PERGAMON

Water Research 32 (2002) 167–177

WATER RESEARCH

www.elsevier.com/locate/watres

Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment

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Received 1 October 2000; received in revised form 1 April 2001; accepted 1 April 2001

Abstract

Sulfate-reducing reactive walls installed in situ in the path of acid mine drainage contaminated groundwater, present a promising passive treatment technology. However, a rigorous and methodical selection of the most appropriate reactive mixture composition still needs to be investigated. The aim of this study was the selection of the most reactive medium using a multiple factor design and the modeling of the sulfate-reduction rate. Reactivity of 17 mixtures was assessed in batch reactors (in duplicates) using a synthetic AMD. Results indicate that within 41 days, sulfate concentrations decreased from initial concentrations of 2000–3200 mg/l to final concentrations of <90 mg/l. Metal removal efficiencies ranged between 51–84% for Ni and 73–93% for Zn. Generated sulfate-reduction rate predictive models which had very satisfactory parameters ($R^2 = 0.86$, $F = 62.38$ (p-level $< 10^{-13}$) and $R^2 = 0.90$, $F = 62.30$ (p-level $< 10^{-13}$)) identified poultry manure and two other carbon sources as the critical variables for sulfate-reduction rate. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Acid mine drainage; Heavy metals; Sulfate reduction; Reactive walls; Stepwise regression; Modeling

1. Introduction

Both surface and underground mining create the potential for acid mine drainage (AMD), a major environmental problem which can cause widespread and often intermittent pollution [1]. AMD generation is a result of bacterial and chemical oxidation of pyrite and other sulfide minerals in mine waste rock and tailings. The complete oxidation reaction of pyrite can be summarized by the following equation [2]:

$$4FeS_{2(s)} + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_{3(s)} + 8SO_4^{2-} + 16H^+. \quad (1)$$

Depending on the host rock geology, the resulting acid runoff can solubilize various heavy metals in its path. Thus, a low pH and alkalinity, and high concentrations of sulfate and heavy metals often characterize mine drainage that may discharge to surrounding surface water.

The acidic drainage may also be transported downward through the tailings where it undergoes a series of acid neutralization and secondary mineral-precipitation reactions. The resulting water, characterized by neutral pH and high concentrations of sulfate, and low to moderate concentrations of heavy metals, can migrate into the groundwater flow system and ultimately discharge into oxygenated surface water bodies [3].

Treatment processes including either ex situ or in situ procedures are able to reduce the potential hazards, using chemical or biological approaches. A heavy metal confinement technique based on sulfide precipitation is more suitable for AMD treatment over the conventional hydroxide precipitation because of the higher degree of metal removal at low pH (pH 3–6). Meanwhile, the sludge characteristics are improved, i.e. chemically more

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PII: S0043-1354(01)00238-X

stable, denser and less voluminous [4]. However, the chemical sulfide precipitation is still an expensive process, which produces heavy metal contaminated sludge that needs treatment and disposal.

Bacterial sulfate reduction has been identified as a potentially valuable process for removing contaminants from mine drainage [5,6]. Due to its ability to convert sulfate to H_2S, an anaerobic group of bacteria is used for inexpensive sulfide generation. Sulfate-reducing bacteria (SRB) obtain energy for growth by oxidation of organic substrates (CH_2O), remove hydrogen atoms from the organic molecule and use sulfate as the terminal electron acceptor [7]:

$$2CH_2O + SO_4^{2-} \overset{SRB}{\rightarrow} H_2S + 2HCO_3^{-}. \qquad (2)$$

Depending on solution pH, hydrogen sulfide and bicarbonate ions equilibrate into a mixture of H_2S, HS^-, S^{2-}, and CO_2, HCO_3^-, CO_3^{2-}. The presence of carbonates and bicarbonates entails a buffering of acid drainage. Soluble sulfide binds with most metals (Me) to form insoluble metal complexes that will precipitate:

$$H_2S + 2Me^{3+} \rightarrow 2Me^{2+} + S^0 + 2H^+, \qquad (3)$$

$$H_2S + Me^{2+} \rightarrow MeS + 2H^+. \qquad (4)$$

The remediation and prevention of acid mine drainage through the use of sulfate-reducing reactive walls is a possible alternative to conventional collection and treatment program [8,9]. The reactive wall consists of a reactive cell installed below the ground surface in the path of AMD contaminated ground waters. The composition of the reactive mixture is crucial for the efficiency of the treatment process [3,10,11]. Waybrant et al. [3] assessed the reactivity of eight organic–carbon reactive mixtures containing four components: an organic source, a bacterial source, a neutralizing agent, and a porous medium. They found that the most reactive mixtures were those containing several organic sources. However, a rigorous and statistically based selection of the most reactive mixture still needs to be investigated.

Therefore, the objective of this study was to select the most appropriate reactive mixture composition using a multiple factor design based on sulfate-reduction rate. For this purpose, upper and lower boundaries were settled for five components (three different organic substrates and two porous supports), while the other mixture components (bacterial source, neutralizing agent and urea) remained constant. Moreover, a predictive model for sulfate-reduction rate was generated in order to identify the variables that significantly affect it.

