Mine Water and the Environment (2006) 25: 163–170 © IMWA Springer-Verlag 2006

Technical Article

Waste from Biodiesel Manufacturing as an Inexpensive Carbon Source for Bioreactors Treating Acid Mine Drainage

K. L. Zamzow, T. K. Tsukamoto, and G. C. Miller

Dept of Natural Resources and Environmental Science, MS 199, Univ of Nevada, Reno, USA 89557; corresponding author's e-mail: gcmiller@unr.edu

Abstract. Alcohol-fed, semi-passive bioreactors have been used to support the growth of sulfate-reducing bacteria (SRB) for treatment of acid drainage from mine sites. An alcohol source not previously examined for use in these reactors is the glycerol-methanol waste remaining after the production of biodiesel fuel. In the laboratory, rock-filled columns were used to investigate biodiesel waste (BDW) as a carbon source for SRB. Columns were provided with water containing 900 mg/L sulfate, and fed reagent-grade glycerol or BDW in sufficient quantity to reduce 50% of the sulfate. Addition of 246 mg/L of reagent-grade glycerol resulted in 50% sulfate reduction and production of up to 59 mg/L of soluble sulfide, while the equivalent of 246 mg/L of glycerol provided as BDW resulted in 55% sulfate reduction and the production of up to 92 mg/L of soluble sulfide. During the initial stages of acclimation, propionic, acetic, formic, and lactic acids were observed. Acid concentrations were reduced over time in the effluent, and organic carbon in the BDW was nearly completely converted to carbon dioxide.

Key words: acid mine drainage; biodiesel; biological sulfate removal; glycerol; sulfate-reducers

Introduction

The production of biodiesel to fuel vehicles and diesel-powered equipment is rapidly increasing. Biodiesel was produced in 23 states as of Jan. 2006 (National Biodiesel Board 2006), and the 30 million gallons (113,562,000 L) produced in the U.S. in 2004 is expected to increase to 125 million gallons (473,177,000 L) in a few years, while Europeans produced 420 million gallons (1,589,873,000 L) in 2003 (McCoy 2005). For each gallon of biodiesel produced, 0.75 lbs (340 g) of glycerin is generated as a waste product. Glycerol is used in lotion, toothpaste, and food products, but the market is unable to absorb the estimated 2.1 million gallons (7,949,000 L) of waste glycerol produced during biodiesel manufacturing in 2004 in the U.S.; due to the amount and caustic nature of this waste, disposal has become an issue, and uses for this waste are needed (McCoy 2005; Soap and Detergent Association 2005).

A potential application is as a carbon source for bioreactors treating acid mine drainage (AMD). The University of Nevada, Reno has been treating AMD from the Leviathan mine, a Superfund site near Markleeville, CA, since 1993 (Tsukamoto and Miller 1999; Tsukamoto et al. 2004). Two lined ponds filled with rock serve as constructed bioreactors for the SRB. Sulfuric acid in mine water is converted to hydrogen sulfide during oxidation of provided ethanol (Equation 1), promoting the precipitation of dissolved metals as metal sulfides (Equation 2).

$$3\ SO_4^{2-} + 2\ CH_3CH_2OH + 2H^+ \rightarrow 3\ H_2S + 4\ HCO_3^- + 2\ H_2O \quad (1)$$

$$H_2S + M^{2+} \rightarrow\rightarrow MS_{(s)} + 2H^+ \quad (2)$$

The cost of ethanol is a factor in the operational costs of alcohol-fed bioreactors. This study was initiated to determine if low cost BDW could serve as a carbon source. Biodiesel is manufactured by adding methanol and sodium- or potassium-hydroxide to vegetable oil, including waste cooking oil, resulting in trans-esterification of the fatty acids to form biodiesel, the methyl ester of the fatty acid component. Glycerol, hydroxides, methanol, and traces of fatty acids remain in the bottom waste fraction. Glycerol and methanol are known to be carbons SRB can utilize.

