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Advances in biotreatment of acid mine drainage and biorecovery of metals: 2. Membrane bioreactor system for sulfate reduction *

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Abstract

Several biotreatmemt techniques for sulfate conversion by the sulfate reducing bacteria (SRB) have been proposed in the past, however few of them have been practically applied to treat sulfate containing acid mine drainage (AMD). This research deals with development of an innovative polypropylene hollow fiber membrane bioreactor system for the treatment of acid mine water from the Berkeley Pit, Butte, MT, using hydrogen consuming SRB biofilms. The advantages of using the membrane bioreactor over the conventional tall liquid phase sparged gas bioreactor systems are: large microporous membrane surface to the liquid phase; formation of hydrogen sulfide outside the membrane, preventing the mixing with the pressurized hydrogen gas inside the membrane; no requirement of gas recycle compressor; membrane surface is suitable for immobilization of active SRB, resulting in the formation of biofilms, thus preventing washout problems associated with suspended culture reactors; and lower operating costs in membrane bioreactors, eliminating gas recompression and gas recycle costs. Information is provided on sulfate reduction rate studies and on biokinetic tests with suspended SRB in anaerobic digester sludge and sediment master culture reactors and with SRB biofilms in bench-scale SRB membrane bioreactors. Biokinetic parameters have been determined using biokinetic models for the master culture and membrane bioreactor systems. Data are presented on the effect of acid mine water sulfate loading at 25, 50, 75 and 100 ml/min in scale-up SRB membrane units, under varied temperatures (25, 35 and 40 °C) to determine and optimize sulfate conversions for an effective AMD biotreatment. Pilot-scale studies have generated data on the effect of flow rates of acid mine water (MGD) and varied inlet sulfate concentrations in the influents on the resultant outlet sulfate concentration in the effluents and on the number of SRB membrane modules needed for the desired sulfate conversion in those systems. The pilot-scale data indicate that the SRB membrane bioreactors systems can be applied toward field-scale biotreatment of AMD and for recovery of high purity metals and an agriculturally usable water.

Introduction and background

Acid mine drainage (AMD) is a common problem for the mining and smelting industries throughout the world. This drainage typically contains dissolved metals of high concentration and more than 3 g/L sulfate. Low pH and presence of heavy metals makes AMD treatment a major concern because of the possible deleterious effects of the effluent on the environment. Conventional AMD treatments use lime to precipitate metals as carbonates and hydroxides. These treatments present some serious limitations in terms of application and effectiveness. They usually result in production of unstable metal hydroxides which also lead to a greater disposal expense. In recent years,

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the use of sulfate reducing bacteria (SRB) to reduce sulfate and precipitate metals in acid mine drainage has been proposed as an alternative to hydroxide precipitation.

One problem with using a biomass for AMD treatment in the same reactor system in which the metals are precipitated, is that the precipitates have to be subsequently recovered from the biomass containing sludge. Govind et al. (1997) separated the biological stage from the precipitation stage for the treatment of acid mine drainage. The bioreactor converted sulfate to hydrogen sulfide gas that was then used in the precipitation units. The water treated was a synthetic mixture of similar composition to Berkeley Pit water. Not only were the metals able to be separated from the biomass, but they were also removed effectively. The copper concentration was reduced 99.9%, the manganese concentration was reduced 98.2%, the zinc concentration was reduced 99.9%, the arsenic concentration was reduced 97.1%, the cadmium concentration was reduced 94.6%, and lead was reduced 81.2%.

Studies on microbial sulfate reduction traditionally involved the use of acetate, lactate, ethanol, methanol and other substrate consuming SRB. Acetate was incorporated in the medium as the source of carbon and energy. There is an extensive literature on biological sulfate reduction using acetate consuming SRB as well as on the treatment of acidic wastewater involving SRB mediated sulfate reduction activity (Shimada 1987; Maree et al. 1989; Du Preez et al. 1991; Dvorak et al. 1992; Van Houten et al. 1996; Kalyuzhnyi & Federovich 1997; Hulshoff-Pol et al. 1998; Sipma et al. 1999; Weijma et al. 2000; Foucher et al. 2001). Accordingly, the biotreatment studies on AMD included the acetate consuming SRB which required appropriate amounts of acetate as energy and carbon source for an effective sulfate reduction in the acidic wastes. Recent studies incorporated hydrogen as a substrate and an electron donor in the SRB mediated sulfate reduction systems. In order to make the AMD biotreatment more cost effective, studies were undertaken to incorporate hydrogen in place of the costly acetate source (acetic acid) in the media as energy source. Studies were undertaken to isolate and characterize SRB that can utilize hydrogen and sulfate as sole energy sources and subsequently use them in the bioremediation of AMD.

There is adequate literature available about the growth of SRB with hydrogen as energy source. Studies of Badzioug & Thauer (1978) reported on isolation and characterization of *Desulfovibrio* species growing on hydrogen plus sulfate and on hydrogen plus thiosulfate as the sole energy sources. Abram & Nedwell (1978) used hydrogen as a substrate for sulfate reduction and methanogenesis as competing systems. Odom & Peck (1981) reported on hydrogen cycling as a general mechanism for energy coupling in the sulfate reducing bacteria and Sorensen et al. (1981) used volatile fatty acids and hydrogen as substrates for sulfate reducing bacteria in anaerobic marine sediment. Brandis & Thauer (1981) reported on the growth of *Desulfovibrio* species on hydrogen and sulfate as sole energy source. Lovley et al. (1982) made a kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments Traore et al. (1983) studied the energetics of growth of a defined mixed culture of sulfate reducer and methanogen with respect to hydrogen transfer in batch and continuous cultures. Lupton et al. (1984) reported on the physiological function of hydrogen metabolism during growth of sulfidogenic bacteria on organic substrates. Robinson & Tiedje (1984) studied competition between sulfate reducing and methanogenic bacteria for hydrogen under resting and growing conditions and Phelps et al. (1985) reported on sulfate-dependent interspecies hydrogen transfer between a methanogen and a sulfate reducer during coculture metabolism of acetare and methanol. Du Preez et al. (1992) were the first to demonstrate that producer gas (as mixture of hydrogen, carbon dioxide and carbon monoxide) can be utilized as carbon and energy source for biological sulfate reduction.

More recent literature about microbial aspects of sulfate reduction with hydrogen and carbon dioxide is presented in two comprehensive reviews by Widdel & Hansen (1991) and Widdel & Bak (1991). Van Houten (1994) studied and optimized biological sulfate reduction process using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. Attention was paid to biofilm formation, sulfide toxicity, sulfate conversion rate, optimization and gas liquid mass transfer limitations. A gas mixture of hydrogen and carbon dioxide (80 and 20%) respectively was used to determine possible toxic effects and for the cultivation of hydrogen consuming SRB. Noguera et al. (1998) developed a unified model describing the role of hydrogen in the growth of SRB under different environmental conditions and Fedorovich et al. (2000) reported on the use of hydrophobic membranes to supply hydrogen to sulfate reducing bioreactors. Battaglia-Brunet et al. (2002) reported on chromate

reduction by fixed films of sulfate-reducing bacteria using hydrogen as an electron source.