2. Materials and methods

2.1. Experimental set-up

Laboratory batch experiments using 500 ml glass reaction flasks were conducted at room temperature (22±1°C), to assess the potential of different reactive mixtures to promote sulfate reduction. A synthetic mine drainage was used to ensure constant quality of water throughout the study. Simulated AMD composition (Table 1) was based on the chemical characterization (unpublished data) of a groundwater plume discharging from a mine site in Abitibi (PQ, Canada). Required quantities of sulfate salts (Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, and Na^+) were added in a 1-l volumetric flask and made up to volume with deionized water (18.2 MΩ cm). 6 N NaOH was slowly added to neutralize proton acidity until final pH was around 5.5–6.0.

Reactive mixtures used in batch tests consisted of a bacterial source, a pH neutralizing material, urea, a carbon source, and a porous media, all proportions considered on a dry basis (required wet weights were determined after water content determination on separate samples).

Urea, a chemical which has a high percentage of nitrogen (65%), was added to reach an appropriate C : N : P ratio of 110 : 7 : 1 to support high SRB activity [12]. The added percentage of urea was constant (3 wt%) for all mixtures.

The SRB source used was a creek sediment (the presence of SRB was suspected by the black color and the H_2S odor of the sediment) sampled from the anaerobic zone of an AMD-affected stream in an inactive mine area (Eastern Townships, PQ, Canada). Enumeration of SRB in the sediment using the Most Probable Number technique [13] showed a SRB concentration of 30×10^3 cells/100 ml. The added percentage of bacterial source was 37 wt%, and was constant for all mixtures.

Table 1
Composition of synthetic acid mine drainage

Element	Desired concentration (mg/l)	Chemical used	Amount of chemical added per liter (mg)
Fe	800	$FeSO_4 \cdot 7H_2O$	3971
Cu	0.08	$CuSO_4$	0.2
Zn	7.3	$ZnSO_4 \cdot 7H_2O$	32
Ni	0.8	$NiSO_4 \cdot 6H_2O$	3.5
Co	0.89	$CoSO_4 \cdot 7H_2O$	4.2
Mn	47	$MnSO_4 \cdot H_2O$	144.5
SO_4	2940	Na_2SO_4	1093
pH	5.5–6.0	—	—

Two weight percent limestone (Calcium Carbonate Light, ACS reagent grade, Anachemia, Canada) was added to each mixture in order to maintain near neutral condition [6].

After the mixtures were added, all flasks were filled with 300 ml of deoxygenated synthetic mine drainage (by sparging with N_2), and the flasks were sealed.

2.2. Experimental design

The input factors chosen for the experimental design were based on the experience gained from previous case studies [3,10,11]. Constraints were imposed for three carbon sources, and for two porous media.

The carbon source for the experiments was prepared using mixtures of cellulosic wastes (wood chips, leaf compost) and poultry manure, because results of previous research indicated that cellulosic waste alone would not sustain SRB growth [10]. Maple wood chips (Bergeron et Fils, Labelle, Canada), leaf compost (Saint-Michel Environmental Complex, Montreal, Canada), and composted poultry manure (Perron W.H., Montreal, Canada) were selected for their ability to sustain sulfate reduction. These substrates contain carbon with varying degrees of liability, either complex or simple organic compounds. Thus, reactive mixtures should have both long term and easily available carbon sources, which should ensure shorter and longer periods of time for degradation. The upper and lower percentages (wt%) for these three components were:

$$0\% < \text{wood chips } (X_1) < 30\%, \tag{5}$$

$$0\% < \text{leaf compost } (X_2) < 30\%, \tag{6}$$

$$10\% < \text{poultry manure } (X_3) < 20\%. \tag{7}$$

Two types of porous support (reactive or inert) were used in order to assess their different influence on the SRB activity, which strongly depends on the presence of a physical support [14]. A readily available porous support (i.e. oxidized tailings) was obtained from the previously mentioned study mine site (Abitibi, PQ), whereas clean silica sand was obtained from a local stone quarry. The particle size distribution (dry wt%) [15] showed that silica sand was composed of 2% gravel (> 4.75 mm), 12% medium sand (2–0.5 mm), 82% fine sand (0.5 mm–75 μm) and 4% fines (< 75-μm). Oxidized tailings were composed of 24% fine sand and 76% fines.

The upper and lower percentages (wt%) for these two components were:

$$0\% < \text{oxidized tailing } (X_4) < 8\%, \tag{8}$$

$$0\% < \text{silica sand } (X_5) < 8\%. \tag{9}$$

In addition of these upper and lower bounds (Eqs. (5)–(9)), the following factor constraints were imposed:

$$30\% < X_1 + X_2 < 40\%, \tag{10}$$

$$5\% < X_4 + X_5 < 8\%. \tag{11}$$

The imposed total for these five factors was:

$$X_1 + X_2 + X_3 + X_4 + X_5 = 58\%. \tag{12}$$

The remaining 42% consisted of 3% urea, 37% creek sediment and 2% limestone.

After all constraints on mixture proportions were processed using the Experimental Design operating module of STATISTICA software [16], the 5 factor mixture design generated 16 vertex and one centroid points to be tested (Table 2). Consequently, 17 reactors were run in duplicates in order to assess reactive mixture efficiencies in terms of sulfate reduction and heavy metal removal.