Materials and Methods

Experimental Design

Seven columns with dimensions 6.4 cm ID and 40.1 cm length were filled with 2 to 5 cm river rock, providing a pore space of 800 mL in each column (Figure 1). The columns were divided into 3 groups based on the carbon received: triplicate columns received biodiesel waste (1A, 1B, 1C) or reagent-grade glycerol (2A, 2B, 2C) and one control column received no carbon. Influent water containing 900 mg/L of sulfate as Na_2SO_4 was held in 20 L carboys and provided to all columns through 0.5" (1.3 cm) ID Tygon tubing. Ports for influent sampling and nutrient

Figure 1. Experimental columns

injections were placed 2 cm and 14 cm, respectively, above the top of the column. A peristaltic pump (Cole-Parmer 1-100 rpm) removed water from the bottom of the column at the rate of 1 ml/minute (1.44 L/day) for a retention time of 13.3 hours; effluent samples were taken from a port in the tubing exiting the bottom of the column. Bacterial inocula were provided as 25 g of sand taken from operating bioreactors at Leviathan mine, which had been acclimated to ethanol as a carbon source. On day 30, 10 ml of horse manure slurry was injected into columns to provide a more complete, non-selected group of sulfate reducers. Columns were sealed at both ends to maintain an anaerobic environment.

Although the highly alkaline nature of BDW is a potential advantage in reactors treating AMD, the pH of the influent in all columns was maintained at near neutrality after the addition of the carbon source. Adjustments were made using diluted NaOH or diluted HCl, as necessary. The pH of all carboys was adjusted to 6.0 for three days, then maintained above 7.0 for 60 days, and then reduced in a stepwise manner to 5.5 over 4 months. This encompasses the range of pH that SRB encounter at the Leviathan reactor.

Three separate nutrient solutions were provided: minerals, trace minerals, and vitamins (Table 1). The mineral solution was a modified Postgate's solution (Postgate 1979) to which iron was added as $FeCl_2 \cdot 4H_2O$. The trace mineral solution provided minerals in concentrations consistent with typical AMD. The concentration of minerals in columns was approximated by assuming injections once every 24 hours with a flow rate of 1.44 L/day. Vitamins were provided as Wolfe's Vitamin Solution (ATCC 2002). All nutrient solutions were introduced through an injection port above the column. A single injection was provided daily for the first 30 days as 1.5 mL mineral, 1.5 mL trace mineral, and 10 µL vitamins. After 30 days injections were given only twice a week, although volumes increased to 3 mL, 3 mL, and 20 µL respectively. Beginning on day 53, injections were provided only once a week (as 3 mL, 3 mL, and 20 µL) until day 86 when they were discontinued. Injections of the mineral solution only (no trace minerals or vitamins) were reinstated on day 125 when it became apparent that column activity was declining.

Reagent-grade glycerol or BDW was added based on the stoichiometric amount of glycerol required to reduce 50% of the 900 mg/L sulfate in the influent water. Although BDW contains some methanol and fatty acids, only the glycerol content was considered

Table 1. Composition of the main mineral and trace mineral solutions

	stock (g/L)	mineral mass in 1.5 mL injection (mg)	approximate concentration of mineral in column (mg/L)
Main Minerals			
$FeCl_2 \cdot 4H_2O$	178.0	78 (Fe)	52
$CaCl_2 \cdot 2H_2O$	50.0	23 (Ca)	16
NH_4Cl	10.0	4 (N)	3
$MgCl_2 \cdot 6H_20$	21.1	4 (Mg)	3
KH_2PO_4	7.3	3 (P)	2
Trace Minerals			
$MnCl_2 \bullet 4H_2O$	7.20	3.0 (Mn)	2
$CuCl_2$-$2H_2O$	0.94	0.52 (Cu)	0.4
$NiCO_3$	0.58	0.43 (Ni)	0.3
$ZnCl_2$	0.52	0.37 (Zn)	0.3
$CoCl_2$-$6H_2O$	0.20	0.07 (Co)	0.1
$Na_2MoO_4 \bullet 2H_2O$	0.004	0.002 (Mo)	0.003
Na_2SeO_3	0.001	0.001 (Se)	0.001
yeast	0.5	not analyzed	not analyzed

when determining the amount of BDW to feed to the columns; that is, BDW was added as the glycerol equivalent of reagent-grade glycerol rather than the carbon equivalent. The reduction of sulfate by glycerol occurs at a 7: 4 ratio (Equation 3); therefore, reduction of 450 mg of sulfate (0.0047 moles) requires 246 mg of glycerol (0.0027 moles).

$$7\ SO_4^{2-} + 4\ C_3H_8O_3 + 2H^+ \rightarrow 7\ H_2S + 12\ HCO_3^- + 4\ H_2O \quad (3)$$

Triplicate columns (2A, 2B, 2C) were each provided with 246 mg/L of reagent grade glycerol. The biodiesel waste contained 64% glycerol; therefore, columns in triplicate (1A, 1B, 1C) were fed 383 mg/L of biodiesel waste fluid to provide 246 mg/L of glycerol. The control column received no carbon.

