Biological treatment of highly contaminated acid mine drainage in batch reactors: Long-term treatment and reactive mixture characterization

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Abstract

Passive bioreactors involving sulphate-reducing bacteria (SRB) are a practical alternative technology to treat acid mine drainage (AMD). Careful selection of the organic carbon source is important to ensure performance and long-term efficiency of the treatment. However, a rigorous and methodical characterization to predict the biodegradability of organic substrates by SRB still needs to be investigated. In the present study, four natural organic materials were thoroughly characterized to assess their ability to serve as substrates and to find a parameter that links organic carbon sources with their biodegradability. Three reactive mixtures were then comparatively evaluated for their performance to treat a highly contaminated AMD in long-term (152 days) batch experiments. All three mixtures were successful for sulphate reduction and metal (Fe, Ni, Cd, Zn, and Mn) removal (91.8–99.8%). Higher efficiencies were observed in the reactors with 30% (w/w) cellulosic wastes (maple wood chips and sawdust) which decreased sulphate concentrations from 5500 mg/L to <1 mg/L, than in reactors with 2–3% cellulosic wastes, where final sulphate concentrations were in the range 2000–2750 mg/L. Organic material characterization indicated that higher C/N ratios, *chemical oxygen demand* (COD)/SO_4^{2-} ratios and *dissolved organic carbon* (DOC)/SO_4^{2-} ratios were associated with better sulphate-reducing conditions and metal removal. This work suggests that C/N and DOC/SO_4^{2-} ratios considered together are key parameters to assess the biodegradability of natural organic wastes under sulphate-reducing conditions.
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1. Introduction

Prolonged exposure of reactive sulphide minerals (e.g. pyrite, pyrrhotite) to oxygen and water, in the absence of sufficient neutralizing minerals, generates acid mine drainage (AMD). AMD is characterized by low pH and high concentrations of sulphates and heavy metals that represent a potential hazard for the environment. Therefore, AMD contaminated waters must be collected and treated before being discharged into the environment [1].

Over the past 20 years, passive bioreactors were successfully used for the treatment of AMD in pilot and field-scale projects on remote sites [2–6]. They rely on sulphate-reducing bacteria (SRB), which are anaerobic microorganisms capable of increasing the pH and alkalinity of water, and of immobilizing dissolved metals by precipitating them as metal sulphides, in the presence of a biodegradable organic carbon source. Under these conditions, organic carbon oxidation is coupled with sulphate reduction:

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \qquad (1)$$
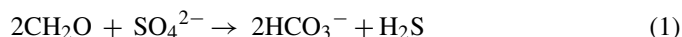
where CH_2O represents a short-chain organic carbon molecule available to SRB.

Soluble sulphides generated in reaction (1) react with metals (Me^{2+}) to form biogenic metal sulphides (MeS), reversing reactions that occurred to produce contaminated waters [4]:

$$H_2S + Me^{2+} \rightarrow MeS + 2H^+ \qquad (2)$$
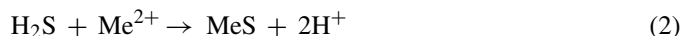
Passive bioreactors utilize a simple passive to semi-passive, flow-through design. AMD is fed horizontally or vertically over a solid reactive mixture into a pond or a tank and is released treated, with higher alkalinity and pH and lower concentrations of heavy metals and sulphates [7]. Reactive mixture composition

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is crucial for the efficiency of the treatment process [8]. Efficient reactive mixtures generally contain an organic carbon source, a bacterial source or SRB inoculum, a solid porous medium, a nitrogen source and a neutralizing agent (e.g. limestone) [8–11]. Recently, consultants such as Golder Associates Ltd. have installed efficient full-scale passive bioreactors containing mixtures of natural organic materials at several former mine sites (e.g. Ferris-Haggarty Mine, Wyoming, USA, and Cadillac Molybdenite, Northern Québec, Canada) [6]. However, efficiencies obtained in laboratory bioreactors are better than in pilot or full-scale bioreactors, none of which have remained operational without significant overhaul or modification for more than three to four years [7]. Assessment of organic material biodegradability in short-term batch experiments can therefore lead to overestimating their capacity to sustain SRB activity and remove metals during long-term operation. Furthermore, for field implementation, selection of locally available organic carbon sources is preferred because they are less expensive than commercialized organic carbon sources such as hydrogen release compounds (Regenesis) or the use of molasses (ARCADIS). Additionally, it was proved that mixtures of several natural organic materials, both organic wastes (animal manure, compost) and cellulosic wastes (wood chips, sawdust) perform better than a single source [8–11]. Careful selection of a suitable carbon source is of great importance to ensure performance and longevity in biological AMD treatment [11]. However, a rigorous and methodical test to predict the biodegradability of organic substrates by SRB is still warranted and needs to be investigated [11,12].