2.3. Sampling and analysis

All sampling sessions were conducted in an anaerobic glove box under nitrogen atmosphere. Mine drainage water samples were removed with a syringe, by piercing the reaction flasks sampling ports fitted with Teflon-lined septa. Reactive mixtures were allowed to settle for approximately 12 h before sampling on day 0. Different volumes of water samples were also collected on days 5,

Table 2
Composition of reactive mixtures (R_1–R_{17}) used in batch experiments (dry wt%)

Variable components[a]	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}	R_{14}	R_{15}	R_{16}	R_{17}
Cellulosic wastes																	
Wood chips	30	30	0	0	30	10	30	10	30	30	3	3	30	30	10	10	17.87
Leaf compost	0	0	30	30	10	30	10	30	3	3	30	30	10	10	30	30	17.87
Organic wastes																	
Poultry manure	20	20	20	20	10	10	10	10	20	20	20	20	13	13	13	13	15.75
Oxidized mine tailings	8	0	8	0	8	8	0	0	5	0	5	0	5	0	5	0	3.25
Silica sand	0	8	0	8	0	0	8	8	0	5	0	5	0	5	0	5	3.25

[a] Constant proportion components: bacterial source 37 wt%, neutralizing agent 2 wt% and urea 3 wt%.

9, 13, 17, 21, 25, 33, and 41. Measurements of pH (ORION ROSS 8175 BN electrode), redox potential (ACCUMET electrode, Ag/AgCl, 4.00 M KCl), alkalinity (2N H_2SO_4 colorimetric titration), and total dissolved sulfide (methylene blue method) were determined immediately after sample collection according to Standard Methods [17]. The pH electrode was calibrated using pH 4.0, 7.0, and 10.0 buffer solutions, and the ORP electrode performance was confirmed using pH 4.0 and pH 7.0 quinhydrone standard solutions. According to the manufacturer's specification, the potential developed by the ORP electrode relative to standard hydrogen electrode at 25°C is 199 mV.

pH and ORP were analyzed directly in the sampling solution while alkalinity and total dissolved sulfide concentrations were determined on a filtered (0.45 μm cellulose acetate filter paper) sample. Filtered samples were also used for Ni, Zn, and SO_4^{2-} determination. Ni, and Zn concentrations were determined by atomic absorption spectrometry (AAS), while sulfate and sulfide concentrations were determined by spectrophotometry using standardized substances and an hach digital spectrophotometer DR/2010 [18]. TOC was determined on a non-filtered sample using a total carbon analyzer (Dorhmann DC-80) by ultra-violet promoted persulfate oxidation [17]. Ni and Zn concentrations were determined in tailings and sediments using inductively coupled plasma atomic emission spectrometry (ICP-AES-TJA, IRIS/Advantage model) after digestion with HNO_3, HF, and $HClO_4$ according to Standard Methods [17].

SRB were enumerated in the mixture suspensions on day 0 and day 41 using the MPN technique [13]. Inoculated tubes were incubated under anaerobic conditions at 30°C for 21 days. The growth of sulfate-reducing bacteria was estimated by the formation of a black FeS precipitate.

3. Results and discussion

3.1. Sulfate-reduction rates

Batch experiments were run until depletion of AMD sulfate concentrations. After 41 days, sulfate concentrations decreased from 2020 to 3254 mg/l to <90 mg/l, sulfate removal being dependent on mixture composition. Sulfate-reducing conditions developed rapidly in all mixtures after an initial acclimation period, which ranged from 0 to 21 days (Fig. 1). Significant populations of SRB were present throughout the experiment suggesting that sulfate removal occurred following bacterial activity. Average SRB concentrations in the 17 mixture suspensions were $5.36 \pm 3.22 \times 10^3$ cells/100 ml on day 0, and $2.54 \pm 2.22 \times 10^4$ cells/100 ml on day 41 of the experiment.

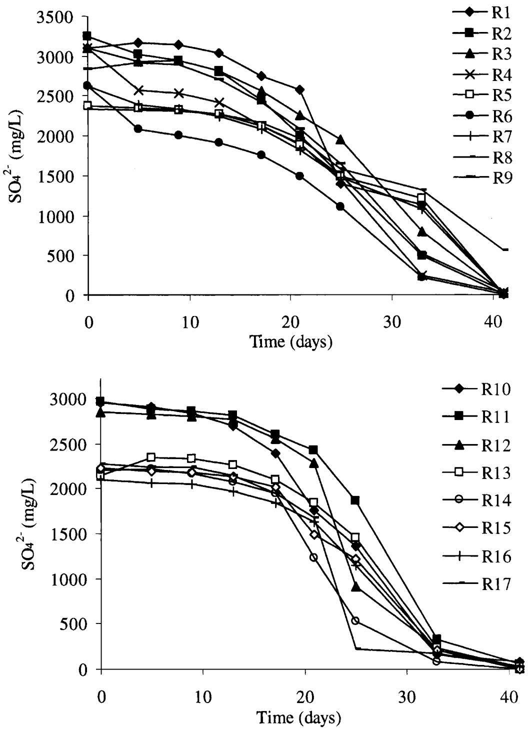

Fig. 1. Sulfate concentration versus time for batch reactive mixtures.