Analytical

Influent and effluent water were analyzed weekly for sulfate, sulfide, alkalinity, pH, glycerol, and metabolic acids. Sample pH was measured with a Beckman 40 pH meter. Alkalinity, as $CaCO_3$ equivalents, was determined by titration with 0.1N HCl, and sulfate concentration was determined by standard gravimetric analysis using barium sulfate precipitation (APHA 1998). Sulfide concentration was determined using the Hach colorimetric method (Hach DR 2000 Direct Reading Spectrophotometer) for sulfide less than 10 mg/L. A modified iodometric titration with sodium thiosulfate and iodine was used to analyze for sulfide concentrations greater than 10 mg/L. On collection of 125 mL of effluent, sodium hydroxide and aluminum chloride were added to remove insoluble matter as an aluminum hydroxide floc, leaving a clear supernatant for analysis (APHA 1998). The supernatant was partitioned into two 50 mL test tubes, where zinc acetate and sodium hydroxide were added to precipitate sulfides for analysis. Sulfide precipitate was allowed to settle for 30 to 90 minutes. The supernatant containing interferences was decanted and distilled water added to resuspend sulfide precipitate. Iodine was pipetted directly into test tubes and titration with sodium thiosulfate proceeded, using a small stir bar to maintain mixing.

Glycerol and metabolic acid products were analyzed by HPLC (HP 1050) equipped with an Aminex HPX-87H column heated to 60°C, using 0.001M H_2SO_4 eluent. Refractive index (RID) and UV detectors were run in sequence. A Waters 510 HPLC was used to pump 0.001M H_2SO_4 through the RID reference cell. A large early eluting peak observed from effluent samples analyzed by UV interfered with the UV analysis of early eluting acids. This was reduced by adding $ZnCl_2$ to precipitate sulfides, removing the supernatant to a separate flask, and adding cation exchange resin briefly to remove any remaining metals. The supernatant was filtered through 0.45 um filter into 2 mL HPLC vials. In previous experiments, the addition of cation exchange resin did not change the detected concentration of alcohols or acids, but was effective in reducing the UV solvent peak. The approximate limits of detection (determined experimentally) were: glycerol, 15 mg/L; lactic and formic acids, 10 mg/L; acetic acid, 20 mg/L; propionic acid, 25 mg/L; and methanol 20 mg/L.

Results

Sulfate reduction

Columns were fed sufficient glycerol to reduce 50% of the influent sulfate. Initially, sulfate reduction increased rapidly in all columns, from 4 or 5% on day 36 (glycerol and BDW, respectively) to 18 and 26% by day 49 (Figure 2). A brief decline was observed in both sets of columns on days 56 and 62, followed by a steady increase to 57% on day 96 for the triplicate BDW columns and 50% on day 102 for the triplicate glycerol columns. A sharp decline in sulfate reduction was observed in all columns after day 102, with a recovery after day 120 to full sulfate reduction by day 158. Triplicate BDW columns showed slightly more variation than the triplicate glycerol columns (Table 2), which may be due to the presence of methanol in BDW (see Discussion). Sulfide was measured as soluble sulfide, and the concentration of undissociated hydrogen sulfide was determined by calculation (Equation 4).

$$[H_2S] = [\text{dissolved sulfide}]/(1 + 10^{pH-pK1}) \text{ for } pK_1 = 7.0 \quad (4)$$

Alkalinity and pH

Alkalinity generation followed sulfate reduction (Figure 2). Except for a temporary decline on day 56 (BDW) and day 62 (glycerol), alkalinity rose steadily in both sets of columns from day 22 through the next two months. BDW columns reached a maximum alkalinity of 465 mg/L on day 96, then declined sharply to 243 mg/L by day 124 before increasing to 428 mg/L on day 158. Glycerol columns followed a similar pattern, reaching a maximum of 389 mg/L on day 102, declining to 260 mg/L on day 124, and increasing to 360 mg/L by day 158. Similar to the pattern in sulfate reduction, variations in alkalinity were observed in BDW columns on day 56 (range 34 to 185 mg/L, mean 105 mg/L), day 82 (range 248 to 414 mg/L, mean 333 mg/L), and day 89 (range 290 to 447, mean 345 mg/L). Except for these dates, variations were within 20% of the mean, and were within 10% of the mean for 6 sample days. Glycerol