Over the past decade, a few studies have attempted to link physicochemical composition of natural organic materials with their ability to promote sulphate reduction and metal removal [8–14]. Results confirmed that the higher the content of lignin and cellulose in the organic substrate, the lower is its biodegradability and its capacity for developing and sustaining bacterial activity [9,10,12,14]. Nevertheless, the recent study of Zagury et al. [11], who assessed the biodegradability of a natural organic substrate mixture versus single substrates, reports a very low efficiency in a bioreactor containing poultry manure as a single organic carbon source, despite its highest DOC and easily available substances (EAS) content. On the other hand, poultry manure was very efficient for sulphate reduction and metal removal when used in a mixture with leaf compost and maple wood chips. Consequently, substrate characterization based solely on EAS and DOC, on an individual basis, does not give a clear indication of its ability to promote sulphate reduction and metal removal [11].

Further, few studies have attempted to link organic material C/N ratios with their efficiency for AMD biological treatment [11,13]. A C/N ratio around 10 is generally considered suitable for biological degradation of complex organic substrates [15,16]. Nevertheless, the C/N ratio taken alone was not a good indicator of the sulphate-reducing ability of a given mixture [11]. Moreover, when lactate is used as substrate, the optimal reported C/N ratios are higher and vary greatly from 15.7 to <45 or from 45 to 120 [17,18].

Finally, other studies have attempted to link COD/SO_4^{2-} ratios with the suitability of a natural organic material to act as a substrate during biological treatment of AMD contaminated waters [19–22]. As in the case of C/N ratios, if the COD/SO_4^{2-} ratio is taken alone, data interpretation must consider the complexity of the organic carbon in the substrate used. The theoretical COD/SO_4^{2-} ratio for SRB is 0.67, while typical observed values range between 0.7 and 1.5, depending on the type of carbon source [23]. Thus, when ethanol is used as the substrate, the optimal COD/SO_4^{2-} ratio is 0.55–0.84 [21,22], whereas when organic wastes (e.g. municipal compost, activated sludge) are used as substrate, not all of the carbon present is used by the SRB and the optimal COD/SO_4^{2-} ratio has been reported to be 1.6 [20] or 5 [19].

In fact, COD does not directly reflect organic carbon availability to anaerobic bacteria because it is determined in aerobic conditions. Additionally, it does not quantify complex dissolved organic carbon because the standard analysis is performed at 150 °C.

Nevertheless, C/N ratios and COD/SO_4^{2-} ratios, eventually coupled with other parameters, are still promising indicators of an organic material's capacity to perform as a substrate in biofilters for AMD passive treatment.

Consequently, the present study is divided in two parts. In the first part, four organic waste materials (maple wood chips, maple sawdust, composted poultry manure, and leaf compost) were thoroughly characterized in terms of biodegradability and ability to serve as organic carbon sources for SRB during AMD treatment in passive bioreactors. The objective was to find the key parameter that links natural organic material composition with its biodegradability. In the second part, two reactive mixtures previously reported as efficient in short-term batch bioreactors (41–71 days), and a third mixture developed for the purpose of this study, were comparatively assessed for their performance to treat a highly contaminated AMD in longer term batch bioreactors (120–152 days). The objective was to select the most efficient reactive mixture in order to test it further in column bioreactors.

2. Materials and methods

2.1. Physicochemical and microbiological characterization of solid organic materials

In the first part of the study, maple wood chips and sawdust (P.W.I. Industries, Canada), composted poultry manure (Fertilo de Fafard, Canada), and leaf compost (city of Montreal) were thoroughly characterized.

These four organic materials as well as creek sediment (Cupra mine site, QC), which was used as a source of acclimated SRB, were refrigerated at 4 °C prior to their analysis. All analyses were performed in triplicate, with wet samples. The reported results were corrected for moisture content.

Solid organic materials were characterized for *physical parameters* (pH and water content), *elemental analysis* (total C, N as total Kjeldahl nitrogen (TKN), and total P), and *biodegradation parameters* (total volatile solids (TVS), total organic carbon (TOC), waxes and resins, easily available substances (EAS), hot water soluble substances, hemicellulose, cellulose,

Table 1
Physicochemical and microbiological characteristics of natural organic materials used in batch reactors

	Cellulosic wastes		Organic wastes	
	Maple wood chips	Maple sawdust	Composted poultry manure	Leaf compost
Physical parameters				
pH	5.75 ± 0.10	5.32 ± 0.01	7.91 ± 0.03	9.32 ± 0.42
Water content (% w/w)	6.5 ± 0.1	5.2 ± 0.0	67.2 ± 1.6	37.4 ± 1.3
Elemental analysis (%w/w dry weight)				
C	47.7	47.9	28.2	15.6
N (TKN)	5.1×10^{-3}	ND	1.3	0.7
P	6.3×10^{-4}	4.3×10^{-4}	1.2	0.1
Biodegradation parameters of solid materials (%w/w dry weight)				
Total volatile solids	99.7 ± 0.4	100.0 ± 0.0	71.4 ± 1.3	25.8 ± 0.9
Total organic carbon (TOC)	44.0	45.3	25.7	1.3
Waxes, resins	1.1 ± 0.5	5.0 ± 0.3	3.5 ± 0.8	7.1 ± 2.4
Easily available substances (EAS)	31.3 ± 0.5	31.9 ± 0.6	25.6 ± 1.2	40.7 ± 15.7
C/N ratio	*8627*	*–*	*20*	*2*
Hot water soluble substances	4.8 ± 0.8	7.4 ± 0.3	11.9 ± 1.0	7.0 ± 0.7
Hemicellulose	24.8 ± 1.6	37.2 ± 1.5	21.5 ± 2.7	10.3 ± 3.5
Cellulose	64.9 ± 2.3	48.4 ± 0.2	25.3 ± 0.8	4.8 ± 0.7
Lignin	2.7 ± 0.0	6.0 ± 1.0	19.5 ± 2.6	41.4 ± 2.3
Ash	2.8 ± 0.1	1.0 ± 0.0	21.8 ± 1.9	36.4 ± 4.4
Water extracts (1:10 solid: liquid ratio) analysis (mg/L)				
Total organic carbon (TOC)	367	892	198	66
Dissolved organic carbon (DOC)	305	692	139	53
Chemical oxygen demand (COD)	842 ± 53	1798 ± 22	182 ± 25	83 ± 5
Microbial counts in solid materials (cells/100mL)				
Heterotrophic anaerobic fermentative bacteria	5.0×10^5	3.3×10^4	$>1.6 \times 10^7$	$>1.6 \times 10^7$
Sulphate-reducing bacteria (SRB)	<2	<2	5.0×10^4	5.0×10^4