Sulfate-removal rate per day (T_{0-41}) was calculated for each reactor using linear least-squares regression analysis for the entire period of study (Table 3). The average sulfate removal rate for the 17 reactors was 70.7 ± 12.1 mg/l d, and the coefficients of determination (R^2) ranged from 0.82 to 0.98. A second sulfate-reduction rate, T_{Modified}, was calculated disregarding early-time data which may have been affected by the acclimation periods, and late-time data which may have been sulfate limited [3]. A 10% variation between two consecutive sulfate concentration values was used as a criterion to establish lag-phase and final sulfate-limited phase. This criterion was chosen since spectrophotometer precision for sulfate analysis was ±9.8%. For this second rate, the average for the 17 reactors was 103.7 ± 26.8 mg/l d, and the coefficients of determination (R^2) ranged from 0.78 to 0.99. Sulfate-reduction rates varied between 35.6 and 87.6 mg/l d for T_{0-41}, and between 45.9 and 156.3 mg/l d for T_{Modified} depending on mixture composition. The results for this second assessed rate (T_{Modified}) are comparable with previously published data [19], where the sulfate-reduction rate varied between 22.5 and 154.4 mg/l d during batch experiments conducted with five different organic mixtures. Both rates will be further considered as dependent variables when generating statistical models.

Table 3
Calculated sulfate-reduction rates (mg/l d) for the 17 batch reactors

Reactor	T_{0-41}		Range	T_{MODIFIED}		Range
	Replicate 1	Replicate 2		Replicate 1	Replicate 2	
1	85.8	77.9	7.9	115.1	104.2	10.9
2	86.5	87.6	1.1	100.3	104.9	4.6
3	85.4	70.8	14.6	128.2	97.2	31.0
4	78.0	79.8	1.8	74.6	83.0	8.4
5	53.2	57.8	4.6	82.3	78.9	3.4
6	66.5	64.6	1.9	68.2	64.3	3.9
7	63.1	58.4	4.7	63.1	79.7	16.6
8	35.6	52.0	16.4	45.9	82.6	36.7
9	77.7	79.1	1.4	100.7	118.1	17.4
10	84.8	84.0	0.8	132.2	125.5	6.7
11	81.6	77.0	4.6	129.4	119.9	9.5
12	83.6	84.3	0.7	153.5	156.3	2.8
13	61.6	64.0	2.4	95.8	97.2	1.4
14	71.7	67.8	3.9	131.8	102.7	29.1
15	63.4	63.0	0.4	93.1	96.1	3.0
16	58.8	58.8	0.0	92.6	107.0	14.4
17	70.7	67.6	3.1	150.2	150.3	0.1

As shown in Table 3, repeatability between duplicates was good with an average range of 4.1 mg/l d for T_{0-41}, and an average range of 11.7 mg/l d for T_{Modified}. Reactors 3 and 8 had a lower repeatability with ranges between duplicates slightly exceeding or approaching upper control limit (UCL), which was 13.3 mg/l d for T_{0-41} and 38.4 for T_{Modified}. The UCL value (UCL = 3.686 process sigma) was calculated according to Montgomery [20].

The experiments in this study were conducted at room temperature (22 ± 1°C). Considering future groundwater remediation, the calculated sulfate-reduction rates may not be directly transferable to lower temperature field conditions since the rates depend on temperature [7]. A lower temperature should slow down reaction kinetics, but is not expected to affect the relative rates of reaction associated with an individual mixture [3]. In fact, SRB tolerate temperatures from −5°C to 75°C and easily adapt to temperature changes [21].

3.2. Mine drainage quality

Ni and Zn concentrations versus time are presented in Fig. 2. Heavy metal removal efficiencies ranged between 51–84% for Ni (with an average of 72%), and 73–93% for Zn (with an average of 88%). Part of Ni and Zn initial concentrations were probably due to supplementary heavy metal input from mixture components like sediment (117 mg/kg Ni and 1383 mg/kg Zn) and oxidized tailings (27 mg/kg Ni and 477 mg/kg Zn). Furthermore, results showed that around 75% of total nickel removal and around 85% of total zinc removal was completed within 17 days, which represent less than half of the entire studied period. At this time, pH was stable near 8.1 ± 0.2 for all mixtures while average ORP was −454 ± 15 mV. Average initial pH was 9.0 ± 0.2 in all reactors (Fig. 3). Therefore, part of Ni and Zn initial removal could be attributed to their low solubility at this pH value. Average final pH and ORP were 7.9 ± 0.1 and −353 ± 96 mV, respectively. Sulfate-reduction rates in addition to SRB populations and very low ORP values, at which the SRB activity is optimum [11], suggest that sulfate reduction and at least part of metal removal was bacterially mediated. The low final iron concentrations (7.0 ± 5.1 mg/l) support the hypothesis of Fe removal by metal sulfide precipitation reactions.

Final average total sulfide concentration was 4.5 mg/l. According to Eqs. (3) and (4), dissolved sulfides bind with most metals to form insoluble metal sulfides. Waybrant et al. [3] also found that dissolved sulfide concentrations were controlled by metal-sulfide precipitation reactions with dissolved sulfide concentrations increasing only after metal concentrations were depleted.

