to U-2, apparently adapted to this solution after several days, as shown by the turnaround in selenium-removal efficiency. Figure 3b shows that H. pseudoflava, indigenous to U-2, did not require this adaptation period.

The final feed solution tested was unspiked U-2 containing 1.6 ppm Se. A. xylosoxydans reduced the selenium concentration to approximately 0.4 ppm over a 2-week period. Selenium reduction by H. pseudoflava gradually decreased; effluent selenium concentrations increased from below 0.25 ppm to about 0.5 ppm at the end of the test.

Over the entire test period, no selenium was removed from the feed solutions in control column A. The selenium reduction observed in columns B and C indicates that biological reduction shows promise as a remediation technique for selenium-contaminated process solutions. Precipitated selenium could be removed from the trickling-column bioreactor by backflushing.

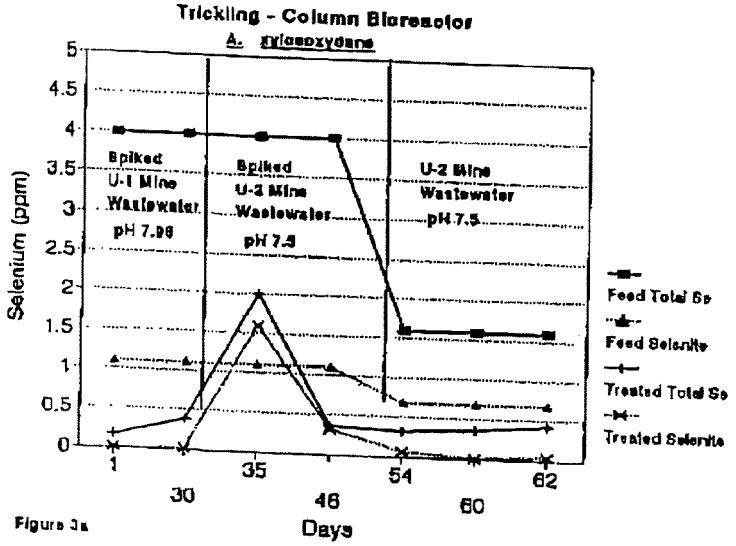


Figure 3a

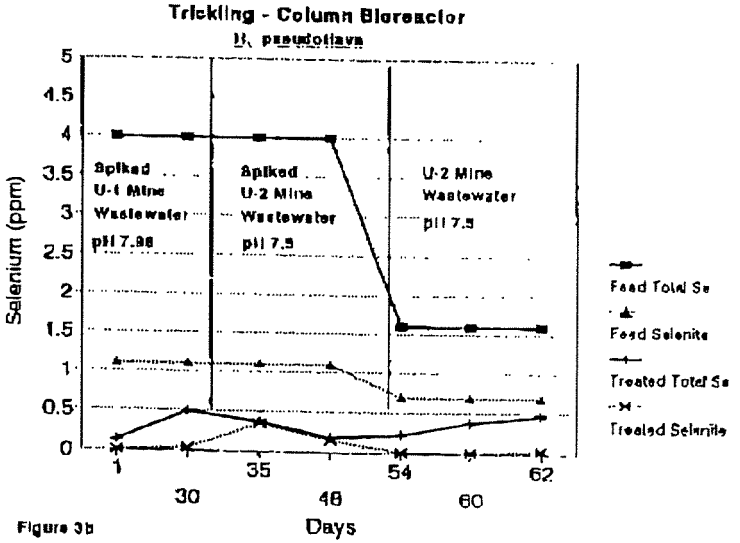


Figure 3b

Figures 3a and 3b - Selenium reduction by Alcaligenes xylosoxydans and Hydrogenophaga pseudoflava in uranium mine wastewater using trickling-column bioreactors.