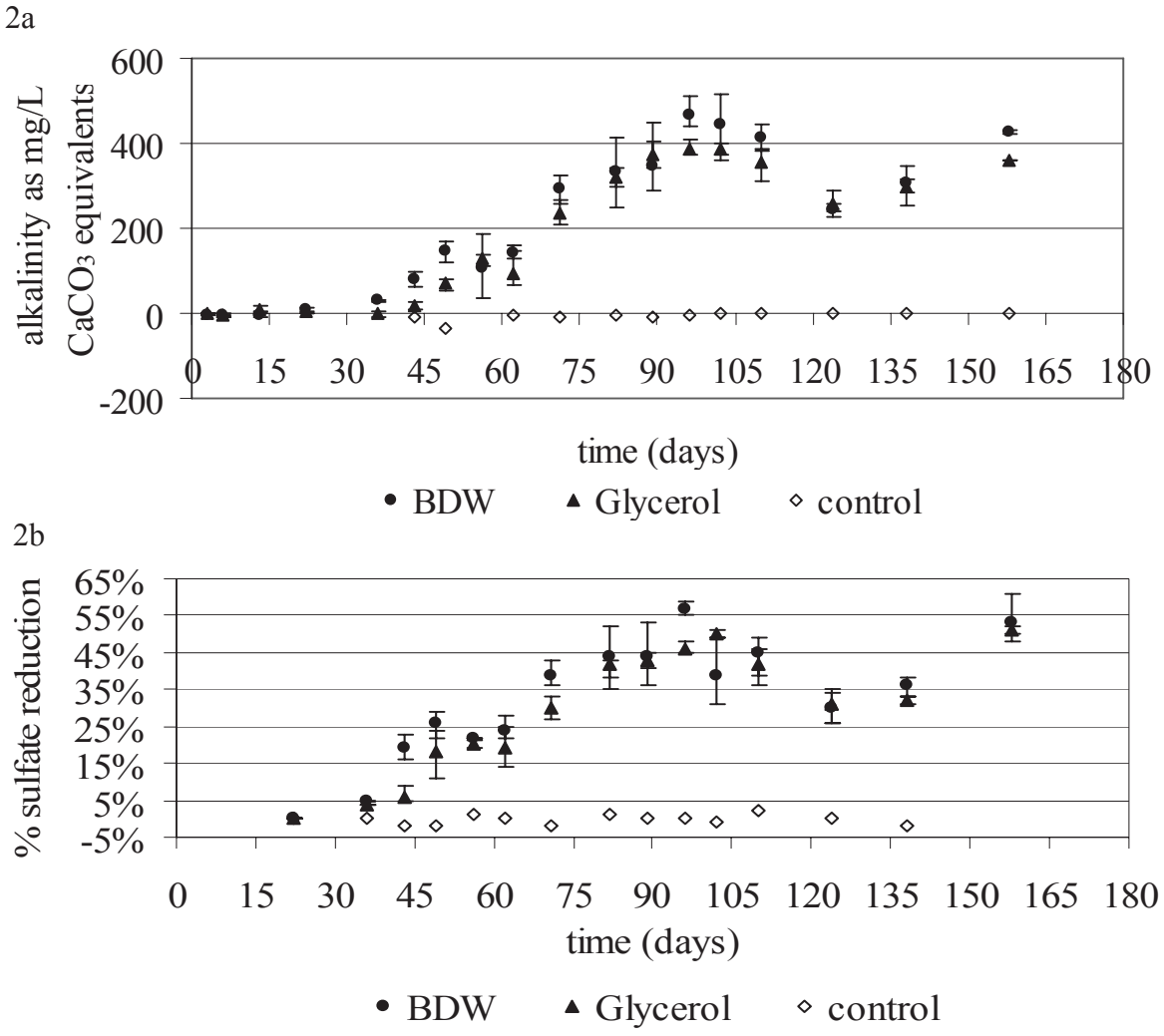

Figure 2. Alkalinity and sulfate reduction correlate closely; variations in sulfate reduction (2a) and alkalinity (2b) observed in triplicate columns are indicated by error bars.