and lignin content). Water extracts (1:10 solid:liquid ratio) were analyzed for total organic carbon (TOC), dissolved organic carbon (DOC), and chemical oxygen demand (COD). Enumeration of heterotrophic anaerobic fermentative bacteria was performed in the solid organic materials, while sulphate-reducing bacteria (SRB) counts were carried out in both the organic materials and the creek sediment. The characterization is presented in Table 1. The pH was determined in deionized water using a solid to liquid ratio of 1:10 according to Method D 4972-95a [24] using a portable pH/mV/temperature meter (HACH, model sensION1) with a gel-filled pH electrode and a combination Ag/AgCl redox potential electrode (HACH, Hampton, NH). Water content was determined at 105 °C according to Method D 2216-92 [25]. Volatile solids were determined at 550 °C according to Karam [26]. Total carbon was measured by combustion with an induction furnace (LECO Corporation, 1975). Total kjeldahl nitrogen (TKN) and total P were determined by Standard Methods 4500-N_{org} and 4500-P, respectively [27]. A phosphoric acid treatment followed by an infrared determination of CO_2 evolved was performed to determine total inorganic carbon [28]. Organic carbon was calculated by the difference between total carbon and total inorganic carbon. Total organic carbon (TOC) and dissolved organic carbon (DOC) in leachate were analyzed after mixing (shaking for 2 h at room temperature with a customized rotary agitator) 20 g (wet weight) of each organic substrate with 200 mL of deionised water (18.2 MΩ). The extracts were then centrifuged (13,800 × g) for 10 min and analyzed for TOC

or filtered (0.45 μm) and then analyzed for DOC. TOC of non-filtered/filtered extract was determined at 680 °C, after acidification of samples with H_3PO_4, according to Standard Method 5310 B [27] using a TOC analyzer (DOHRMAN, model DC-190). COD analysis was performed using the dichromate reflux method with a COD reactor (HACH Procedure Manual, 1998) and a spectrophotometer (HACH model DR/2010). Easily available substances (EAS) and waxes and resins were analyzed by a modified forage fibre analysis (FFA), as per Zagury et al. [11]. Hot water soluble substances, hemicellulose, cellulose, and lignin content were determined according to Harper and Lynch [29]. Enumeration of heterotrophic anaerobic fermentative bacteria and of SRB in organic materials and the creek sediment was performed using the Most Probable Number technique as per Standard Methods [27] and ASTM [30], respectively.

2.2. Batch experiment description

The capacity of maple wood chips, maple sawdust, composted poultry manure, and leaf compost to promote sulphate reduction and metal removal was assessed during a 150-day batch experiment. The study was performed with three reactive mixtures, in duplicate, in 2 L glass reaction flasks, at room temperature (22 ± 1 °C). The mixture proportions (%w/w, dry weight) are given in Table 2. Mixture #1 and mixture #2 contain three organic carbon sources (maple wood chips, composted poultry manure, and leaf compost) and have been previously

Table 2
Composition of three reactive mixtures assessed in batch reactors

Component	%w/w dry weight		
	Mixture #1 [8]	Mixture #2 [11]	Mixture #3 (this study)
Organic carbon sources			
Maple wood chips	3	2	10
Maple sawdust	0	0	20
Composted poultry manure	20	18	10
Leaf compost	30	30	20
Nitrogen source			
Urea	3	3	3
Bacterial source			
Creek sediment	37	15	15
Porous medium			
Sand	5	30	20
pH neutralizer			
Calcium carbonate	2	2	2