Recently, studies were reported in the literature on the use of SRB as biofilms in their activities in wastewater systems and in their sulfate reduction activity. Nielsen (1987) studied the biofilm dynamics and kinetics during high-rate sulfate reduction under anaerobic conditions. Ramsing & Jorgensen (1993) elaborated on the distribution of sulfate reducing bacteria, oxygen and hydrogen sulfide in photosynthetic biofilms determined by oligonucleotide probes and microelectrodes. Santegoeds et al. (1998) reported on structural and functional dynamics of sulfate reducing populations in bacterial biofilms, while Okabe et al. (1999) analyzed spatial distributions of sulfate reducing bacteria and their activity in aerobic wastewater biofilms. Ito et al. (2002) have reported on the successional development of sulfate-reducing bacterial populations and their activities in a wastewater biofilm under microaerophilic conditions.

Studies on the kinetics of sulfate reduction, undertaken to provide kinetic coefficients of SRB growth and of sulfate reduction, are very important in the development of biotreatment strategies for acidic waste streams. The kinetic parameters are very helpful for prediction of the success of the bioremediation approach for a particular acid mine water and for developing alternate biotreatment processes. The most relevant kinetic data were generated recently from the following research studies on the kinetics of SRB sulfate reduction (Brandis & Thauer 1981; Traore et al. 1982; Robinson & Tiedje 1984; Lupton & Zeikus 1984; Widdel & Pfennig 1982; Widdel 1987; Okabe et al. 1995; Ingvorsen & Jorgensen 1984; Ingvorsen et al. 1984; Nielsen 1987; Brandt et al. 2001; Nanninga & Gottschall 1987; Rinzema & Lettinga 1988; Kalyuzhnyi & Federowich 1997).

Traditional methods of using hydrogen and carbon dioxide gas mixture for sulfate reduction include the use of gas sparged reactors (Buisman et al. 1989; Paques Bio Systems BV, Balk, The Netherlands, 2000), as shown in Figure 1. The cheapest available hydrogen/carbon dioxide source for this system were effluents from steam reforming process of natural gas. The gas mixture is bubbled through the reactor liquid, with the liquid bubbles rising through the liquid containing active SRB. The gases dissolve and diffuse to the active cells, resulting in the formation of sulfides. Since hydrogen is rather insoluble in water, the unreacted gases exiting the reactor are repressurized and recycled. The hydrogen sulfide gas formed and

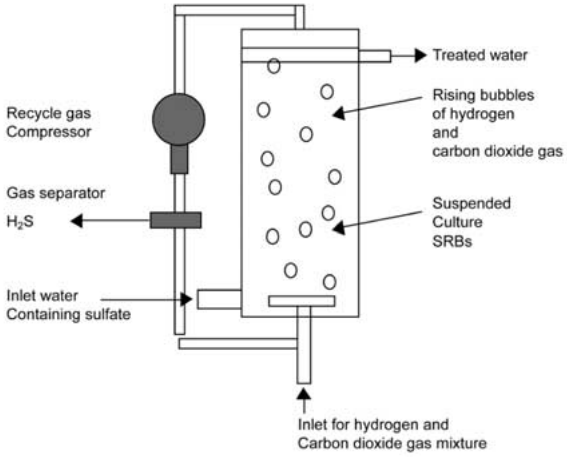

Figure 1. Schematic of a gas-sparged reactor system for treatment of acid mine drainage.

stripped from the liquid, should be separated from the recycle gas stream, to prevent its accumulation.

The main disadvantages of the sparged gas reactor system are as follows: (1) large hydrogen gas mixture recycle, since hydrogen gas has very low aqueous solubility; (2) substantial gas-phase pressure drop, which results in the use of large recycle gas compressors; (3) safety issues resulting from hydrogen gas compression for recycle; (4) poor mass transfer of the hydrogen gas to the active hydrogen-consuming SRB; and (5) the use of free flowing active SRB population subjected to a possible washout of cells.

The use of a membrane reactor system schematically shown in Figures 2 and 3, overcomes the problems of using gas sparged reactors. The main advantages of the membrane reactor system are: (1) the microporous membrane surface presents a very large surface area to the liquid phase, resulting in high mass fluxes, compared to the surface area of the much larger rising gas bubbles in the sparged reactor system; (2) the hydrogen sulfide gas is formed outside the membrane and hence does not mix with the pressurized gas inside the hollow fibers; (3) there is no requirement of a gas recycle compressor, which is major advantage especially with the safety issues concerned with hydrogen gas compression; (4) the membrane surface provides a suitable support surface for immobilization of active SRB resulting in the formation of biofilms; the concentration of active SRB (present as biofilms) is substantially greater than the concentration of SRB that can be achieved in suspended culture gas-sparged reactors, resulting in substantially higher sulfate reduction rates; (5) formation of biofilms pre-

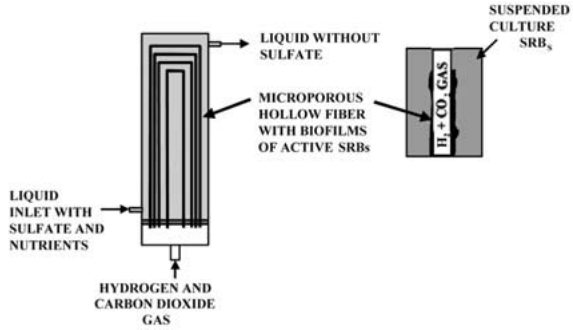

Figure 2. Schematic of the membrane reactor system using hydrogen-consuming sulfate reducing bacterial biofilms outside the membrane hollow fibers.

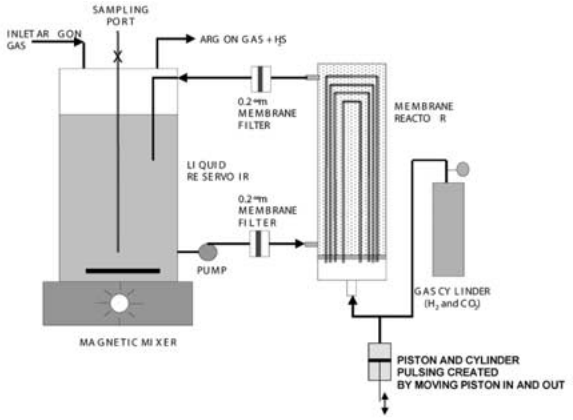

Figure 3. Schematic of the membrane reactor experimental set-up.

vents washout problems associated with suspended culture reactors; and (6) the investment and operating cost of the proposed reactor are significantly lower than a tall liquid-phase sparged reactor system. Due to mass transfer limitations, sparged gas reactors will have a significantly higher volume than membrane reactors, and the operating costs of sparged reactors is higher compared to membrane systems mainly due to gas recompression and recycle costs.

Membrane reactors have been used in a variety of applications, including wastewater treatment, chemical processing and air pollution control. Recent literature provides information on the use of membrane bioreactors in wastewater treatment, and in biological sulfate removal as alternate systems to the conventional bioreactors (Pankhania et al. 1994; Choo & Lee 1996; Mizuno et al. 1998; Federovich et al. 2000). The membrane bioreactors used in those studies employed hydrophobic microporous membranes, either as gas permeable fluoroplastic membrane plates for biological sulfate reduction or as bubbleless membrane aeration systems for the treatment of wastewater. Information on microbial hollow fiber reactors and on the use of hollow fiber membranes in an activated sludge aeration tank for direct solid-liquid separation was reported by Vick et al. (1983) and Yamamoto et al. (1989), respectively. Ruthemond et al. (1994) and Brindle & Stephenson (1996) reported on the use of biofilms in the membrane bioreactors, where the biofilms, are attached to the membranes and actually grow in the pores of membranes. Cote et al. (1988) reported on bubble-free aeration using membrane process analysis. Ghyoot & Verstraete (1991) studied the coupling of membrane filtration to anaerobic primary sludge digestion. Greben (1999) reported on biological sulfate reduction in a membrane bioreactor.