CANMET Studies

Flask Tests. A series of screening tests were conducted in 2 L Erlenmeyer flasks with one liter of medium (pH 7.0) to investigate the selenium-reducing capabilities of E. coli and P. stutzeri. Flasks were incubated in triplicate on a rotary shaker (150 rpm) at 30° C with initial selenium concentrations of 343.2 ppm. Test data, presented in Figures 4a-4c, indicate that P. stutzeri reduced both selenate and selenite ions; selenate and selenite reductions from 343.2 ppm and 304.8 ppm to 28.6 ppm and 29.2 ppm, respectively, were obtained. E. coli reduced only selenite and at a much slower rate than P. stutzeri; selenite was reduced from 321.7 ppm to approximately 23.4 ppm over 48 h. Selenite reduction using E. coli has been known for years (37); whereas, the reduction of selenite and selenate using pseudomonads, such as P. stutzeri, is a more recently reported phenomenon (40).

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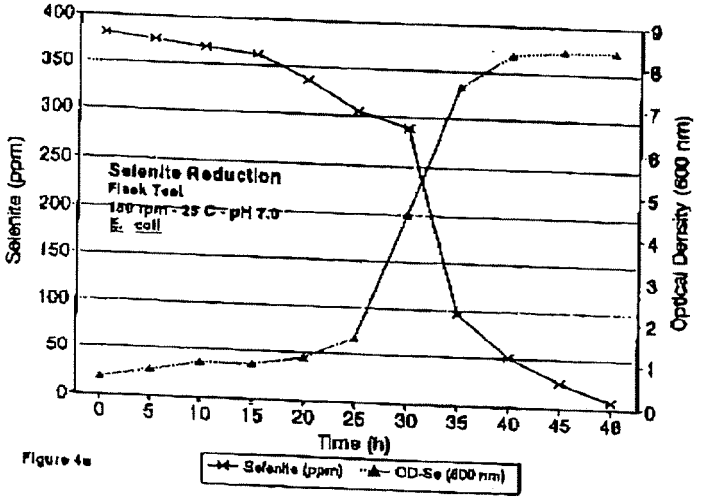


Figure 4a

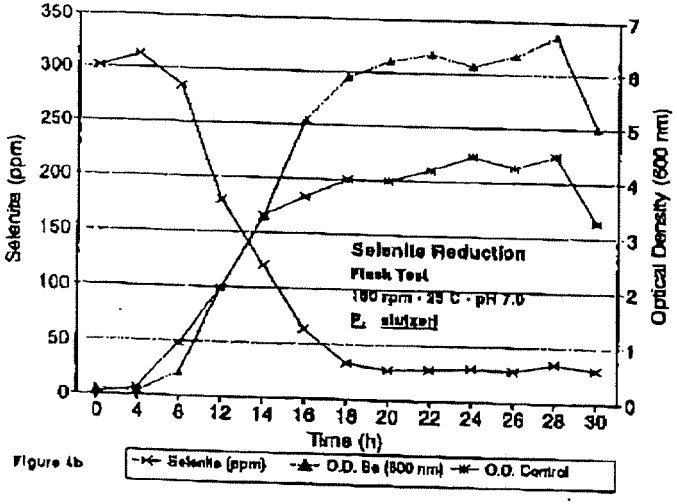


Figure 4b

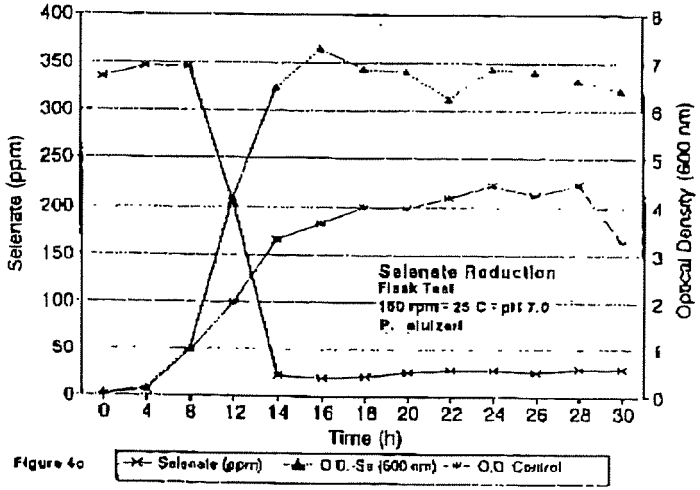


Figure 4c

Figures 4a, 4b, and 4c - Selenite and selenate reduction by E. coli and Pseudomonas stutzeri.