Table 2. BDW and glycerol columns have similar levels of sulfate reduction and sulfide production, and standard deviations show triplicate BDW columns had similar variations to triplicate glycerol columns.

Sulfate Reduction					Sulfide Production			
	BDW		Glycerol		BDW		Glycerol	
Day	mean	std dev	mean	std dev	mean	std dev	mean	std dev
36	5%	0%	4%	0%	16	3	4	1
43	19%	4%	6%	2%	42	5	4	1
49	26%	4%	18%	8%	na*		na	
56	22%	0%	20%	1%	na		na	
62	24%	2%	15%	1%	67	5	22	3
72	39%	5%	30%	3%	na		na	
84	44%	9%	42%	3%	na		na	
89	44%	9%	43%	3%	na		na	
96	57%	2%	46%	2%	na		na	
102	39%	9%	50%	1%	70	20	58	8
110	45%	5%	42%	5%	na		na	
124	30%	4%	31%	5%	64	8	62	12
138	36%	3%	32%	1%	42	9	58	8
158	53%	7%	53%	4%	71	9	55	3

*na= not analyzed

columns showed variation on days 43 (range 11 to 28 mg/L, mean 18 mg/L) and day 62 (range 67 to 145 mg/L, mean 95 mg/L), with remaining days having variations within 20% of the mean and 7 sample days within 10% of the mean.

Influent sulfate water was started at pH 6.0 for the first 3 days, maintained at pH 7.3 through day 13, and reduced to 7.0, 6.5, 6.0, and 5.5 on days 14, 63, 82, and 99 respectively (Figure 3). The effluent pH declined temporarily when the influent pH was decreased. In general, the effluent pH rose steadily from day 30 on. Between day 71 and day 82, the effluent pH increased significantly in both sets of columns, from 6.8 to 7.8, and remained above 7.0 for the remainder of the experiment, despite the decreasing influent pH. A net increase in pH was observed from day 71 on.

Sulfide production

Soluble sulfide in BDW columns increased from 4 mg/L on day 22 to 67 mg/L on day 62 (Figure 4). Sulfide in glycerol columns increased less rapidly, from 4 mg/L on day 22 to 31 mg/L on day 62. No measurements were taken between day 62 and day 102. Except for a drop on day 138, columns remained near 70 mg/L between days 102 and 158. Glycerol columns remained near 60 mg/L from day 102 to day 158. Triplicate BDW columns showed similar variation

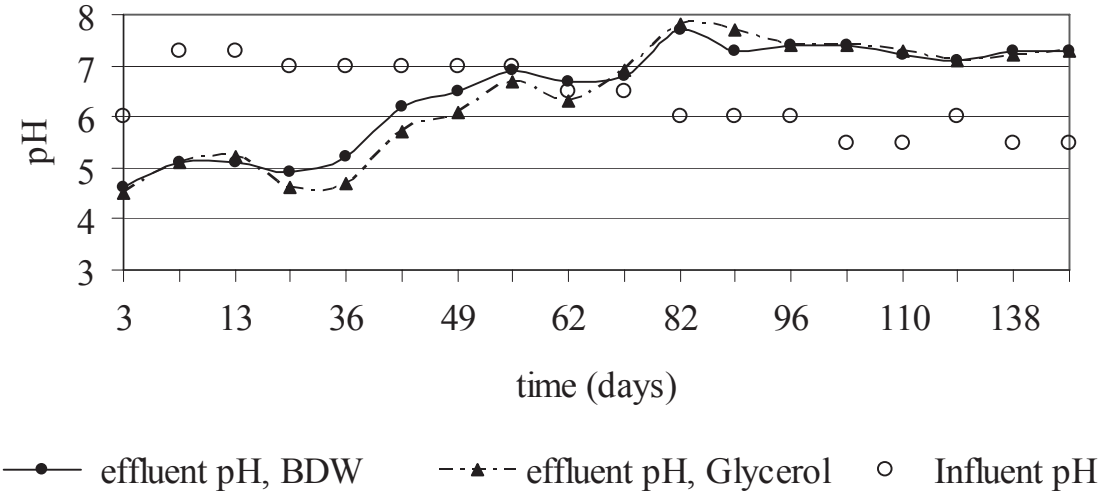

Figure 3. Column influent and effluent pH. The pH increased steadily after day 30, with brief declines as influent pH was reduced on days 14 and 63; a net pH increase was observed from day 71 on, despite decreasing influent pH.