Furthermore, final average alkalinity was 187.2 mg/l $CaCO_3$, and the average final TOC was 3044 ± 693 mg/l showing that all reactors had some carbon left (potentially available for SRB).

3.3. Statistical analysis

Statistical analysis was carried out using Quality Control, and Multiple Regression operating modules of STATISTICA. Among the 17 studied mixtures, the most reactive one was chosen after realizing the control

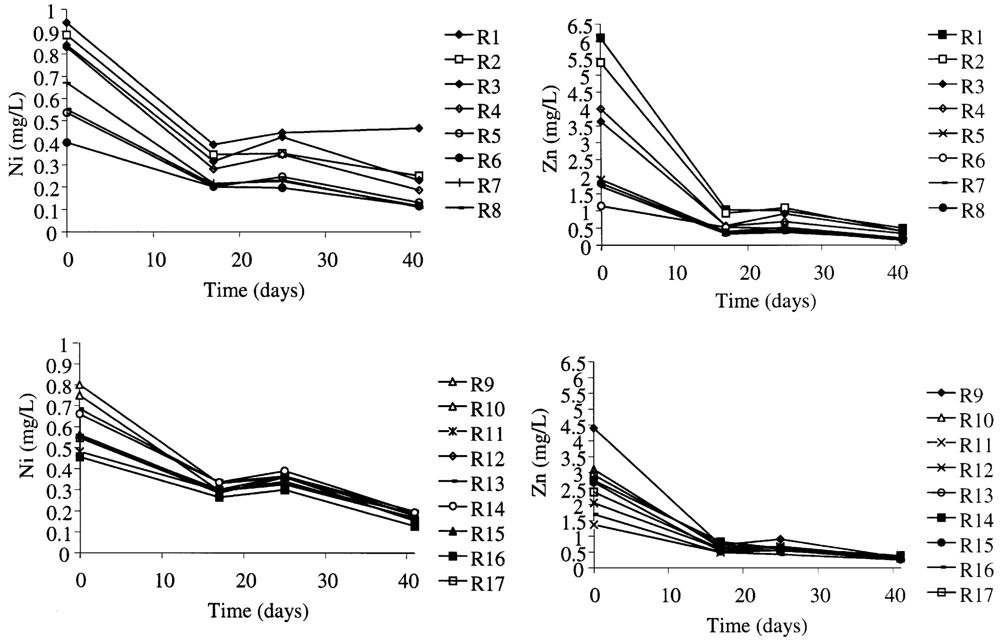

Fig. 2. Metal concentrations versus time.

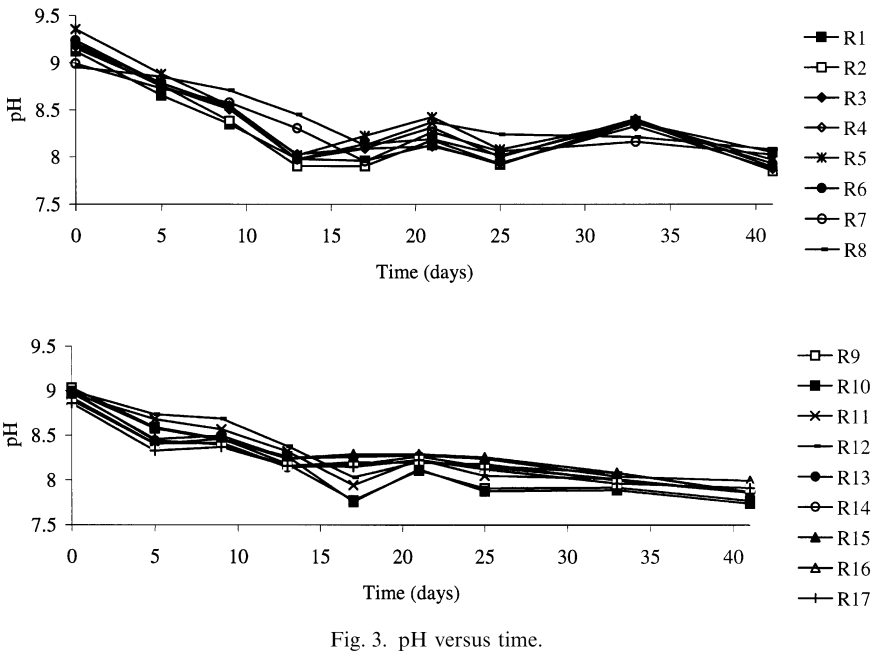

Fig. 3. pH versus time.

charts for the dependent variables. Imposed variables with measurements to produce the X-bar charts (Fig. 4) were sulfate-reduction rates, either T_{0-41} or T_{Modified}, while variables with sample identifiers were the 17 mixtures.