tested in short-term batch experiments [8,11]. Mixture #3 contains maple sawdust as a fourth organic carbon source. Mixture #1 was added in reactor R1, mixture #2 in reactor R2, and mixture #3 in reactor R3. Synthetic AMD was then added to the six reactors (in duplicates for each reactive mixture). The reactors contained 250 g or 300 g (dry weight) of reactive mixture, for a final solid:liquid ratio of 1:3 in R1 and of 1:4 in R2 and R3. Reactors were then sealed and thoroughly shaken. Synthetic AMD preparation has been described in detail in the study of Zagury et al. [11]. AMD characterization is presented in Table 3. Sampling was performed every 8–16 days for a total period of 120 days (R3) or 152 days (R1 and R2). Batch reactors contained sampling ports fitted with Teflon-lined septa. All sampling was performed under anaerobic conditions ($N_{2(g)}$ high purity atmosphere) in a glove bag. SRB counts and analysis of pH, oxidation reduction potential (ORP), alkalinity, TOC, DOC, COD, sulphate, Fe^{2+}, total sulphides, and metals (Fe, Mn, Cd, Ni, Zn) were also carried out. Measurements of pH and the redox potential (HACH electrode, Ag/AgCl) were determined directly in the sampling solution (within the glove box) immediately after collection. Except for TOC and SRB counts, all other parameters were determined on separate 0.45 μm filtered samples. Sulphides, sulphate, and ferrous iron were determined

during the first 1–2 h after collection, using a spectrophotometer (HACH, model DR/2010) and Standard Methods [27]. Alkalinity was analyzed using Standard Method 2320-B [27]. TOC of non-filtered extract and of filtered extract (DOC) was determined after acidification of samples with H_3PO_4, according to Standard Method 5310 B [27] using a TOC analyzer (DOHRMAN, model DC-190). COD analysis was performed by the dichromate reflux method using a COD reactor (HACH Procedure Manual, 1998) and a spectrophotometer (HACH, model DR/2010). Metal concentrations were determined using an atomic absorption spectrometer (PerkinElmer, model AAnalyst 200) (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) after sample acidification at pH 2 with concentrated HCl. Samples for SRB enumeration were taken every 16–32 days.

To avoid H_2S accumulation due to dropping of metal concentrations, a 10 mL spike of metals (24 g/L Fe, 0.6 g/L Cd, 0.7 g/L Mn, 0.9 g/L Ni, and 0.9 g/L Zn), was added at day 74. Metal concentrations in the spike were calculated in order to reach the initial concentrations in the reactors (Table 3). After the spike, the first sampling was performed on day 75 (the day after the spike) and then sampling was performed every 8–16 days until the end of the experiments.

All laboratory ware used during the analytical procedures was cleaned sequentially with a phosphate-free detergent, soaked in 10% (v/v) nitric acid for 24 h, then in distilled water, and finally rinsed three times with deionized water (18.2 MΩ). Unless otherwise stated, all reagents were of analytical grade (ACS) or better.

2.3. Geochemical modeling

Geochemical modeling using the thermodynamic chemical equilibrium model VMINTEQ version 2.51 was performed on supernatant samples collected on days 0, 8, 16, 24, 32, and 40 to help in the assessment of the metal removal mechanisms observed during the early phase of the batch experiment. For the mixtures where sulphate reduction was not evident, additional samples were collected later in the experiment.

Table 3
Composition of synthetic AMD added in batch reactors

Component	Concentration (mg/L)	Source
Ca^{2+}	487.8 ± 10.5	$CaSO_4 \cdot 2H_2O$
Cd^{2+}	12.6 ± 0.9	$CdSO_4 \cdot 8/3H_2O$
Fe^{2+}	1670 ± 66	$FeSO_4 \cdot 7H_2O$
K^+	67.1 ± 1.4	K_2SO_4
Mg^{2+}	98.9 ± 5.0	$MgSO_4$
Mn^{2+}	13.5 ± 1.2	$MnSO_4 \cdot H_2O$
Na^+	87.1 ± 1.7	Na_2SO_4
Ni^{2+}	16.8 ± 1.8	$NiSO_4 \cdot 6H_2O$
Zn^{2+}	18.9 ± 1.1	$ZnSO_4 \cdot 7H_2O$
$SO_4{}^{2-}$	5500 ± 250	–
pH	5.45–5.51	–

VMINTEQ calculates saturation indices of various mineral phases taking into account geochemical processes such as dissolution/precipitation, complexation, oxidation/reduction, ion exchange and gas equilibrium. However, this model does not take into account SRB activity and subsequent precipitation of metal sulphides.

3. Results and discussion

3.1. Physicochemical characterization of natural organic materials

Table 1 presents the characterization of the natural organic materials used in the batch experiment. For the purpose of discussion, natural organic materials are divided in two groups: cellulosic wastes (maple wood chips and maple sawdust) and organic wastes (composted poultry manure and leaf compost). As indicated in Table 1, the pH of the cellulosic wastes was slightly acidic (5.32–5.75), whereas the organic wastes had an alkaline pH ranging from 7.91 to 9.32. Elemental analysis indicated that total carbon (TC) was lower in leaf compost (15.6%) and composted poultry manure (28.2%) than in cellulosic wastes (up to 47.9%). Similarly, TOC was lower in leaf compost (1.3%) and in composted poultry manure (25.7%), while higher values were measured in cellulosic wastes (up to 45.3%). However, organic wastes were characterized by a higher P content (0.1–1.2%) and N content (0.7–1.3%) than cellulosic wastes (10^{-3}–10^{-4}%). One may calculate a C/N ratio (expressed as TOC/TKN), which gives values of 2 in leaf compost, 20 in composted poultry manure, and >9×10^3 in cellulosic wastes. A C/N ratio less than 10, together with the highest EAS content of leaf compost (40.7%) might indicate this organic substrate as the most available for SRB [13]. Nevertheless, leaf compost alone was not successful when used as single substrate for AMD treatment in batch and column bioreactors [11,12,31]. One explanation could be related to a lower TC of leaf compost compared to other organic wastes (e.g. animal manure, municipal compost). Additionally, in leaf compost, high percentages of TC can be in the form of carbonates and bicarbonates (TIC). Leaf compost used in the present study contained 15.6% TC, from which 1.3% was TOC and 14.3% was TIC. Such a high percentage of TIC can interfere during an EAS analysis and give misleading results about the capacity of leaf compost to act as a good substrate for bacterial activity.

