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# PART I: Acid Mine Drainage

## Chapter 1

### SHORT-TERM AND LONG-TERM BIOREACTORS FOR ACID MINE DRAINAGE TREATMENT

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**Abstract:** Passive biological treatment of acid mine drainage (AMD) relies on sulfate-reducing bacteria (SRB) supported by a biodegradable organic carbon source. However, long-term treatment performance can be limited by the degradation rates of organic carbon available to SRB, and by low metal stability in spent reactive mixtures. The first part of this study focused on characterization of six natural organic materials and their short-term effectiveness in sulfate-reduction and metal removal from synthetic AMD. In the second part, long-term performance and metal precipitates in the reactive mixtures were assessed. Maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure and conifer sawdust were analyzed in terms of their carbon and nitrogen content, as well as their easily available substances content (EAS). Single substrates and substrate mixtures were tested in a 70-day batch experiment (2-L reactors) and in an extended batch study for up to 350 days. Geochemical modeling and scanning electron microscopy (SEM) was used to assess the minerals present in the solid phase. The highest EAS content and the lowest C/N ratio suggested poultry manure would be the best substrate. Nevertheless, the lowest efficiency was found in the poultry manure reactor, whereas the mixture of three organic materials was the most effective. After 350 days, the mixture of organic carbon sources was still efficient for AMD treatment. SEM analysis of the solid phase indicated the presence of iron sulfides. Substrate characterization provided insight on organic carbon availability but did not elucidate their ability to promote sulfate-reduction and metal removal. Further research is needed to accurately predict long-term carbon availability and to assess the metal precipitates in spent reactive mixtures.

**Key words:** acid mine drainage, sulfate-reducing bacteria, long-term passive bioreactors, mineralogical analysis.

## 1. INTRODUCTION

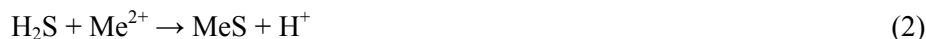
Prolonged exposure of reactive sulphide minerals (e.g. pyrite, pyrrhotite) from mine tailings to oxygen and water, in the absence of sufficient neutralizing minerals, generates acid mine drainage (AMD) (Tsukamoto et al., 2004). AMD contaminated waters are mainly characterized by low pH and high concentrations of sulfates and heavy metals. Therefore, AMD must be collected and treated to remove metals and to increase the pH before being discharged into the environment (Neculita et al., 2006).

Over the past 20 years, passive bioreactors have been successfully used for the treatment of AMD contaminated waters in pilot and full-scale projects (Reisinger et al., 2000; Reisman et al., 2003; URS Report, 2003; Kuyucak et al., 2006). They rely on sulfate-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 sulfides, provided there exists a biodegradable organic carbon source and a favourable biogeochemical environment. Under these conditions, organic carbon oxidation is coupled with sulphate reduction:



where  $\text{CH}_2\text{O}$  represents a short-chain organic carbon molecule available to SRB. The soluble sulfides generated in reaction (1) react with metals to form metal sulfide precipitates, essentially reversing the reactions that occurred to produce the AMD contaminated waters (Reisinger et al., 2000):



Passive bioreactors use a simple flow-through design, with an AMD feed over a solid reactive mixture acting as a source of carbon for SRB and as a physical support for microbial attachment and metal sulphide precipitation (Tsukamoto et al., 2004). Efficient reactive mixtures contain an organic carbon source (several organic/cellulosic wastes), a bacterial source or SRB inoculum (river sediment/animal manure), a solid porous medium (gravel/sand), a nitrogen source (urea) and a neutralizing agent (limestone) (Waybrant et al., 1998; Cocos et al., 2002; Zagury et al., 2006). The efficiency of passive bioreactors depends on the activity of SRB, which is mainly controlled by the composition of the reactive mixture. The most important component is the organic carbon source (Neculita et al., 2006). Many studies have attempted to predict the biodegradability of complex organic substrates by using chemical extractions (Prasad et al., 1999; Gibert et al., 2004; Zagury et al., 2006); however, they have not been totally successful.

An important objective of passive bioreactor efficiency is represented by the stability of the final wastes, which contain metal precipitates. Waste stability depends on metal speciation, which is related to metal removal mechanisms and on the form of sulphur, including sulfides and sulfates.