Subsequently, the effect of various anions, such as nitrate, nitrite, sulfate, and sulfite, on selenium reduction using these bacterial strains was investigated. Tests were conducted in flasks containing 100 mL TSB medium to which filter-sterilized salt solutions and filter-sterilized sodium selenite or sodium selenate had been added. Test data, presented in Table III, indicate that selenite reduction using E. coli was inhibited to some degree by nitrate, nitrite, and sulfite. Selenite reduction using P. stutzeri was inhibited only by sulfite; selenate reduction was inhibited to a minor degree by sulfite.

Table III Effect of various anions on selenium reduction by two different bacterial isolates

Bacterial Isolate	Selenium Species	Anion	Concentration (M)	Decrease in Selenium Reduction (%)
E. coli	$(SeO_3)^=$	SO_4	10^{-2}	4
		NO_3	10^{-2}	30
		NO_2	10^{-2}	38
		SO_3	10^{-3}	38
P. stutzeri	$(SeO_3)^=$	SO_4	10^{-2}	3
		NO_3	10^{-2}	10
		NO_2	10^{-2}	7
		SO_3	10^{-3}	90
P. stutzeri	$(SeO_4)^=$	SO_4	10^{-2}	3
		NO_3	10^{-2}	1
		NO_2	10^{-2}	3
		SO_3	10^{-2}	10

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Overall, these test results show that P. stutzeri would be more effective than E. coli for remediation of selenium-contaminated process solutions because (1) it reduced both selenite and selenate and (2) its selenium-reducing capabilities were not affected by a number of anions common to process solutions.

Rotating Biological Contractor Tests. A pilot plant study was conducted in which E. coli was used to treat a base metal smelter weak acid effluent containing 33 ppm Se. A rotating biological contractor (RBC) with a 9.2 m^2 disk surface and 30-L capacity was used as the bioreactor. Initial test results indicated that 97 pct of the selenium was removed from the contaminated solution with a 4 h retention time.

Summary

Cost-effective, off-the-shelf technology is not currently available for selenium removal from large-volume wastewaters. However, laboratory-scale testing, completed at SIRC and CANMET, demonstrated the feasibility of using biological selenium reduction for remediation of selenium-contaminated process solutions; significant selenium removal was obtained from seven different process solutions and wastewaters. Selenium reduction was obtained using a variety of bacteria isolated from agricultural and mining wastewaters; tests indicate that indigenous bacteria might be used for selenium removal if overgrowth by indigenous non-selenium reducers can be prevented. In all tests, the selenium removed from solution did not redissolve.

Ammonium sulfate and various sugars, used as bacterial nutrient supplements, resulted in significant selenium reductions; both selenate and selenite were reduced in the wastewaters tested. This suggests that (1) ammonium sulfate and sugar may be used to maintain an established selenium-reducing bacterial population, (2) ammonium sulfate plus sugar (dextrose was the primary sugar tested) may be an effective alternative to peptone-based nutrients, and (3) bioreactor operation procedures, such as air addition, can be simplified.

It is important to note that these were preliminary tests to demonstrate the feasibility of biological selenium reduction in actual process wastewaters. Operating parameters and nutrient supplements would need to be optimized for each site-specific application. These tests indicate that cost-effective biological selenium reduction from mining process waters may be obtainable; single stage treatment removed selenium, in all but one solution tested, to levels significantly below EPA BADT criterion of 1.0 ppm for discharge of wastewaters.