Hot water soluble substances showed little variation from 4.8% in maple wood chips to 11.9% in composted poultry manure. As expected, higher contents of hemicellulose (up to 37.2%) and cellulose (up to 64.9%) were found in cellulosic wastes.

Water extract analysis indicated that a higher content of TOC and of DOC characterized cellulosic wastes compared to organic wastes. The lowest (53 mg/L) and the highest (692 mg/L) DOC contents were found in leaf compost and maple sawdust, respectively. Organic carbon from sawdust is not easily available for SRB and long acclimatization periods can be required for passive bioreactors filled with the sawdust as the sole organic carbon source before becoming efficient for AMD treatment [32]. However, after an acclimatization period, better results in terms of metal and sulphate removal were reported with sawdust alone [32,33] than with compost alone [11,12,31]. Therefore, sawdust can be a good source of organic carbon for SRB during long-term operation of passive bioreactors.

3.2. Microbial enumeration

Bacterial counts (Table 1) showed a high density of heterotrophic anaerobic fermentative bacteria in all solid organic materials: 10^4–10^5 cells/100 mL in cellulosic wastes to 10^7 cells/100 mL in organic wastes. Counts also showed the presence of SRB in organic wastes (10^4 cells/100 mL), which confirmed previous assumptions about their presence in natural organic wastes [11]. However, SRB were not detected in maple wastes (<2 cells/100 mL). Creek sediment, which is generally used as a potential bacterial inoculum, showed a fair count of SRB (10^2 cells/100 mL), which is lower compared with the sediment used by Cocos et al. [8] (3.0×10^4 cells/100 mL) but higher compared to the sediment used by Zagury et al. [11] (25 cells/100 mL).

3.3. Batch experiments

3.3.1. Long-term efficacy

There was little difference between the duplicate reactors throughout the experiment (Figs. 1–3): results are therefore presented as the average between bioreactors that contained the same reactive mixture. Generally, the graphs indicate two patterns in physicochemical quality of treated water. First, a lag period of about 80 days was observed before the occurrence
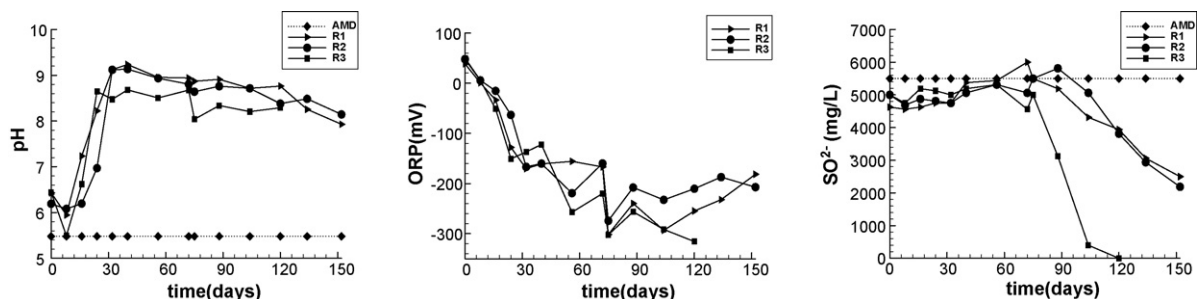

Fig. 1. Variation of pH, ORP and sulphate in batch reactors containing three different reactive mixtures (R1, R2, and R3).
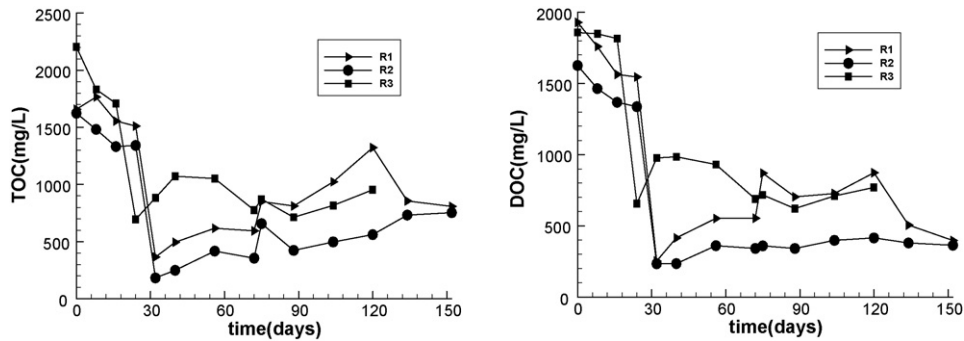
Fig. 2. Variation of total organic carbon (TOC) and dissolved organic carbon (DOC) in batch reactors containing three different reactive mixtures (R1, R2, and R3).

of sulphate reduction and the increase in SRB counts for all mixtures. This could be related to higher initial ORP values compared to previously published studies [8,10,11] that retarded the start of sulphate-reduction. Second, the evolution of principal water quality parameters indicated three phases of AMD treatment: one between day 0 and day 32, a second phase between day 32 and day 75, and the last phase between day 75 and the end of the experiments (day 120–152).