Research of Govind et al. (2000) generated a USEPA Report on Membrane Reactor Studies for Treatment of AMD in which results are presented on the use of hollow fiber membrane reactor systems for biotreatment of acid mine water and biorecovery of metals. The hollow fiber membrane bioreactors, developed in our laboratory, were used for the treatment of AMD via sulfate reduction to hydrogen sulfide gas by the hydrogen consuming SRB biofilms. The biogenic hydrogen sulfide gas was then used to precipitate the metals from AMD. The use of a hollow fiber membrane reactor system for the biotreatment of AMD, schematically shown in Figures 2 and 3, overcame the problems of using gas sparged reactor

Objective

The main objective of this research was to operate the membrane reactor system to determine the rate of sulfate reduction using hydrogen-consuming SRB biofilms. The specific objectives of this research are outlined as follows: (1) conduct a membrane material test to determine which membrane polymers are capable of withstanding the low acidity of the acid mine water from Berkeley Pit, Butte, MT and resistant to pore plugging by the metals, SRB biomass and organic matter; (2) assemble bench-scale hollow fiber membrane bioreactor systems using the most suitable membrane polymer; (3) Setup and operate a series of master culture reactors. using various sources of SRB, such as anaerobic digester sludge and marine sediments; (4) determine the optimum nutrient media composition which is capable of culturing hydrogen consuming SRB; (5) conduct batch biokinetic tests to determine the suspended culture kinetic coefficients for sulfate reduction; (6) conduct sulfate reduction

Table 1. Dissolved metal concentrations in Berkeley Pit mine water

Compound	Concentration (mg/L)
AI^{+3}	293
Cu^{+2}	223
Mn^{+2}	223
Fe^{+2}	514
Zn^{+2}	630
Cd^{+2}	1.38
Ni^{+2}	2.14
As^{+3}	0.512
Co^{+2}	1.23
$SO4^{2-}$	2400
CL^{-}	16
Na^{+1}	213

Table 2. Composition of water after metal precipitation

Composition of AMD water after precipitation and filtering	Concentration (mg/L or ppm)
Al	2.66
Cu	0.42
Mn	129.0
Fe	0.04
Zn	7.39
Cd	0.45
Ni	0.60
As	–
Co	0.50
$SO4^{2-}$	5400
CL^{-}	132.4
Na	128.1
Temperature	25 °C
pH	8.35

studies with SRB biofilms in the bench-scale membrane bioreactor systems using cultures isolated from the anaerobic digester sludge and sediment master culture reactors and determine the biokinetic parameters in those systems; (7) study the effect of acid mine water sulfate loading at various temperatures in scale-up SRB membrane bioreactors on sulfate conversion; and (8) develop the design of a full-scale SRB multi-module membrane bioreactor system to treat acid mine water. Acid mine water from the Berkeley Pit, Butte, MT, was obtained to conduct this study. The average composition of the acid mine water is summarized in Table 1.

Materials and methods

Precipitation of metals

A large batch of acid mine water was mixed with calcium hydroxide and hydrogen sulfide gas mixture (50% hydrogen sulfide and 50% carbon dioxide) and was than bubbled to precipitate the metal sulfides and hydroxides as well as calcium sulfate, resulting in acid mine water at pH = 8.0. At this pH of 8.0, the majority of copper, zinc, aluminum, iron, manganese and other metals were precipitated as either sulfides and/or hydroxides, resulting in water with high sulfate content. At the end of the batch precipitation process,, after a pH of 8.0 has been attained, the water was filtered to separate the precipitates. The composition of the acid mine water after filtering is given in Table 2.

Development of master cultures

Anaerobic digester sludge as a source of SRB

Sludge was withdrawn from a local municipal (City of Cincinnati, OH) plant's anaerobic digester. Approximately 200 g of wet filtered sludge was mixed with 1.5 L of a basal medium, which had the following composition (per liter of distilled water): 5.3 g ammonium sulfate, 2 g sodium acetate, 0.5 g potassium phosphate, 1 g sodium chloride, 0.2 g magnesium sulfate, 0.1 g calcium chloride, 1 ml reazurin solution (0.2% in water) and 10 ml of trace element solution. The trace element solution contained per liter of distilled water, the following chemicals: 12.8 g nitrilotriacetic acid neutralized to pH 6.5 with sodium hydroxide, 300 mg ferrous chloride, 20 g cuprous chloride, 100 mg manganese chloride, 170 mg cobalt chloride, 100 mg zinc chloride, 10 mg boric acid and 10 mg of sodium molybdate. The medium was autoclaved, cooled and the following components were added from sterile stock solution: 50 ml of 8% sodium carbonate, 5.5 ml of 25% hydrochloric acid and I ml of sodium thiosulfate solution (0.5 M in water). The pH of the basal medium was 7.2 after preparation. The gas phase used was 80% hydrogen/20% carbon dioxide or nitrogen gas. The pH of the culture was maintained by adding sulfuric acid. All the chemicals used in our studies were purchased from Sigma-Aldrich Chemicals.

Sediment as a source of SRB

Another source of active SRB cultures was a sediment collected from New York/New Jersey Harbor and from East River, NY, containing SRB species that have been shown to biodegrade polycyclic aromatic hydrocarbons (PAHs) (the major pollutants in addition to metals in those sediments). Sediment samples (500 g) were mixed with the above basal medium and trace elements solution and additional chemicals described above were added to small culture reactors to grow the hydrogen consuming SRB. After the sediment culture had been grown for four weeks, the culture liquid was filtered through a coarse filter to separate the sediment coarse particles. The culture liquid was decanted to prevent all the sediment particles from being filtered. The turbid liquid was then added as an inoculum to the SRB master culture reactors.

Three master culture reactors were set up to grow sulfate-reducing bacteria from each of the two sources for operating the membrane reactors. The volume of each reactor was 2 L and the working volume of the culture was about 1800 ml. The reactors were gas-tight so that there was no oxygen contamination. All the reactors were operated in the incubator at a temperature of 30 °C and the contents of the reactors were stirred using the magnetic stirrers. Hydrogen and carbon dioxide gas mixture in a 50/50 ratio was bubbled through the reactors. The gas exiting the reactor was bubbled through zinc acetate solution and thus the production of hydrogen sulfide was measured. The pH of the master culture was measured periodically and was found to vary between 7 and 8.

Batch studies to determine kinetics of sulfate reduction

The master culture reactors were spiked with known sulfate concentration of 3200 ppm. The sulfate concentrations in each of the master culture reactors was measured at a regular time interval of three hours using a Dionex Ion Chromatograph. The amount of biomass was measured by drying a known volume of sample and measuring the dry weight of the SRB cells. Analysis was performed on the culture growth, sulfate conversion, hydrogen sulfide formation and acetate consumption. The hydrogen consumption by the SRB was measured by chemical methods (Robinson et al. 1984; Lovley et al. 1982; Van Houten et al. 1994; Noguera et al. 1998) as well as by respirometry (Holder & Tabak 2002). The measurement of the biomass at appropriate time intervals provided biomass yield data for the determination of the SRB growth kinetics as well as the kinetics of SRB-mediated sulfate reduction.