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Electronic Acknowledgement Receipt

EFS ID:	7162414
Application Number:	11996384
International Application Number:	
Confirmation Number:	6480
Title of Invention:	Apparatus and Method for Treating Fgd Blowdown or Similar Liquids
First Named Inventor/Applicant Name:	Jeffrey Gerard Peeters
Customer Number:	68508
Filer:	Scott Raymond Pundsack/Leslie Taggart
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Attorney Docket Number:	218465-9
Receipt Date:	08-MAR-2010
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Application Type:	U.S. National Stage under 35 USC 371

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APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

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Independent (37 CFR 1.16(h))	* 2	Minus	***4	= 0	X $ =		OR	X $220=	0
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☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

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☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/Katischa R. Wanzer/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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NOTICE OF ALLOWANCE AND FEE(S) DUE

68508 7590 05/04/2010

BERESKIN & PARR (MISSISSAUGA) / GE
SCOTIA PLAZA
40 KING STREET WEST, 40TH FLOOR
TORONTO, ON M6H-3V2
CANADA

EXAMINER	
PRINCE, FRED G	
ART UNIT	PAPER NUMBER
1797	

DATE MAILED: 05/04/2010

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/996,384	01/22/2008	Jeffrey Gerard Peeters	218465-9	6480

TITLE OF INVENTION: APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	08/04/2010

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 08/07) Approved for use through 08/31/2010.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

68508 7590 05/04/2010

BERESKIN & PARR (MISSISSAUGA) / GE SCOTIA PLAZA
40 KING STREET WEST, 40TH FLOOR
TORONTO, ON M6H-3V2
CANADA

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/996,384	01/22/2008	Jeffrey Gerard Peeters	218465-9	6480

TITLE OF INVENTION: APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	08/04/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
PRINCE, FRED G	1797	210-615000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PTOL-85 (Rev. 08/07) Approved for use through 08/31/2010. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/996,384	01/22/2008	Jeffrey Gerard Peeters	218465-9	6480

68508 7590 05/04/2010

BERESKIN & PARR (MISSISSAUGA) / GE
SCOTIA PLAZA
40 KING STREET WEST, 40TH FLOOR
TORONTO, ON M6H-3V2
CANADA

EXAMINER	
PRINCE, FRED G	
ART UNIT	PAPER NUMBER
1797	

DATE MAILED: 05/04/2010

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 167 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 167 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 08/07) Approved for use through 08/31/2010.

Notice of Allowability	Application No.	Applicant(s)	
	11/996,384	PEETERS ET AL.	
	Examiner	Art Unit	
	Fred Prince	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *March 8, 2010*.

2. ☒ The allowed claim(s) is/are *1-8 and 10-16 (renumbered as 1-15)*.

3. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☒ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☒ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 03/08/10, 03/08/10
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/Fred Prince/
Primary Examiner, Art Unit 1797

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance:

The claims are allowed for the reasons presented by applicant.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Fred Prince whose telephone number is (571)272-1165. The examiner can normally be reached on M-Th 8:00-5:30; Alt. Fridays 8:00-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Duane Smith can be reached on (571)272-1166. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Fred Prince/
Primary Examiner
Art Unit 1797

FP
4/27/10

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11996384	PEETERS ET AL.
	Examiner	Art Unit
	Fred Prince	1797

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	09/03/2009	12/04/2009	04/27/2010						
1	1	÷	✓	=						
2	2	÷	✓	=						
3	3	÷	✓	=						
4	4	÷	✓	=						
5	5	÷	✓	=						
6	6	÷	✓	=						
7	7	÷	✓	=						
8	8	÷	✓	=						
	9	÷	✓	-						
9	10	÷	✓	=						
10	11	÷	✓	=						
11	12	÷	✓	=						
12	13	÷	✓	=						
13	14	÷	✓	=						
14	15	÷	✓	=						
15	16	÷	✓	=						
	17	÷	-	-						
	18	÷	-	-						
	19	÷	-	-						
	20	÷	-	-						
	21	÷	-	-						
	22	÷	-	-						

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11996384	PEETERS ET AL.
	Examiner	**Art Unit**
	Fred Prince	1797

ORIGINAL		INTERNATIONAL CLASSIFICATION	
CLASS	SUBCLASS	CLAIMED	NON-CLAIMED
210	615	C 0 2 F 3 / 30 (2006.0)	