3.3.1.1. First phase (0–32 days). Important changes in water quality were recorded in this phase. The pH increased sharply from 5.5 to between 8 and 9, as well as alkalinity (results not shown), which rose from 6–40 mg/L $CaCO_3$ to around 2500 mg/L $CaCO_3$. ORP decreased from 33–50 mV to values as low as −177 mV. TOC and DOC decreased from around 2000 mg/L to about 500 mg/L in R1 and R2, whereas values around 1000 mg/L were recorded until day 56 in R3. Sulphate concentrations varied around 5000 mg/L, whereas metal concentrations dropped in all bioreactors. On day 32, metal removal varied from 98.5% to 99.9% (Cd, Fe, Mn, and Zn) and from

94.7% to 98.4% (Ni). As modeling results showed, early metal removal might be explained by (oxy)hydroxide and carbonate mineral precipitation. Saturation indices calculated with VMINTEQ using water chemistry from day 0, day 8, and day 16 indicated that metal removal could be attributed to the precipitation of (oxy)hydroxide minerals such as ferryhydrite, goethite, K-jarosite, Na-jarosite, lepidocrocite, maghemite, magnetite, as well as siderite ($FeCO_3$). Between day 16 and day 40, precipitation of carbonate minerals such as calcite ($CaCO_3$), magnesite ($MgCO_3$), rhodocrosite ($MnCO_3$), otavite ($CdCO_3$), smithsonite ($ZnCO_3$), and $NiCO_3$ was also indicated. These results are in agreement with other batch experiment studies [10,11].

3.3.1.2. Second phase (32–75 days). Water quality was less variable during this phase. Alkalinity increased steadily and reached the highest values on day 75 (12–13 g/L $CaCO_3$). The ORP dropped to values around −300 mV on day 75, while sulphate concentrations increased to initial concentrations (5500 mg/L) or more. Before the spike on day 74, metal concentrations remained stable. However, after the spike, metal
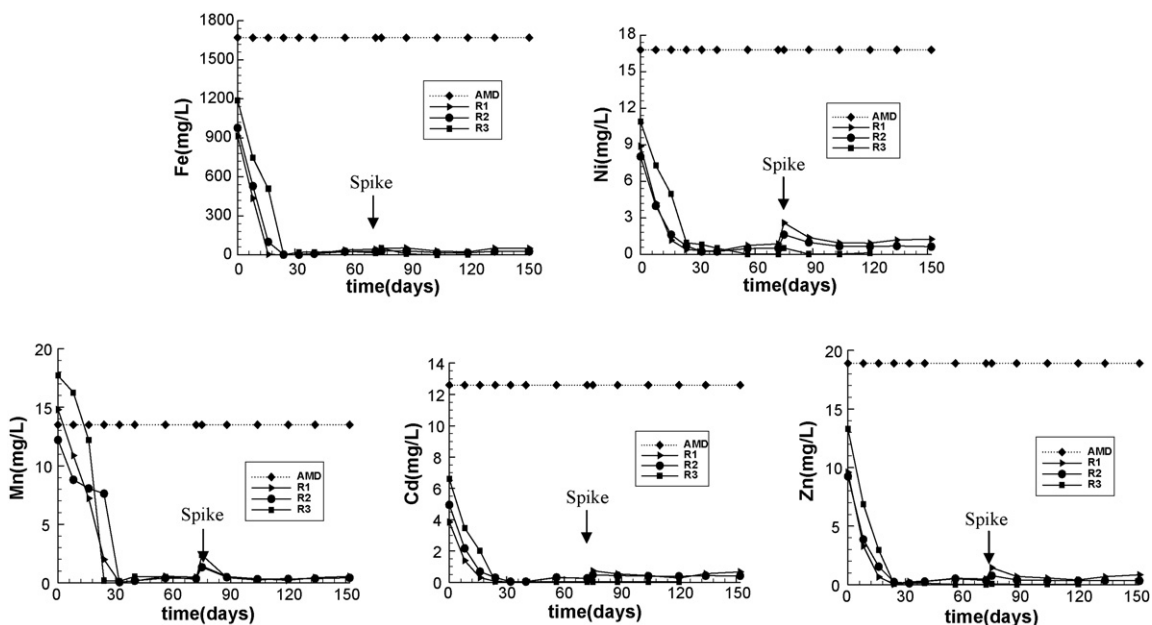

Fig. 3. Metal concentrations in batch reactors containing three different reactive mixtures (R1, R2, and R3) as a function of time.

concentrations decreased rapidly and remained low until the end.