Membrane polymer material tests

Membrane material tests were performed on representative membranes to determine their resistance to acidity, uptake of metals and fouling (chemical and biological) in order to select an appropriate hollow fiber membrane polymer for the construction of the membrane bioreactor for sulfate reduction and AMD biotreatment by hydrogen consuming SRB. The membrane polymers tested for the construction of the SRB membrane bioreactor module were: glass, polypropylene, polystyrene, polysulfone, polyphenylsulfone, ethylene propylene, silicone rubber, viton, xylon, acetyl copolymer and teflon. The percentage uptake of metals: iron zinc, copper, aluminum and manganese was measured for each membrane polymer for a period of 6 weeks.

Operation of the SRB membrane bioreactor process

The acid mine water obtained after filtration from the batch metal precipitation step, was pumped through the membrane module (Figures 2 and 3). The membrane module consisted of a FibreFlo hollow fiber capsule filter with a surface area of 0.0557 cm^2. The specifications of the membrane module used in the bench-scale experiments are given in Table 3. The membrane was made of polypropylene, which was found to have the least uptake of metals. Three membrane modules were initially loaded with the hydrogen-consuming sulfate reducing bacterial culture from each of the two sources by pumping the water containing culture from the master culture reactor through the shell-side of the membrane module for several hours. As the culture flowed through the shell side of the membrane module, sufficient amount of biomass became attached to the outside surface of the hollow membrane fibers to form an immobilized culture (biofilm).

The metal-free, filtered acid mine water was placed in three 500 ml reservoirs. Nutrient solution was added to this water. After the culture was attached to the outside of the membrane hollow fibers, the filtered acid mine water was pumped through the shell-side of the membrane module using a multi-channel peristaltic pump. The hydrogen-carbon dioxide gas mixture, used for culturing the inoculum in the master culture reactors was used to pressurize the inside of the membrane hollow fibers. The bubble pressure inside the membrane module was 30 psig. The gas mixture

Table 3. Specifications of a membrane module

Shell diameter	5.08 cm
Shell height	16.51 cm
Fiber diameter	0.1 mm
Number of fibers/shell	600
Surface area per module	0.557 m^2
Type	Fibreflow® L-I-200-D
Manufacturer	Mimitech Fibercor
	1045 28th Avenue North
	Minneapolis, MN 55447

at a pressure of 24 psig was used to pressurize the insides of the membrane, so the biomass on the outer side can avail it. A 0.2-micron inline filter was used to prevent the biomass from the membrane reactors from contaminating the AMD water in the reservoirs. After every 4 hours, samples from the reservoirs were taken and analyzed for sulfate concentrations using a Dionex ion chromatograph. A total of 21 samples were collected for each experimental run from each of the membrane units. The following operating conditions were used: (1) initial gas pressure in the gas reservoir = 24 psi; (2) composition of gas in the reservoir = 50 mole% hydrogen and 50 mole% carbon dioxide; (3) flow rates of acid mine water = 25, 50, 75 and 100 ml/min; and (4) temperatures: 25, 35 and 40 °C.

Results and discussion

Results of membrane material selection tests

Experiments were conducted to test the structural integrity of several polymeric materials in acid mine water, obtained from the Berkeley Pit, Butte, MT. Twelve polymeric materials were selected and soaked in acid mine water under argon atmosphere to prevent atmospheric oxidation of ferrous salts in the water. Results shown in Figure 4 and tabulated in Table 4 were obtained. Both polypropylene and viton materials had percentage metal uptake of less than 5 by weight percent after 6 weeks of exposure, indicating that changes in polymer properties were small due to exposure to acid mine drainage. The membrane material test data illustrate the superiority of the polypropylene and viton membranes over the other membrane polymers tested in resisting the acidity of the acid mine water and the fouling by the metal sulfide deposits as well as by the SRB immobilized cultures on the membrane surfaces. The polypropylene membrane was selected as the membrane polymer for the construction of the SRB membrane bioreactor systems.

Culture growth characteristics

Growth of SRB on hydrogen and carbon dioxide alone was slow at first. However growth occurred when acetate was added to the basal medium in trace amounts. Similar results with regard to growth of the hydrogen consuming SRB were obtained by other investigators. Acetate is used by SRB as a carbon source and hydrogen is used as an electron donor for the conversion of sulfate to hydrogen sulfide by SRB. Slowly the hydrogen consuming SRB were beginning to grow at higher rates on the surfaces of the membrane and with less acetate needed as carbon source to support their growth This was a result of an adaptation process to the consumption of hydrogen and selection of species from the general SRB population which have adapted themselves to the consumption of hydrogen as energy source in addition to sulfate. Results of culture growth, sulfate reduction, hydrogen sulfide formation and acetate consumption are shown in Figure 5.

Based on the experimental data, the yield of hydrogen consuming SRB μg cells per g dried weight of sulfate consumed (Y_{S04}) was calculated to be 0.04 ± 0.003. Literature yield data vary in the range of 0.03–0.06 g/g. The value of yield is about 10% of the yield for acetate consuming SRB mixed cultures, which demonstrates that the SRB in the master culture reactors were hydrogen consuming SRB rather than acetate consuming SRB.

Results from batch test studies in master culture reactors

In the batch test studies, sulfate concentration in the master culture reactor decreased with time as shown in Figure 6. The sulfate reduction data was analyzed using the Monod model:

$$-\frac{dC_{S04}}{dt} = \frac{\mu_{S04} C_{S04} X_{SRB}}{Y_{S04}(K_{S04} + C_{S04})} \qquad (1)$$

where C_{S04} is the sulfate concentration in the batch reactor; u_{S04} is the maximum specific growth rate for the SRB culture; Y_{S04} is the yield of the SRB culture; K_{S04} is the half-saturation constant for the SRB culture; and X_{S04} is the dry weight concentration of SRB culture in the reactor.