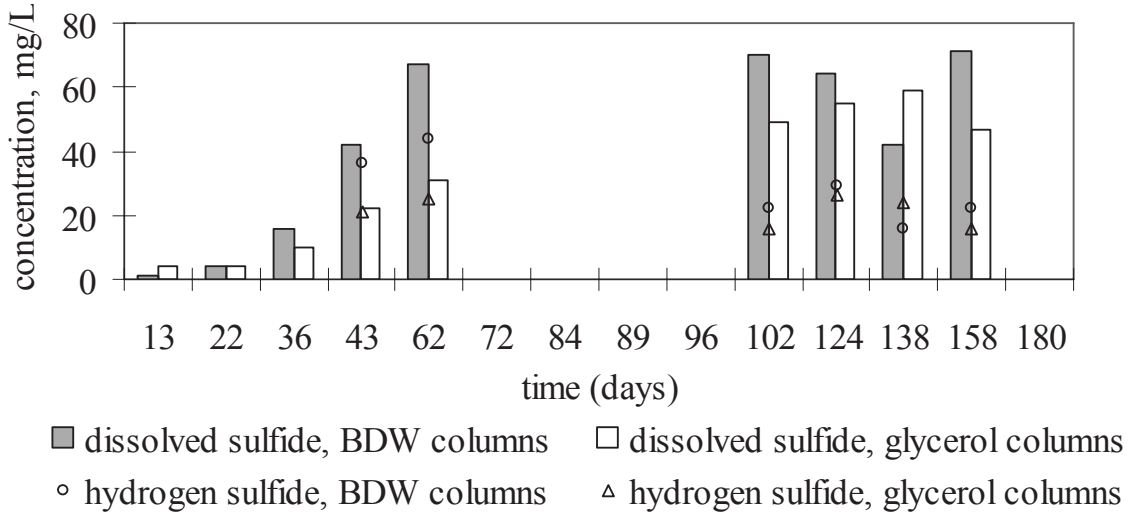

Figure 4. BDW columns produced more dissolved sulfide than glycerol columns initially, but soluble sulfide and hydrogen sulfide remained relatively consistent through the latter part of the experiment. Hydrogen sulfide was determined by calculation (Equation 4).

as triplicate glycerol columns (Table 2). Hydrogen sulfide in the glycerol columns remained at 16 to 26 mg/L of S in the effluent throughout the experiment (although fluctuations may have occurred in the data gap), while hydrogen sulfide in the BDW columns was initially higher at 36 to 44 mg /L of S, then dropped to 16 to 26 mg/L of S at day 102, where it remained through the latter months of the experiment.

Metabolic products

Full analysis was not conducted between days 15 and 62, or on day 138, due to instrument problems. Residues of propionic acid were observed in BDW columns on day 62 and in glycerol columns on days 71 and 82 (Table 3), and qualitative analysis indicated this was the primary metabolic acid observed in the first two weeks (data not shown). Complete resolution of lactic and formic acid peaks was not obtained; the combined concentrations remained below 25 mg/L, and were most commonly observed below 10 mg/L. Tentatively, more lactic acid was observed during the initial weeks and more formic acid observed later.

Although some unmetabolized glycerol was observed in BDW columns on day 62, by day 71 all glycerol was consumed to below the limit of detection (15 mg/L), as was propionate. Between days 62 and 102, there was little change in the total metabolic acid production. Acids reached a low of 34 mg/L on day 96 coincident with the highest sulfate reduction, but otherwise remained between 45 and 60 mg/L until day 124. On day 102, coincident with declines in sulfate reduction and alkalinity production, acid production increased. By day 110, SRB were no longer able to metabolize the glycerol provided, as indicated by the glycerol observed in the effluent water. After mineral injections were reinstated on day 125, glycerol consumption increased, and by day 158, all glycerol had been consumed with no apparent production of carboxylic acids.

The glycerol columns followed essentially the same pattern as the BDW columns. There was little change between day 71 and 110, with the sum of metabolic acids remaining between 35 mg/L and 45 mg/L, except for a low of 15 mg/L on day 89. Initial residual propionate (data not shown) was followed by residual acetic and formic acids until loss of column activity on day 124. There was a consequential rebound of glycerol consumption as minerals were injected. Acid concentrations were not measured in either set of columns on day 138; the decrease in effluent glycerol suggests that acids would have been observed on day 138. Neither acids nor glycerol were detected in column effluent on day 158, indicating full recovery of column activity.