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
210	617	630	631	150	914

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1		17												
2	2		18												
3	3		19												
4	4		20												
5	5		21												
6	6		22												
7	7														
8	8														
	9														
9	10														
10	11														
11	12														
12	13														
13	14														
14	15														
15	16														

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	15	
/Fred Prince/ Primary Examiner.Art Unit 1797	4/27/10	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	2

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11996384	PEETERS ET AL.
	Examiner	Art Unit
	Fred Prince	1797

SEARCHED

Class	Subclass	Date	Examiner
210	603, 605, 610, 615-617, 630, 631, 150, 911, 914	12/4/2009	/FP/
435	262, 262.5	12/4/2009	/FP/
	Above Updated.	4/27/2010	/FP/

SEARCH NOTES

Search Notes	Date	Examiner
Inventor and text search in EAST.	12/4/2009	/FP/

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner
	Interference searched in EAST.	4/27/2010	/FP/

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United States Patent and Trademark Office
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BIB DATA SHEET

CONFIRMATION NO. 6480

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/996,384	01/22/2008 RULE	210	1797	218465-9

APPLICANTS

Jeffrey Gerard Peeters, Toronto, ON, CANADA;
William A. Bonkoski, Wilmington, DE;
Pierre Lucien Cote, Dundas, ON, CANADA;
Hidayat Husain, Oakville, ON, CANADA;
Timothy Michael Pickett, Salt Lake City, UT;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/CA06/01220 07/24/2006
which claims benefit of 60/701,996 07/25/2005
and claims benefit of 60/736,859 11/16/2005

**** FOREIGN APPLICATIONS ***************************

CANADA 2,517,322 08/26/2005

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

07/03/2008

Foreign Priority claimed ☑ Yes ☐ No 35 USC 119(a-d) conditions met ☑ Yes ☐ No Verified and Acknowledged /FRED PRINCE/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		ON	9	~~22~~ 15	~~4~~ 2

ADDRESS

BERESKIN & PARR (MISSISSAUGA) / GE
SCOTIA PLAZA
40 KING STREET WEST, 40TH FLOOR
TORONTO, ON M6H-3V2
CANADA

TITLE

Apparatus and Method for Treating Fgd Blowdown or Similar Liquids

FILING FEE RECEIVED		
1240	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

BIB (Rev. 05/07).

EAST Search History

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L2	9670	(selenium or slenite or selenate).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:32
L3	7	2 and ((flue or exhaust or gas) near5 (de$1sulfuriz $3 or de$1sulfurization or de$1sulphuriz$3 or de $1sulphurization)).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:34
L4	0	3 and (soften$3).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:34
L5	1	3 and (hardness or calcium or magnesium or scale).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:35
L6	9	2 and (((flue or exhaust or gas) near5 (de$1sulfuriz $3 or de$1sulfurization or de$1sulphuriz$3 or de $1sulphurization)) or fgd).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:40
L7	4	6 and (lime or sulfide).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2010/04/27 14:41

4/27/2010 4:06:02 PM
C:\Documents and Settings\fprince\My Documents\EAST\Workspaces\11996384.wsp

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	11996384
Filing Date	2008-01-22
First Named Inventor	Jeffrey Gerard Peeters
Art Unit	1797
Examiner Name	Fred G. Prince
Attorney Docket Number	4320-957

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3617540		1971-05-13	Bishop et al.	
	2	3957632		1976-05-18	Knopp et al.	
	3	4839052		1989-06-13	Maree	
	4	5185080		1993-02-09	Boyle	
	5	5192441		1993-03-09	Sibony et al.	
	6	5271831		1993-12-21	Oremland	
	7	5556536		1996-09-17	Turk	
	8	5895576		1999-04-20	Yamasaki et al.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /F.P./

Receipt date: 03/08/2010	Application Number	11996384	11996384 - GAU: 1797
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-01-22	
	First Named Inventor	Jeffrey Gerard Peeters	
	Art Unit	1797	
	Examiner Name	Fred G. Prince	
	Attorney Docket Number	4320-957	

	9	5976376		1999-11-02	Ogushi et al.	
	10	6692642		2004-02-17	Josse et al.	
	11	7025885		2006-04-11	Cote et al.	