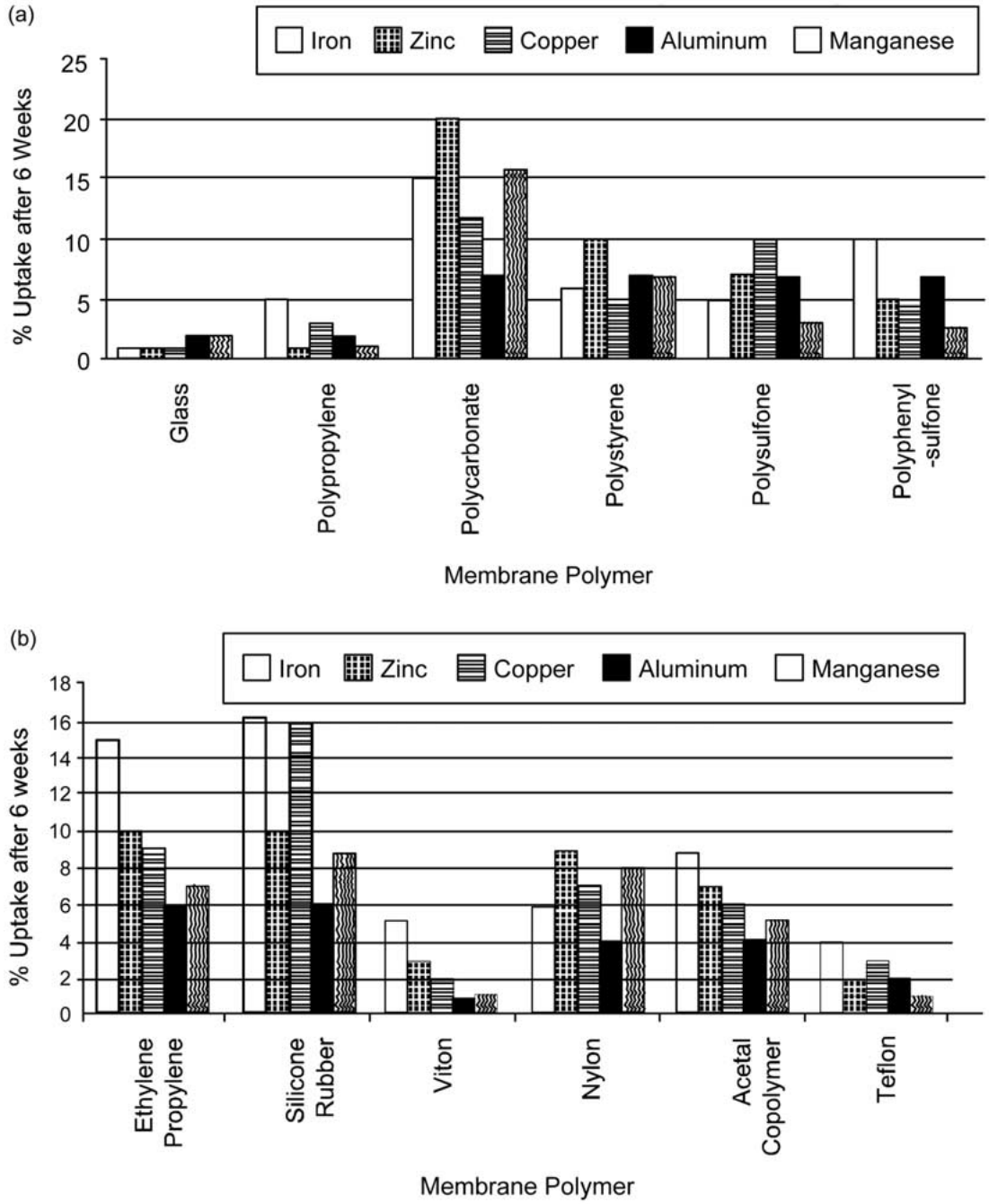

Figure 4. (a) Net uptake of the metals present in acid mine drainage by membrane polymers. (b) Net uptake of the metals present in acid mine drainage by membrane polymers.

Table 4. Percentage uptake of metals in acid mine drainage by the membrane polymers after 6 weeks of exposure

Polymer	Iron (%)	Zinc (%)	Copper (%)	Aluminum (%)	Maganese (%)
Glass	<1	<1	<1	<2	<2
Polypropylene	<5	<1	<3	<2	<1
polycarbonate[1]	>15	>20	>12	>7	>16
Polystyrene[1]	>6	>10	>5	>7	>7
Polysulfone	<5	<7	>10	>7	<3
polyphenylsulfone[1]	>10	<5	<4	>7	<3
Ethylene propylene[1]	>15	>10	>9	<6	<7
Silicone rubber[2]	>17	>10	>16	<6	<9
Viton	<5	<3	<2	<1	<1
Nylon[1]	>6	>9	>7	<4	<8
Acetyl copolymer[1]	>9	>7	>6	<4	<5
Teflon	<4	<1	<3	<2	<1

[1] Polymer swelled in acid mine water (pH = 2.7) after 1.5 months.
[2] Only available in dense rubber membranes and hence unsuitable due to low flux.

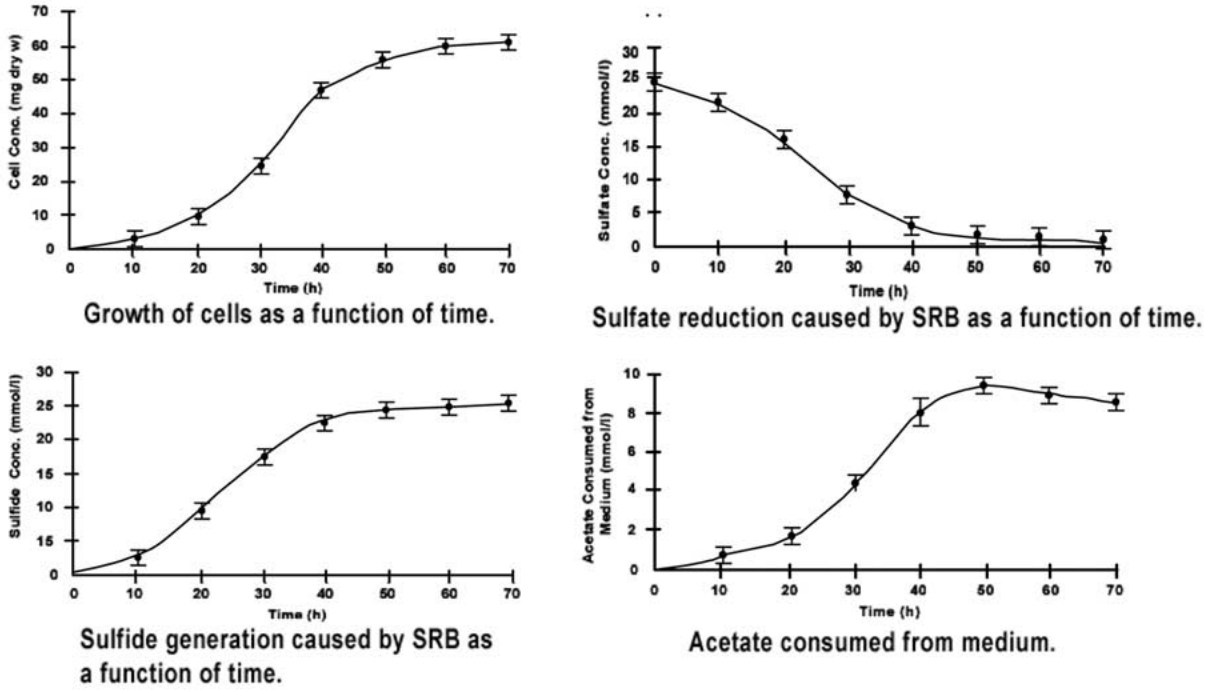

Figure 5. Results on the growth of cells, sulfate reduction, hydrogen sulfide production and acetate consumption.

Using the above model, the biokinetic parameters were determined by plotting dt/dC_{S04} versus $1/C_{S04}$, as shown in Figure 7 to determine the maximum specific growth rate and the half saturation constant. The following values of the biokinetic parameters were calculated after determining the dry weight SRB biomass concentration (X_{SRB}) in the reactor to be 1.11×10^5 ppm.

$$K_{S04} = 4695 \pm 23 \text{ ppm and } (Y_{S04}\mu_{S04}X_{SRB}) = 0.0121 \pm 0.002 \text{ hr/ppm.}$$

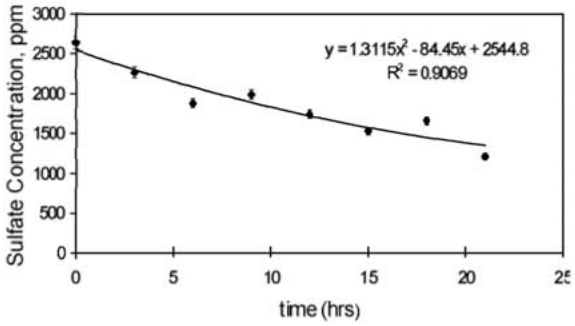

Figure 6. Sulfate reduction in batch tests in the master culture reactor.