Discussion

In all respects, columns fed BDW responded similarly to those fed glycerol, indicating that SRB will use the carbon available in BDW to reduce sulfate, produce alkalinity, and raise pH. As acclimation in the columns was occurring, alkalinity increased and consumption of the produced metabolic acids was complete at the end of the experiments.

Nutrient addition was found to alter the SRB performance. Nutrients were injected daily until day 30, after which they were introduced only twice a week but at increased concentration. This change did not affect column activity. Reducing injections to once a week slowed the rate of increase of alkalinity production and sulfate reduction. Removal of all nutrients had no impact for ten days, after which declines were observed in all columns; resumption of column activity did not occur until injections of the main mineral solution were reinstated. The main mineral solution contained primarily iron, with some calcium and small concentrations of nitrogen, phosphorous, and magnesium.

BDW columns reached higher maximum reduction rates and outperformed glycerol in sulfide and alkalinity production, suggesting that BDW columns reduced more sulfate than columns fed reagent-grade glycerol, although the amounts of glycerol provided to each were identical. This may be accounted for by

Table 3. Influent glycerol and metabolic acids observed in columns; na = not analyzed; nd = below the limit of detection

Carbon concentration (mg/L)	Day 62	Day 71		Day 82		Day 89		Day 96		Day 102		Day 110		Day 124		Day 138		Day 158	
Influent	B	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G
glycerol	na	218	206	218	161	283	272	248	257	247	239	241	259	267	246	272	247	242	228
Effluent																			
propionic acid	30	nd	4	nd	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	na	na	nd	nd
acetic acid	22	53	24	35	19	30	11	9	36	36	33	7	17	14	13	na	na	nd	nd
lactic, formic acids	8	7	6	16	15	15	4	21	24	18	10	6	14	nd	nd	na	na	nd	nd
glycerol	16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	25	nd	90	127	33	24	nd	nd

the methanol and carboxylic acids present in the initial biodiesel waste solution. Although SRB can utilize both fatty acids and methanol, electron accounting determined that methanol alone could account for the increased sulfate reduction. While the fatty acid content of the BDW was not measured, the biodiesel waste solution contained 8% methanol, so that BDW contained, on a percent basis, a ratio of eight glycerol (64%) to one methanol (8%). Four moles of methanol will reduce three moles of sulfate (Equation 5).

$$3\,SO_4^{2-} + 4\,CH_3OH + 2H^+ \rightarrow 3\,H_2S + 4\,HCO_3^- + 4\,H_2O \quad (5)$$

With 14 moles of sulfate reduced per 8 moles of glycerol, and 0.75 moles of sulfate reduced per one mole of methanol, there is combined reducing power of glycerol and methanol of 14.75 moles of sulfate, compared to 14 moles of sulfate reduced by 8 moles of glycerol alone; i.e., it is expected that the glycerol-methanol BDW solution would be able to reduce 5% more sulfate than glycerol alone. BDW columns reduced an average of 4.7% more sulfate than glycerol columns from day 36 to 158.

As alcohol was consumed in the initial stages, the produced metabolic acids decreased the pH of the system, keeping the effluent pH lower than influent. As indicated in Table 3, SRB were able to consume glycerol and acids at least to day 89. The trend from day 62 to 89 suggests that all carbon sources would have been completely consumed shortly after day 89, had nutrients not been withheld. From day 89 to 102, the SRB were still able to consume glycerol, but were not able to consume all acids produced. By day 124, they lost the ability to consume all glycerol. A steep decline in alkalinity generation and a correlated but lesser decline in the ability to raise pH were observed between day 110 and day 124. By day 158 (after resumption of nutrient addition on day 125), all of the alcohols and acids had been consumed below the limit of detection, and sulfate reduction and alkalinity had fully recovered.

Hydrogen sulfide is toxic to SRB and may have been involved in the reduced activity of SRBs in the columns. The concentration of hydrogen sulfide causing toxicity varies in the literature from a low of 40 mg/L to a high of 124 mg/L (Kaksonen et al. 2004; Luo 2004; Li et al. 1996; Maillacheruvo et al. 1993; McCartney and Oleszkiewicz 1991; Okabe et al. 1995). In similar column experiments, activity was found to decline when sulfide concentrations exceeded 40 mg/L as H_2S, and stabilized at 20 mg/L to 40 mg/L over time (Luo 2004). Injection of iron can reduce soluble sulfide concentrations, by formation of insoluble FeS.