3.3.1.3. Third phase (75–152 days). From day 75 until the end of the experiment, all parameters showed very little variation, except for sulphate, which drastically decreased from about 5500 mg/L to <1 mg/L in R3 (day 120), and to values in the range 2250–2750 mg/L in R1 and 2000–2375 mg/L in R2 (day 152). A sharp decrease in sulphate concentrations such as observed in R3 have already been reported in other batch experiment studies, where concentrations up to 5000 mg/L SO_4^{2-} decreased to <163 mg/L in only 35 days or less [10,11]. In addition, sulphide concentrations up to 2.7 mg/L were measured. The results of sulphate reduction were well correlated with SRB counts, which grew progressively during the experiment. Initial (day 0) SRB counts in the reactors were lower than 2 cells/100 mL in R1, 80 cells/100 mL in R2, and 230 cells/100 mL in R3. However, after the lag period, SRB counts increased in all reactors, despite there being no evidence of sulphate removal in reactors R1 and R2. On day 88, SRB counts were 8.0×10^4 cells/100 mL (R1), 2.3×10^4 cells/100 mL (R2), and 2.3×10^6 cells/100 mL (R3). Final SRB counts (day 120) were 1.3×10^5 cells/100 mL (R1), 2.3×10^4 cells/100 mL (R2), and 5.0×10^4 cells/100 mL (R3). As results indicated, SRB counts yielded the highest values in R3, which contained the most reactive mixture in terms of sulphate-reduction and metal removal.

In all reactors, metal removal efficiencies were generally high (Fig. 3). Metal concentrations were lowest in reactor R3, with total Fe final values around 3 mg /L. Metal removal was up to 99.8% for all metals, except for Mn that reached 96.9%. In reactors R1 and R2, metal concentrations were higher, especially Fe, with final values around 27 mg/L in R2, and around 51 mg/L in R1. In these reactors, metal removal yielded values ranging from 91.8% (Ni) to 98.1% (Zn). In a related study performed with a very similar reactive mixture, a mineralogical analysis using scanning electron microscopy and X-ray elemental mapping on a spent solid mixture after 350 days of batch AMD treatment clearly indicated the presence of iron sulphides [34]. This finding supports the removal of metals through formation of metal sulphides once the sulphate-reducing conditions are fully established.

3.3.1.4. Sulphate-reduction rates. Sulphate reduction rates were calculated using the least squares regression method (as per Cocos et al. [8] and Zagury et al. [11]) on data from the period when bioreactors demonstrated sulphate-reduction. The data between day 88 and day 152 (R1 and R2), and between day 56 and day 120 (R3) were used. Sulphate reduction rates were 39–43 mg/L per day in R1, 55–59 mg/L per day in R2, and 80–86 mg/L per day in R3. The sulphate reduction rates measured in R3 are comparable to the rates previously reported [8,11].

3.3.1.5. Comparative efficiencies of the three reactive mixtures. In terms of sulphate and metal removal, mixture #3 was the most efficient and mixture #1 was the least efficient. The differences between reactors were in the solid:liquid ratio used, which was 1:3 (R1) and 1:4 (R2 and in R3). More importantly, mixture #3 contained 30% of each group of organic materials (organic and cellulosic), whereas mixtures #1 and #2 contained 48–50% organic wastes and only 2–3% cellulosic wastes (Table 2). It is worth noting that organic wastes had a higher EAS content than cellulosic wastes, which had the highest content of recalcitrant organic carbon (e.g. hemicellulose, cellulose) (Table 1). As a result, mixtures #1 and #2 had a higher content of easily available organic carbon than mixture #3 and could be expected to perform better. Moreover, TOC and DOC contents calculated from characterization data (Table 1) showed values of 20.6 g and 52.9 mg (R1), of 13.4 g and 47.0 mg (R2), and of 40.7 g and 483.5 mg (R3), respectively. Batch mixture #3 had much higher contents of TOC and of DOC than mixtures #1 and #2. Additionally, more than half of the organic carbon in reactor R3 was released from cellulosic wastes, and this type of organic carbon is reportedly not easily available to SRB [8,10]. However, in long-term batch tests, mixture #3 with 30% cellulosic wastes yielded higher efficiencies. Therefore, the usual characterization parameters could not predict the most efficient reactive mixture. These findings are different from Cocos et al. [8] who reported that a higher percentage of poultry manure entails a better reactivity. It must be noted that the experiment of Cocos et al. [8] only lasted 40 days.

Furthermore, in all bioreactors, AMD treatment started in the first 80 days, when pH increased to 8-9, alkalinity increased to 12–13 g/L, and heavy metal removal reached values as high as 99.9%. Modeling results indicated that up to day 40, metal removal mechanisms can be attributed to precipitation of (oxy)hydroxide and carbonate minerals. Sulphate reduction occurred after this lag period of about 80 days, as indicated by increased SRB counts and by decreasing sulphate concentrations.

Long-term batch experiments can thus lead to a more realistic evaluation of a reactive mixture's potential efficiency in long-term AMD treatment. However, batch results must be confirmed by continuous flow column experiments.

3.3.2. Role of C/N, COD/SO$_4$$^{2-}$, and DOC/SO$_4$$^{2-}$ ratios
C/N and COD/SO_4^{2-} ratios were calculated using data available from natural organic material characterization and from water quality during batch experiments.