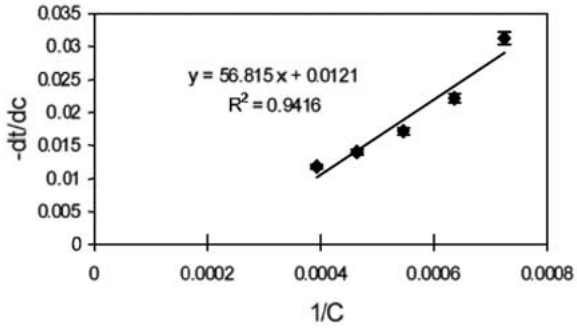

Figure 7. Determination of biokinetic parameters for the batch culture.

Results from membrane reactor studies

Membrane reactor studies were conducted by sampling the liquid from the reservoir periodically and determining the sulfate concentration. The hydrogen and carbon dioxide gas mixture was supplied to the inside of the hollow fibers during this study. As sulfate reduction occurred due to biological activity of the hydrogen-consuming SRB, liquid samples were withdrawn and analyzed for sulfate using the EPA standard method 4110. The results of this study are shown in Figure 8.

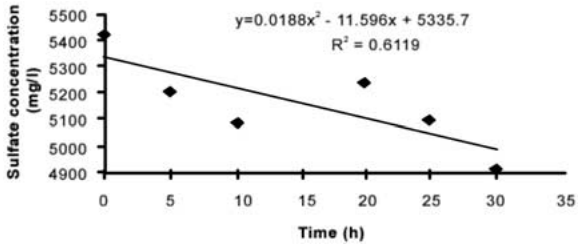

Figure 8. Sulfate resuction as a function of time in the reservoir of the membrane reactor experimental system.

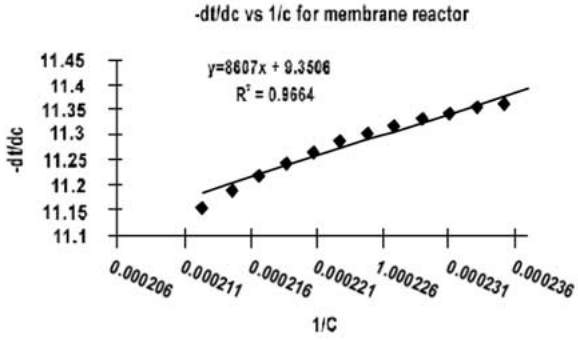

Figure 9. Plot to determine the biokinetic parameters for the membrane reactor sytem.

The above experimental data were analyzed using a well-mixed membrane reactor model. Because the membrane reactor module was small compared to the reservoir volume the liquid residence time in the membrane bioreactor was also small. Hence, the reduction of sulfate concentration in the reservoir was caused cumulatively by sulfate reduction in the membrane bioreactor. Since the SRB culture could not exit the membrane module due to the 0.2 um filter, sulfate reduction occurred only due to the SRB activity in the membrane module.

Using a well mixed model fir the system, the following equation is obtained:

$$-V_{reservoir}\frac{dC_{S04}}{dt} = V_{membrane} = \frac{\mu_{S04,membrane}C_{S04}X_{SRB,membrane}}{Y_{S04}(K_{S04,membrane} + C_{S04})} \quad (2)$$

where $V_{reservoir}$ = the volume of the liquid reservoir; $V_{membrane}$ = the volume of the membrane module (shell side); $K_{S04,membrane}$ = the half saturation constant for the membrane module; $X_{SRB,membrane}$ = the concentration of SRB biomass in the membrane module; $\mu_{S04,membrane}$ = the maximum specific growth rate of the SRB biomass in the membrane module.

Using the above equation for the sulfate reduction experimental data and plotting dt/dC_{S04} versus $1/C_{S04}$, as shown in Figure 9, the biokinetic parameters can be obtained. The biokinetic parameters were calculated from the above plot and were determined as follows:

$$K_{S04,membrane} = 920.50\,\text{ppm and } Y_{S04}/\mu_{S04} \times \text{membrane} \cdot X_{S04,membrane}) = 0.88\text{hr/ppm}.$$

These values are different than the values obtained for the batch reactor, mainly due to the fact that in the

membrane module there is a combination of both suspended culture and immobilized culture (biofilms) on the outside surface of the hollow fibers. Hence, the use of a well mixed model is not entirely appropriate, although it provides a simple way to obtain a scale-up design estimate.

Results from scale-up membrane bioreactor studies

Sulfate reduction at higher flow rates

The membrane modules were scaled-up in order to study the effect of sulfate loading at four different acid mine water flow rates and at three different temperatures. The membrane modules were operated at 25, 50, 75 and 100 ml/min flow rates, using a peristaltic pump, and at temperatures of 25, 35 and 40 °C for a period of 36 hours. Samples of acid mine-water were collected at appropriate time intervals during the 36 hour run and analyzed for sulfate concentration. Figure 10 provides data on sulfate concentration in the membrane bioreactors at 25 °C temperature for the flow rates of 23, 50, 75 and 100 ml/min; at 35 °C temperature for the flow rate of 50 ml/mine, and at 40 °C temperature for the flow rate of 50 ml/min.

Data on sulfate concentration at 25 °C temperature indicate that at 25 ml/min flow rate, the biofilm integrity on the surfaces of the hollow fiber membrane is well maintained and that a higher rate of sulfate reduction is obtained at 50 ml/min flow rate without any deleterious effect on biofilm integrity. However, at 75 ml/min and 100 ml/min flow rates, the rate of sulfate reduction begins to decrease. This decrease is mainly due to the sloughing off of the biofilms from the outside surfaces of the hollow fibers of the membrane bioreactor. The rate of sulfate conversion at 35 °C temperature for the flow rates of 25 and 50 ml/min of acid mine water is higher when compared to data for the same flow rates at 25 °C. Higher temperature is expected to increase the rate of sulfate reduction due to increased reaction rates. It was found that sulfate reduction was only 1.5% higher at 35 °C than at 40 °C, thus indicating that the effect of temperature higher than that of 35 °C is insignificant as compared to the effect of the flow rates in the temperature range of 25–40 °C. The percent of sulfate reduction in the reservoirs of the SRB membrane bioreactor systems operated at 25 °C temperature for the flow rates of 25, 50, 75 and 100 ml/min was shown to be approximately 13.0, 16.6, 14.5 and 10.5 for the respective flow rates. Figure 11 illustrates a plot of rate of sulfate reduction versus the inverse of sulfate concentration. The plot shows experimental data at different flow rates of acid mine water ranging from 25 ml/min to 100 ml/min. From this Lineweaver-Burke plot, biokinetic parameters are determined for sulfate conversion at the different flow rates of acid mine water and biofilm thickness on the membrane surface is ascertained for each of these flow rates.

Large-scale membrane reactor performance

The large-scale membrane reactors were operated at various Reynolds Numbers. The membrane reactor performance relating sulfate removal efficiency in biofilm reactors, biofilm/mixed reactors and mixed reactors (Paques design) to the Reynolds number (a dimensionless number derived from the flow rate of the acid mine drainage) is illustrated in Figure 12. The sulfate removal efficiency in biofilm reactors is highest at lower Reynolds Number values, followed by significant reduction of sulfate conversion efficiency at higher Reynolds Number values in the biofilm/mixed and fully mixed reactors. At Reynolds Number less than 500, the membrane bioreactor behaves as a biofilm system, where the SRB biofilms are retained on the outside surface of the hollow fibers within the module. However, as the Reynolds Number increases, which is achieved by increasing the liquid flow rate through the shell side of the membrane module, the biofilm begins to slough-off, resulting in a combination of a biofilm and mixed reactors in which the active biomass consists of immobilized cells (biofilms) on the surface of the membrane as well as suspended cells in the shell side of the membrane unit. As the Reynolds Number is increased further, the system behaves as a mixed reactor and at higher liquid flow rates, significant amount of the biomass is washed out of the membrane reactor. The sulfate conversion efficiency declines as the membrane reactor changes from a biofilm system to a mixed reactor system. Figure 13 illustrates the membrane SRB biofilm reactor performance in recycle mode with respect to percent sulfate conversion in the SRB membrane reactor at a Reynolds number value of 300 using a biofilm model with a constant biofilm thickness. As time increases, the sulfate conversion efficiency increases, until all of the sulfate in the reservoir is converted to sulfides.