The concentration of total carbon may have affected sulfide levels. Carbon was added to influent sulfate water as glycerol equivalents, not as carbon equivalents. The BDW contains methanol and fatty acids in addition to glycerol, so that the BDW columns received higher concentrations of total carbon. Dissolved sulfide levels in the BDW columns were initially higher than in glycerol columns, as might be expected if more carbon was provided to the BDW columns, and in the first two months of the experiment, sulfide concentrations increased with increasing sulfate reduction. Except for a drop in BDW sulfides on day 138 (42 mg/L), both sets of columns remained between 55 and 70 mg/L soluble sulfide through the latter part of the experiment, with hydrogen sulfide at 15 to 30 mg/L as calculated (Equation 4). Sulfate reduces to soluble sulfides (speciation as S^{2-}, HS^-, and H_2S dependent on pH), thiosulfates, and possibly polysulfides (Langmuir 1997). This study measured only the sum of soluble sulfides. The consistent concentration of soluble sulfide observed in columns after day 102, despite changing sulfate concentrations, suggests that fluctuations may have been occurring in one of the sulfide forms not measured (i.e. polysulfides, thiosulfates, or FeS).

Summary

Biodiesel waste shows potential for use in semi-passive bioreactor systems treating AMD. Sulfate is reduced, pH raised, and there is no apparent acid buildup following acclimation. BDW is available at little or no cost, and the indication that the carbon may be completely used by SRB make this an attractive carbon source. Further study is needed to determine the species of sulfide produced, the diversity of the bacterial community that develops, and the sensitivity of the system to fluctuations of environmental parameters, such as changes in flow and temperature that may be experienced in a natural setting.

References

ATCC (American Type Culture Collection) (2002) Vitamin supplement MD-VS. www.atcc.org/common/products/MicrobialMediaSupplements.cfm

APHA (1998) Standard Methods for the Examination of Water and Wastewater. 20th edit, American Public Health Assoc, Washington, DC, USA, Part 4000, p 4:162– 4:167

Kaksonen AH, Franzmann PD, Puhakka JA (2004) Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized bed reactor. Biotech and Bioeng 86 (3): 332-343

Langmuir D (1997) Aqueous Environmental Geochemistry. Prentice Hall, NY, USA, 576 p

Li YY, Shirley L, Fang HHP (1996) Interactions between methanogenic, sulfate-reducing, and syntrophic acetogenic bacteria in the anaerobic degradation of benzoate. Wat Res 30 (7): 1555-1562

Luo Q (2004) Sulfate reducing bioreactors: efficiency of three carbon sources and removal of arsenic and selenium from mine water. MS thesis, Univ of Nevada, Reno, USA, 79 pp

Maillacheruvu KY, Parkin GF, Chen YP, Kou WC, Oonge ZI, Lebduschka V (1993) Sulfide toxicity in anaerobic systems fed sulfate and various organics. Wat Environ Res 65 (2): 100-108

McCartney DM, Oleszkiewicz JA (1991) Sulfide inhibition of anaerobic degradation of lactate and acetate. Wat Res 25 (2): 203-209

McCoy M (2005) An unlikely impact: the growth of biodiesel has big implications for the oleochemical industry. Chemical and Eng News, Feb 21

National Biodiesel Board (2006) Active plants – production map and table. www.biodiesel.org/resources/fuelfactsheets

Okabe S, Nielsen PH, Jones WL, Characklis WG (1995) Sulfide product inhibition of *Desulfovibrio desulfuricans* in batch and continuous cultures. Wat Res 29 (2): 571-578

Postgate J (1979) The sulphate-reducing bacteria. Cambridge Univ Press, 151 pp

Soap and Detergent Association (2005) SDA Glycerine Innovation Award. http://cleaning101.com/whatsnew/05-02-05.html

Tsukamoto T and GC Miller (1999) Methanol as a carbon source for bioremediation of acid mine drainage. Wat Res 33 (6): 1365-1370.

Tsukamoto T, Killian H, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage; low temperature, low pH, and matrix investigations. Wat Res 38 (6): 1405-1418

Submitted July 6, 2006; revised July 29, 2006; accepted August 4, 2006