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20060272198		2006-12-07	Yoon et al.	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2477333	CA		2006-02-12	Zenon Environmental Inc.		☐
	2	2517322	CA		2007-01-25	Zenon Environmental Inc.		☐
	3	2615945	CA		2007-02-01	Zenon Technology Partnership		☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /F.P./

Receipt date: 03/08/2010	Application Number	11996384	11996384 - GAU: 1797
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-01-22	
	First Named Inventor	Jeffrey Gerard Peeters	
	Art Unit	1797	
	Examiner Name	Fred G. Prince	
	Attorney Docket Number	4320-957	

	4	0692458	EP	A1	1996-01-17	Eniricerche S.p.A.		☐
	5	0769479	EP	A1	1997-04-23	N.V. Kema		☐
	6	0773192	EP	A1	1997-05-14	Mitsubishi Jukogyo Kabushiki Kaisha		☐
	7	1270513	EP	A1	2003-01-02	Aquafin N.V.		☐
	8	105789	JP		1998-01-13			☐
	9	8323391	JP		1996-12-10	Kurita Water Ind Ltd		☐
	10	200162486	JP		2001-03-13	Electric Power Dev Co et al.		☐
	11	2005016826	WO	A2	2005-02-24	Zenon Environmental Inc.		☐
	12	2007012181	WO	A1	2007-02-01	Zenon Technology Partnership		☐
	13	8700161	WO	A1	1987-01-15	EPOC Limited		☐
	14	2004071973	WO	A1	2004-08-26	Zenon Environmental Inc.		☐

Receipt date: 03/08/2010

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	11996384 11996384 - GAU: 1797
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Attorney Docket Number	4320-957

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	1	KURITA WATER IND LTD, English Language Abstract of JP 8323391. 12/1996	☐
	2	ELECTRIC POWER DEV CO et al., English Language Abstract of JP 2001062486. 03/2001	☐
	3	ZENON TECHNOLOGY PARTNERSHIP, European Search Report of EO 1910234 dated July 9, 2008.	☐
	4	APPLIED BIOSCIENCES CORPORATION, "Biological Treatment of FGD Wastewaters, presented January 27, 2005, Raleigh, N.C., U.S.A. Publication Date Unknown	☐
	5	SONSTEGARD et al., "ABMet Biological Selenium Removal from FGD Wastewater", Industrial Water Conference, Orlando, Florida, October, 2005.	☐

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(Not for submission under 37 CFR 1.99)

Application Number	11996384
Filing Date	2008-01-22
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Art Unit	1797
Examiner Name	Fred G. Prince
Attorney Docket Number	4320-957

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Receipt date: 03/08/2010	Application Number	11996384	11996384 - GAU: 1797
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-01-22	
	First Named Inventor	Jeffrey Gerard Peeters	
	Art Unit	1797	
	Examiner Name	Fred G. Prince	
	Attorney Docket Number	4320-957	