Minimization of membrane fouling

In membrane bioreactor systems, the active SRB biofilms on the outside membrane surface need to be maintained to provide a proper biomass yield for an

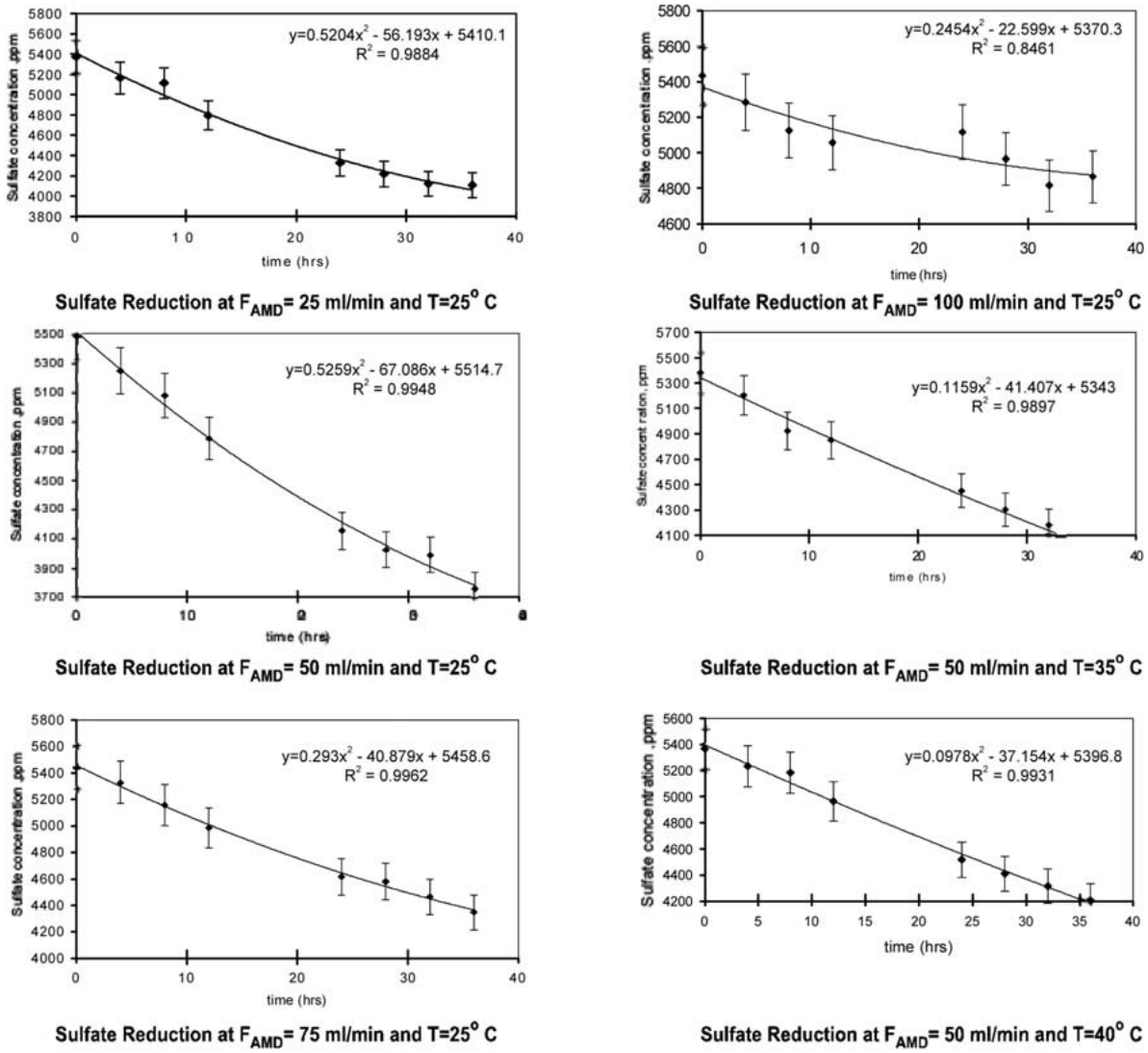

Figure 10. Sulfate conversion data for 25, 50, 75 and 100 ml/min at 25 °C, for 50 ml/min at 35 °C and for 50 ml/min at 40 °C.

effective AMD biotreatment process and an optimum rate of sulfate conversion. At the same time conditions need to be set up to prevent pore closure on the surface of the membrane due to growth of the active SRB culture biofilms. Amplitude is defined as the size of the wave of sinusoidal pressure due to the velocity of sulfate containing acid mine water flow outside the hollow membrane fibers. Frequency is defined as the measure of how fast is the pressure change due to the velocity of the flow. Lower frequency and amplitude of pressure back pulsing was achieved by the implementation of a back pulsing system, developed in our laboratory (under patent protection) and using a cylinder and piston arrangement which is attached to the inlet gas flow line of the membrane module, as shown in Figure 3. Appropriately low amplitude/bubble pressure and a low back pulsing frequency to the membrane bioreactor system promotes appropriate liquid particle-particle collision, removes excess biomass and leaves a thin active biofilm on the membrane surface and at the same time keeps the membrane surface clean from biomass fouling.

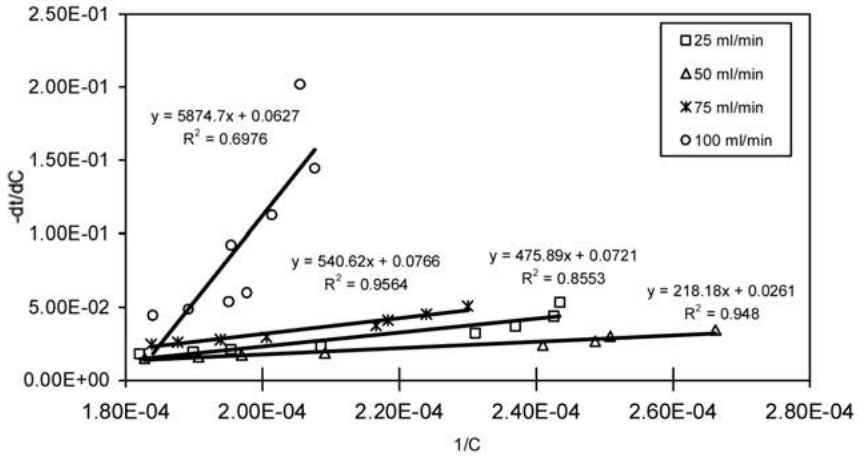

Figure 11. A plot of inverse rate of sulfate conversion versus the inverse sulfate concentration to determine biokinetic parameters of sulfate reduction and biofilm thickness at the different flow rates of acid mine water.