Initial C/N ratios (expressed as TOC/TKN) were 4.0, 3.5, and 10.1 for mixture #1, mixture #2, and mixture #3, respectively. Therefore, mixture #3, which performed the best, had the highest C/N ratio. These results are in agreement with other studies, which indicated that a C/N ratio around 10 is suitable for biological degradation of complex organic substrates [15,16] but not with the findings of Zagury et al. [11], who reported the best efficiencies for a C/N ratio around 3. However, mixture #3 contained a proportion of cellulosic wastes (30%) 10-fold higher compared to the 3% used in the study of Zagury et al. [11]. As already mentioned, cellulosic wastes release a higher content of DOC in water (Table 1 and Fig. 2). As a result, the choice of the organic carbon source for an efficient biological AMD treatment cannot be made based solely on the C/N ratio.
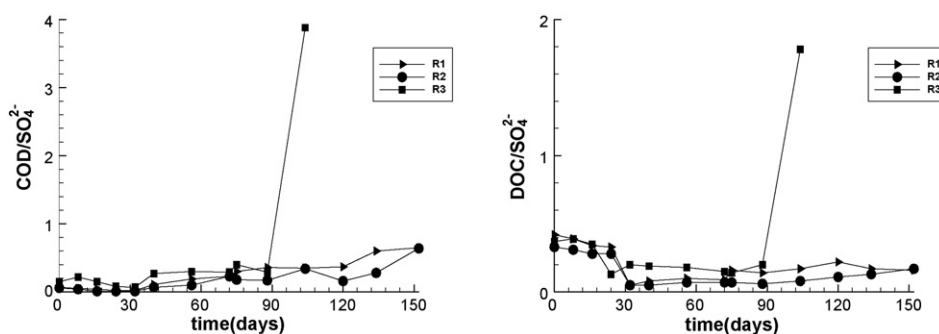
Fig. 4. Chemical oxygen demand (COD)/SO$_4^{2-}$ ratio and dissolved organic carbon (DOC)/SO$_4^{2-}$ ratio in batch reactors containing three different reactive mixtures (R1, R2, and R3) as a function of time.

Initial COD/SO$_4^{2-}$ ratios were then calculated, giving relatively similar values of 0.07, 0.06, and 0.15, for mixture #1, mixture #2, and mixture #3, respectively. The COD/SO$_4^{2-}$ ratios were also calculated over the duration of the batch tests (120–152 days; Fig. 4). The results showed similar trends for all three mixtures with COD/SO$_4^{2-}$ ratios that decreased slightly until day 32 and then increased to values up to 0.65 (R1 and R2) or to 3.88 (R3) on day 104, due to the decrease in sulphate concentrations to 400 mg/L. It is worth noting that the initial COD/SO$_4^{2-}$ ratios were less than the theoretical value (0.67) [23] in all reactors. However, sulphate reduction was observed even at COD/SO$_4^{2-}$ ratios less than 0.67. This can be explained by the presence of cellulosic wastes in mixture compositions that released complex dissolved organic carbon, which was not quantified during the COD determination. Additionally, the high alkalinity generated (up to 13 g/L) could interfere during the test analysis. Furthermore, COD is not an accurate measure of organic carbon availability to anaerobic bacteria because it is determined under aerobic conditions.

From these findings, we suggest that DOC could be a more appropriate indicator for organic carbon availability to SRB than COD. The DOC/SO$_4^{2-}$ ratio was therefore calculated to confirm this hypothesis. Initial DOC/SO$_4^{2-}$ ratios were similar in all bioreactors and varied from 0.32 to 0.38. DOC/SO$_4^{2-}$ ratios calculated over the duration of the batch tests showed similar trends as in the COD/SO$_4^{2-}$ ratio (Fig. 4), with DOC/SO$_4^{2-}$ ratios that decreased until day 32 and then increased slowly to values up to 0.17 (R1 and R2) or more sharply to 1.78 (R3) on day 104. Therefore, higher ratios of both COD/SO$_4^{2-}$ and DOC/SO$_4^{2-}$ seemed to be better correlated with sulphate-reducing conditions. However, DOC is more easily and accurately quantified in the complex system of a passive bioreactor than the chemical oxygen demand (COD). For that reason, C/N and DOC/SO$_4^{2-}$ ratios taken together are suggested as key parameters that link an organic mixture composition with its biodegradability under anaerobic conditions.

4. Conclusions

The three reactive mixtures tested were successful in promoting sulphate reduction and metal removal (91.8–99.8%) but higher efficiencies were observed in the reactor (R3) that contained a mixture of equal proportions (30%, w/w) of organic and

cellulosic wastes. A lag period of about 80 days was observed before the occurrence of sulphate reduction stressing the drawbacks of short-term (less than three months) batch experiments for mixture selection. Organic material characterization indicated that for C/N, COD/SO$_4^{2-}$ and DOC/SO$_4^{2-}$ ratios, higher values were associated with better sulphate-reducing conditions. In addition, C/N and DOC/SO$_4^{2-}$ ratios taken together are easily measurable parameters that link natural organic material composition with its biodegradability under anaerobic conditions.

Because hydraulic retention time is an essential parameter for an efficient design of a full-scale passive bioreactor, the most efficient reactive mixture (#3) is currently being tested in column bioreactors that will be operated at different hydraulic retention times for more than a year.

Acknowledgements

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