As shown in Fig. 4, the three most reactive mixtures in terms of sulfate reduction for T_{0-41} were R_2 (87.0 mg/l d), R_{10} (84.4 mg/l d) and R_{12} (83.9 mg/l d), while the least reactive was R_8 (43.8 mg/l d). For T_{Modified}, the most reactive mixture was R_{12} (154.9 mg/l d), the least reactive being also R_8 (64.3 mg/l d). Since the second calculated sulfate-reduction rate better reflects the sulfate reduction process by excluding initial and final phases, and since R_{12} reactor had a greater

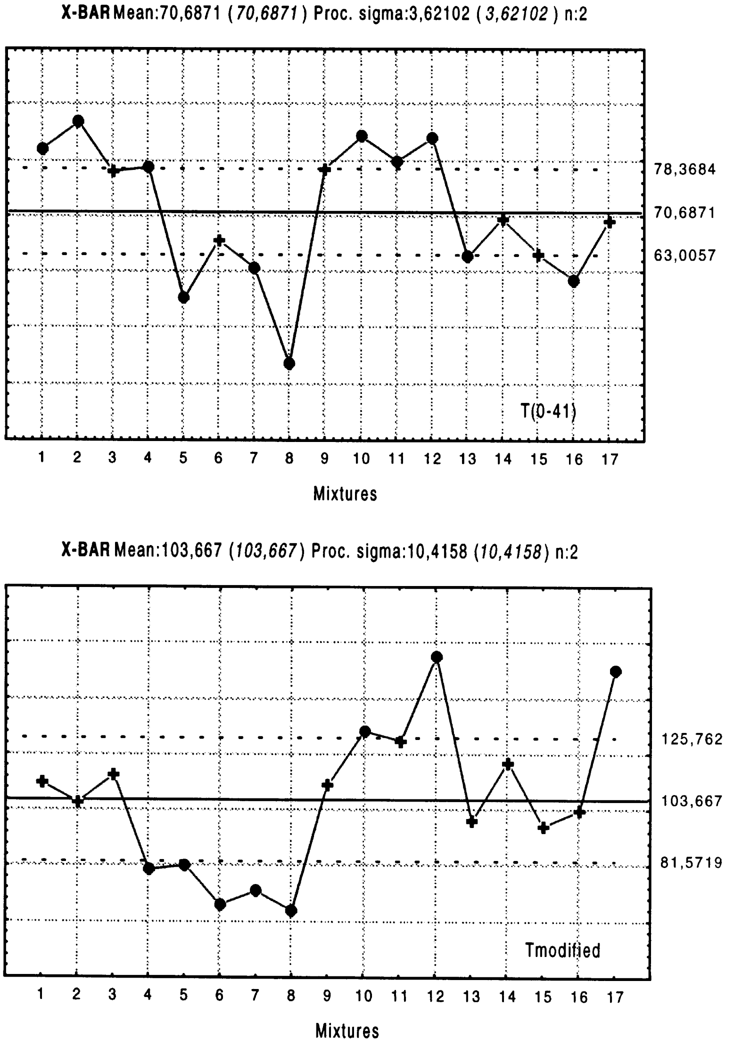

Fig. 4. Mean values of sulfate-reduction rates (mg/l d).

reactivity for both calculated rates, it could be considered the most reactive mixture. Mixture proportions of R_{12} are 3% wood chips, 30% leaf compost, 20% poultry manure, 0% oxidized mine tailings, and 5% silica sand (Table 2). Among the carbon sources tested, poultry manure (X_3) and leaf compost (X_2) had the highest considered percentages (Eqs. (6) and (7)), and porous support ($X_4 + X_5$) had the lowest percentage according to Eq. (11). It should be noted that the least reactive mixture (R_8) contains the lowest proportion of poultry manure (X_3) tested in this experimental design.

3.4. Modeling of sulfate-reduction rate

The statistical modeling of sulfate-reduction rate must take into account the nature of the experimental space defined by Eqs. (5)–(12). Mixture designs were modeled with first- or second-degree polynomials. Because of the linear dependence between the components (Eq. (12)), the polynomials were forced to go through the origin, that is with a zero intercept. As an alternative, ratios of components were used in order to break the linear dependence [22]. In the latter case, the polynomials were not subjected to have a zero intercept.

Two possibilities were considered for the modeling of sulfate-reduction rate (Y):

$$Y = \sum_{i=1}^{5} \alpha_i X_i + \sum \sum_{i \neq j}^{5} \alpha_{ij} X_i X_j, \qquad (13)$$

a response function with the mixture components (X_i), where subscripts i,j indicate the particular component $i, j = 1, 2, 3, 4, 5$, α being the partial regression coefficient, and

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i Z_i + \sum_{i=1}^{5} \beta_{ii} Z_i^2 + \sum \sum_{i<j}^{5} \beta_{ij} Z_i Z_j,$$

$$i, j = 1,\ 2,\ 4,\ 5, \tag{14}$$

a response function with mixture components ratios (Z_i), where β represents the partial regression coefficient, and

$$Z_i = \frac{X_i}{X_3}, \quad i = 1,\ 2,\ 4,\ 5. \tag{15}$$

Independent variable relationships in the form of ratios of their proportions are often considered in some areas of mixture experimentation [22]. The X_3 variable was chosen as a denominator among the five variables because it varies between 10% and 20% (Eq. (7)), being the only one that is never zero.