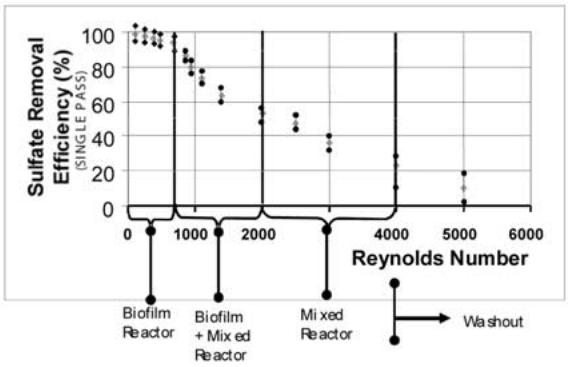

Figure 12. Effect of Reynolds number on sulfate removal efficiency for the larger-scale membrane bioreactor module.

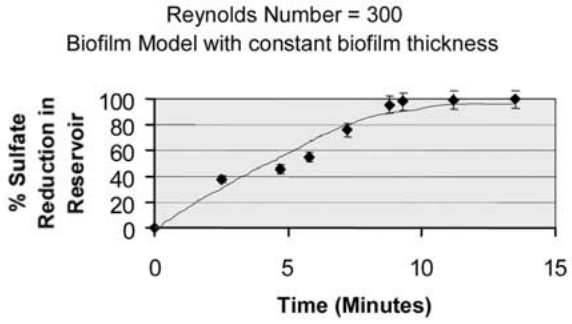

Figure 13. Percentage sulfate reduction in the membrane reactor as a function of time.

Scale-up design of the membrane bioreactor unit

For scale-up of the AMD biotreatment process, a plug flow reactor model was assumed to treat the acid mine water. For the scale-up of the membrane unit three scale-up criteria were proposed: (1) the shell-side liquid velocity is to be maintained the same way as in the bench-scale experiments. This allows the hydrodynamics of the large-scale unit to be maintained the same way as in the bench-scale unit, since the liquid velocity will control the thickness of the immobilized cells on the surface of membrane hollow fibers; (2) the hollow fiber packing density between the bench-scale and the large-scale membrane unit should be of the same value. This also ensures hydrodynamic similarity between the bench-scale and the large-scale membrane units; and (3) the Monod biokinetic parameters. namely K_{S04} and $(\mu_{S04}X_{SRB}/Y_{S04})$ as used for the bench-scale membrane unit, are the same as for the large-scale membrane unit.

These three scale-up conditions can be stated mathematically as follows:

$$\nu_{\text{shell,bench}} = \nu_{\text{shell,large-scale}}$$

$$\phi_{\text{bench}} = \phi_{\text{large-scale}}$$

where $\phi = (N_{\text{hollow-fibers}}\pi d^2_{\text{fiber}}L_{\text{fiber}})/(4V_{\text{shell}})$ = packing density of the hollow fibers in the membrane module; $\nu_{\text{shell,bench}}$ = liquid velocity in the shell side of the bench-scale membrane module unit; $\nu_{\text{shell,large-scale}}$ = liquid velocity in the shell side of the large-scale membrane module unit; (K_{S04}, membrane) bench-scale = (K_{S04}, membrane) large-scale;

$$\left(\frac{\mu_{S04,\text{membrane}}X_{SRB,\text{membrane}}}{Y_{S04}}\right)\text{bench-scale}$$

$$= \left(\frac{\mu_{S04,\text{membrane}}X_{SRB,\text{membrane}}}{Y_{S04}}\right)\text{large-scale.}$$

Table 5. Results of the scale-up design calculations

Flow rate of acid mine water (MGD)	Inlet sulfate concentration (ppm)	Outlet sulfate concentration (ppm)	Number of racks (100 units/ rack)	Length of membrane unit (m)	Diameter of membrane unit (m)
1	8000	100	18	18.8	11.6
1	5000	100	14	15.3	11.6
1	3000	100	12	12.5	11.6
3	8000	100	53	28.2	14.6
3	5000	100	43	23.0	14.6
3	3000	100	35	18.7	14.6
5	8000	100	88	37.6	14.9
5	5000	100	72	30.7	14.9
5	3000	100	58	24.9	14.9

The plug flow model for the reactor system scale is given by

$$F_{AMD} \int_0^L \frac{dC_{S04}}{dx} = -\frac{\mu_{S04,batch} \cdot X_{SRB} \cdot C_{S04}}{Y_{S04,batch}(K_{S04,batch}+C_{S04})} \times 2n\pi D_f L_f.$$

The cross-sectional area and the diameter of the membrane module can be calculated using the following equations:

$$A_{membrane} = \frac{F}{V_{shell,large-scale}}$$

$$D = 2\sqrt{\frac{A_{membrane}}{\pi}}.$$

Using the flow rates of the acid mine water as 1 million gallons per day (MGD), 3 MGD and 5 MGD and assuming the inlet sulfate concentrations to be 3000 mg/l, 5000 mg/I and 8000 mg/l, membrane modules were designed using the bench-scale kinetic parameters and the three scale-up design criteria discussed above. The packing density (ϕ) for both the bench-scale and large-scale membrane unit is taken to be 0.383. Similar to the commercial membrane units available, the membrane units were designed as 22.32 cm diameter and 152.88 cm in length These units were assembled in racks containing 100 units each The number of such racks for each flow capacity and respective sulfate concentrations are given in Table 5. The results from these studies can be used to design membrane modules for the field-scale biotreatment of acid mine water at Berkeley Pit. Butte, MT.

Conclusions

Membrane material tests showed that polypropylene and viton materials had minimal metal uptake and were only slightly affected by AMD. Polypropylene polymer was used for the construction of the SRB membrane bioreactors. Master culture reactor studies showed that hydrogen consuming SRB could be cultured from anaerobic digester sludges and from marine/estuarine sediments containing SRB which have been shown to biodegrade PAH contaminants present in the sediment. The nutrient media used in the master culture reactors were adequate for growing hydrogen consuming SRB and biokinetic studies showed that the yield of the SRB biomass, although low at first, increased significantly with time. The membrane reactor studies conducted using the hydrogen consuming SRB showed that the SRB membrane bioreactors are capable of converting sulfate efficiently in a short residence time. The sulfate reduction rates of approximately 14–15% were considered feasible for treating the acid mine water within the time limits of the biotreatment. It is proposed that the full-scale membrane modules should be operated in series with such lower rates in order to reduce the sulfate content appreciably in the acid mine water within a prescribed length of time. Data have been generated on sulfate conversion in scale-up SRB membrane units at temperatures of 25, 35 and 40 °C for flow rates of acid mine water of 25, 50, 75 and 100 ml/min. The SRB membrane bioreactors were shown to be capable in producing very active hydrogen consuming SRB biofilms on the surfaces of the hollow fiber membranes. The produc-

tion of SRB biofilms (immobilized cultures) active in sulfate conversion and thus in the biotreatment of AMD, prevents a washout of cultures occurring in bioreactors using free flowing or suspended cultures.

The integration of the membrane bioreactor system using hydrogen consuming SRB biofilms, with the selective sequential metal precipitation, recovery and purification process, as separate and parallel running systems, is a unique and advanced process for biotreatment of AMD through an efficient microbial sulfate conversion and the biorecovery of metals from the acidic metal bearing water, using the biogenic hydrogen sulfide formed from sulfate reduction in membrane bioreactors. Such integration of SRB membrane bioreactor AMD treatment system and the selective metal precipitation, recovery and recycle process improves significantly the process operability for metal recovery and cost for the treatment of AMD

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