	1	TWIDWELL, et al., "Technologies and Potential Technologies for Removing Selenium from Process and Mine Wastewater", Undated, Butte, Montana, U.S.A. Publication Date Unknown	☐
	2	SCHEURMAN, "Selenium Reduction Issues in a Coal Fired Power Plant", Industrial Water Conference, Orlando, Florida, October 2005.	☐
	3	OREMLAND, "Selenate Reduction to Elemental Selenium by Anaerobic Bacteria in Sediments and Culture: Biogeochemical Significance of a Novel, Sulfate-Independent Respiration", Applied and Environmental Microbiology, September 1989, Vol. 55, No. 9, pp. 2333-2343.	☐
	4	STOLZ, et al., "Microbial transformation of elements: the case of arsenic and selenium", Int. Microbial (2002) 5, pp. 201-207.	☐
	5	OREMLAND et al., "In Situ Bacterial Selenate Reduction in the Agricultural Drainage Systems of Western Nevada", Applied and Environmental Microbiology, February 1991, vol. 57, No. 2, pp. 615-617.	☐
	6	HAZTECH NEWS, "Biological Process Proved Most Consistent in Demonstration for Removing Se from Water", The Newsletter of Hazardous Waste Treatment Technology, Vol. 16, 2001.	☐
	7	WAHLQUIST et al., "Effective Biological Water Treatment Through Biological Process Control", Tailings and Mine Waste Conference, Vail Co., 2003.	☐
	8	ADAMS et al., "Microbial and Cell-Free Selenium Bioreduction in Mining Waters", Ch. 24 of Frankenberger et al., Environmental Chemistry of Selenium, Marcel Dekker, Inc., 1998, pp. 479-499.	☐
	9	LOVLEY, "Dissimilatroy Metal Reduction", Annu. Rev. Microbiol. 1993, 47, pp. 263-290.	☐
	10	ADAMS et al., "Biotechnologies for Metal and Toxic Inorganic Removal from Mining Process and Waste Solutions", Randol Gold Forum 1996, pp. 143-146.	☐
	11	ADAMS et al., "Bioreduction of Selenate and Selenite", from A.E. Torma et al., Biohydrometallurgical Technologies, The Minerals, Metals and Materials Society, 1993, pp. 755-771.	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /F.P./

Receipt date: 03/08/2010	Application Number	11996384 11996384 - GAU: 1797
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	First Named Inventor	Jeffrey Gerard Peeters
	Art Unit	1797
	Examiner Name	Fred G. Prince
	Attorney Docket Number	4320-957

	12	MSE TECHNOLOGY APPLICATIONS, INC. et al., "Selenium Treatment/Removal Alternatives Demonstration Project", Mine Waste Technology Program, Activity III, Project 20, June 2001, Bute, MT, U.S.A.	☐

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(Depositor's name)
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(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/996,384	01/22/2008	Jeffrey Gerard Peeters	218465-9	6480

TITLE OF INVENTION: APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	08/04/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
PRINCE, FRED G	1797	210-615000

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☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

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1 Bereskin & Parr LLP/S.E.N.C.R.L., s.r.l.
2
3

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(A) NAME OF ASSIGNEE: Zenon Technology Partnership
(B) RESIDENCE: (CITY and STATE OR COUNTRY): Wilmington, Delaware

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

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Authorized Signature Scott R. Date Aug 3/10

Typed or printed name Scott R. Pundsack Registration No. 47,330

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Application Number:	11996384
Filing Date:	22-Jan-2008
Title of Invention:	APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS
First Named Inventor/Applicant Name:	Jeffrey Gerard Peeters
Filer:	Scott Raymond Pundsack/Hetal Patel
Attorney Docket Number:	218465-9

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U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1810**

Electronic Acknowledgement Receipt

EFS ID:	8144273
Application Number:	11996384
International Application Number:	
Confirmation Number:	6480
Title of Invention:	APPARATUS AND METHOD FOR TREATING FGD BLOWDOWN OR SIMILAR LIQUIDS
First Named Inventor/Applicant Name:	Jeffrey Gerard Peeters
Customer Number:	68508
Filer:	Scott Raymond Pundsack/Hetal Patel
Filer Authorized By:	Scott Raymond Pundsack
Attorney Docket Number:	218465-9
Receipt Date:	03-AUG-2010
Filing Date:	22-JAN-2008
Time Stamp:	14:33:21
Application Type:	U.S. National Stage under 35 USC 371

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Warnings:					
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2	Fee Worksheet (PTO-875)	fee-info.pdf	32292 f06f65b849e8d758e12f8c1060dd7c5b2edda447	no	2
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/996,384	09/07/2010	7790034	218465-9	6480

68508 7590 08/18/2010

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Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

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APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Jeffrey Gerard Peeters, Toronto, CANADA;
William A. Bonkoski, Wilmington, DE;
Pierre Lucien Cote, Dundas, CANADA;
Hidayat Husain, Oakville, CANADA;
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APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	REQUEST ID
11/996,384	7790034	1797	36411

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