These two classes of models were fitted by linear least square with STATISTICA software. The second order terms X_iX_j were not statistically significant and not needed in the model described by Eq. (13). A reduced first order model with X_i components only was further adjusted but again, some components were not statistically significant. These results can be explained by the quasi-linear dependence of the components induced by the linear restrictions of Eqs. (10) and (11). As a final iteration trying to fit a second order model with the original components X_i, a forward stepwise regression was used. Forward selection starts by choosing as the one variable subset, the independent variable that accounts for the largest amount of variation in the dependent variable, being the one having the highest simple correlation with Y [23]. The second variable is chosen by seeking the maximum of the partial correlation of the remaining independent variables with the dependent variables, adjusted for the variable already chosen. Selection process stopped after three variables entered the model. The stepwise regression of T_{0-41} rate on the original variables generated the following equation:

$$Y = 35.93 + 2.205X_3 - 0.057X_2X_5 + 0.065X_3X_5. \tag{16}$$

This predictive model has a multiple determination coefficient (R^2) of 0.86, all regression coefficients being highly significant. According to the analysis of variance (Table 4), the F-criterion, which is the sum of squares due to regression over the sum of squares for error divided by the respective degrees of freedom was highly significant (62.38, p-level $< 10^{-13}$) meaning that the two groups (effects and residuals) are significantly different from each other.

The most important variable in the model is X_3, the poultry manure. This mixture component explains 78% of the sulfate-reduction rate variability and induces a sulfate-reduction rate increase of 2.205 for each 1% increase of its proportion. This can be explained by the faster decomposition of poultry manure simple organic compounds. Moreover, this fresh organic carbon source contains a higher concentration of short-chain, single-carbon-bond aliphatic, which are the preferred substrate of sulfate-reducing bacteria [8].

On the opposite, the addition of the two other terms in the model (X_2X_5 and X_3X_5) increased the R^2 by only 0.08 (from 0.78 to 0.86). The silica sand component (X_5) interacts positively with poultry manure (X_3) and negatively with leaf compost (X_2) but its overall contribution to the model is minimal.

Although the physical support properties are important for the SRB activity [14], these research results attest the minimal influence of the tested porous supports (either oxidized tailings (X_4) or silica sand (X_5)) on sulfate-reduction rate. Therefore, the use of oxidized mine tailings could be considered in mixture composition, because they are readily available on mining sites. However, mine tailings addition could entail side effects like decrease of reactive wall permeability, heavy metal addition to groundwater, or ferric minerals addition—which will delay the onset of sulfate reduction. Thus, in order to minimize side effects, clean silica sand is likely to be used.

As an alternative, the fitted ratio model for T_{0-41} rate previously described by Eq. (14), was

$$Y = 79.045 - 9.345Z_1Z_2 - 11.155Z_2Z_5 + 23.357Z_5 + 5.051Z_2Z_4. \tag{17}$$

This equation has a slightly higher value of R^2 (0.90) and an F value of 62.30 (p-level $< 10^{-13}$), all five components (X_1–X_5) being involved through their ratios defined by Eq. (14). Using a forward stepwise regression method, the first term selected ($R^2 = 0.76$) was

$$Z_1Z_2 = \frac{X_1X_2}{X_3^2}, \tag{18}$$

Table 4
Analysis of variance for original variables model

	Sums of squares	df	Mean squares	F	p-level
Regression	4351.06	3	1450.36	62.38	$< 10^{-13}$
Residual	697.47	30	23.25		
Total	5048.53				

which also confirms the supposition that the critical variables for sulfate-reduction rate were the three carbon sources: wood chips (X_1), leaf compost (X_2) and poultry manure (X_3). Furthermore, considering this term's negative sign, the smallest its value the higher the sulfate-reduction rate. A small Z_1Z_2 value implies either a high X_3 or a low X_1X_2 value. It should be noted that X_3 (poultry manure) is a second-degree term, its impact on sulfate-reduction rate being therefore much more important.

In accordance with the predictive model previously generated with original variables, the addition of the other terms (involving the porous support) in this model increased the multiple determination coefficient R^2 by only 0.14 (from 0.76 to 0.90).

Potential clusters of both models which are not well predicted were identified by the plot of the predicted versus observed values (Figs. 5a and b). The two important outliers are the first replicate of mixture R_8 and the second replicate of mixture R_3, for which the predicted T_{0-41} values compared to observed values were 49.6 mg/l d (compared to 35.6) and 80.0 mg/l d (compared to 70.8) respectively for original variables model and 43.0 mg/l d (compared to 35.6) and 82.1 mg/l d (compared to 70.8) for the ratio variables model. This difference was allocated to poor repeatability, which was already discussed.

A final comparison was made between Eqs. (16) and (17) by computing the predicted sulfate-reduction rate values for all possible combinations of X_1–X_5 in the experimental space. Both models gave very similar results.

The regression models generated for the second calculated sulfate-reduction rate (T_{Modified}) had less satisfactory multiple determination coefficients (R^2) being either 0.73 for the model using original variables or 0.54 for the ratio variables model. Although these second-degree polynomial models showed a lack of fit, both were equations with two variables, the first one sorted for the ratio variables model being also Z_1Z_2 ($R^2 = 0.35$), while the first term sorted for the original variables model was also X_3 ($R^2 = 0.35$).

In summary, the poultry manure proportion (X_3), which is the critical variable for sulfate-reduction rate should be high, while a smaller proportion should characterize the product of the two more complex carbon sources. These statements are in accordance with the selection of R_{12} as the most reactive mixture and of R_8 as the least reactive.

The high number of reactive mixtures tested simultaneously justified the use of 500 ml batch reactors. However, laboratory column studies should be performed before extrapolating the results to dynamic flow conditions.

Another important aspect, which should be considered for barrier reactive mixture efficiency is its longevity. The best sulfate-reduction rate criterion used in this study for a 41 day period could be insufficient to choose the best substrate for long-term treatment. It is expected that treatment performance will slowdown after highly labile carbon is depleted. Cellulosic carbon sources, either wood chips or leaf compost, should at this point provide carbon release and therefore growth energy for SRB population already developed. Therefore, further research is needed to study the impact of mixture composition on long-term treatment effectiveness. Supplementary precautions should be taken for physical factors within the reactive barrier such as contaminated groundwater contact time and reactive mixture permeability.

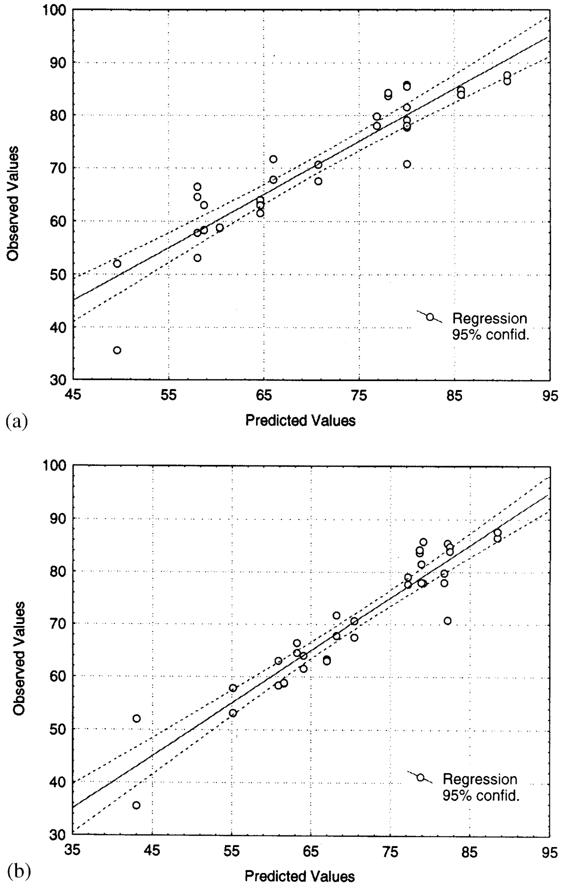

Fig. 5. Predicted versus observed values for T_{0-41} models. (a) Original variables model; (b) ratio variables model.

4. Conclusions

The following conclusions can be drawn from the foregoing study:

- Final concentrations of sulfates in the synthetic AMD were lower than 90 mg/l and the average rate of sulfate-reduction was either 70.7 ± 12.1 mg/l d for T_{0-41}, or 103.7 ± 26.8 mg/l d for T_{Modified}. Sulfate reduction rates varied between 35.6 and 87.6 mg/l d for T_{0-41}, and between 45.9 and 156.3 mg/l d for T_{Modified}, indicating a strong influence of mixture composition on its reactivity.

- Heavy metal removal efficiencies ranged between 51% and 84% for nickel and 73% and 93% for zinc depending on the reactive mixture.
- The most reactive mixture in terms of sulfate reduction contained 3% wood chips, 30% leaf compost, 20% poultry manure (maximum proportion tested in the study), 0% oxidized mine tailings and 5% silica sand as a porous support. The constant proportions components were 37% bacterial source, 2% limestone and 3% urea (all proportions given in wt%).
- Modeling of sulfate-reduction rates was carried out using a forward stepwise regression in order to generate a statistical model for sulfate-reduction rate and to assess its critical variables. The generated sulfate-reduction rate predictive models which had very satisfactory parameters ($R^2 = 0.86$, $F = 62.38$ (p-level $< 10^{-13}$) and $R^2 = 0.90$, $F = 62.30$ (p-level $< 10^{-13}$)) identified poultry manure and the two other carbon sources as the critical variables. They also showed a minimal influence of the tested porous supports (either oxidized tailings or silica sand) on sulfate-reduction rates.
- The sulfate-reduction rate predictive models generated in this study can be useful to determine the initial barrier composition for further use in porous sulfate-reducing reactive walls. However, further research is needed to study the impact of mixture composition on long-term treatment effectiveness.

Acknowledgements

The authors acknowledge the financial support of the Chair partners: Alcan, Bell Canada, Canadian Pacific Railway, Cambior, Centre d'expertise en analyse environnementale du Québec, EDF-GDF, Hydro-Québec, Ministère des Affaires municipales et de la Métropole, Petro-Canada, Natural Science and Engineering Research Council (NSERC), Solvay, Total Fina Elf, and Ville de Montréal. The authors also wish to thank Sandra Estrela for her valuable comments during the manuscript preparation.

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