Metal removal mechanisms include sulfide precipitation, which is the desired mechanism; however, many mechanisms including adsorption and precipitation of metal carbonates and hydroxides occur in passive bioreactors (Zagury et al., 2006; Neculita et al., 2006). Experience has shown that metal removal mechanisms change during the life span of passive bioreactors. At the beginning of the treatment, in an oxidized or slightly reduced geochemical environment, metals are mainly removed by sorption onto organic matter and precipitation as (oxy)hydroxides and carbonates. After SRB acclimation and sulfide generation, metals are removed mainly as less soluble sulfide precipitates (Machemer et al., 1993; Herbert et al., 1998; Song, 2003; Gibert et al., 2005). Further, the more reduced the environment, the more reduced are the forms of sulfides. Amorphous FeS, greigite ( $\text{Fe}_3\text{S}_4$ ), and mackinawite (FeS) are very common metastable iron sulfides that act as precursors in the formation of pyrite ( $\text{FeS}_2$ ) in very reducing environments (Machemer et al., 1993). The following equations represent possible pathways of sulfide transformations (Machemer et al., 1993):



Sometimes pyrite formation might be limited by the rate of  $\text{SO}_4^{2-}$  reduction, which generates sulfides, and by Fe availability (Machemer et al., 1993).

Spent mixture analysis can provide information about how efficient the system is in removing metals, how available the metals are to remobilization, how long the system will last, and the stability of the environment over time (Machemer et al., 1993). Mineralogical analysis of wastes can provide information about the form of sulfides present. There are only a few techniques appropriate for waste mineralogical analysis because they are amorphous or sulfide concentrations are below detection limits. Scanning electron microscopy (SEM) with X-ray microanalysis is a common technique to locate and identify the metal elements in solids. In backscattered scanning electron microscopy (SEM-BSE), structures composed of heavier elements appear brighter than those of lighter ones. With the addition of Energy Dispersive X-ray Spectroscopy (EDS), microanalysis can give atomic percentage of the tested spots and then the elemental composition of the sample can be obtained with high spatial resolution.

Only a few studies have performed mineralogical analysis of wastes generated in wetlands (Machemer et al., 1993; Song, 2003) or passive bioreactors used for AMD passive treatment (Gibert et al., 2005). Using SEM-BSE, no clear evidence of crystalline sulfides was found in the study of Machemer et al. (1993). However, amorphous iron and lead sulfides were reported by Song (2003), while mackinawite and greigite were found in the study of Herbert et al. (1998). Nevertheless, a very recent study did not detect the presence of sulfides in wastes at the end of a 158 day AMD passive biological treatment (Gibert et al., 2005).

The first part of this study focused on characterization of six natural organic materials and their short-term effectiveness (over 70 days) in sulfate-reduction and metal removal from synthetic AMD. Results obtained during this first part will be very briefly presented.

In the second part, long-term performance and metal precipitates in the reactive mixtures were assessed. The objective was a physicochemical and mineralogical analysis of treated water and solids after 350 days of passive treatment in batch bioreactors, in order to assess the presence of metal sulfides and to gain insight into the long-term stability of generated wastes.

## **2. MATERIALS AND METHODS**

### **2.1 Sampling and Preparation**

In the first part of the study, maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure, and conifer sawdust were evaluated. Nine reactive mixtures, in duplicate, containing single substrates and substrate mixtures were tested in a 70-day batch experiment. Bioreactors were 2 L glass reaction flasks, filled with 200 g of reactive mixture and 800 mL of synthetic AMD. Sampling and analyses of treated water was performed every 5-9 days for the first 70 days. More details on the first part of the study can be found in Zagury et al. (2006).

In the second part of the study, the 18 batch bioreactors were kept sealed, at room temperature ( $22\pm 1^\circ\text{C}$ ), for a long-term assessment until day 350, when the final sampling and analyses of treated water were performed. Unfortunately, in bioreactors filled with sphagnum peat moss (2 & 2D) no more liquid was available for sampling. At the same time (day 350), the solid waste from reactor 8D, which contained (% w/w) maple wood chips (2%), leaf compost (30%), poultry manure (18%), urea (3%), sand (30%), creek sediment (15%), and calcium carbonate (2%) was separated and dried at  $60^\circ\text{C}$  until a constant weight was attained. After drying, solid samples were mounted on sample stubs and conductively coated by thin-film deposition of carbon tape for mineralogical analysis by SEM or used for metal content analysis after acid digestion with  $\text{HNO}_3/\text{Br}_2/\text{HF}/\text{HCl}$ .

### **2.2 Physicochemical and Mineralogical Analysis**

The six organic matter sources were analyzed in terms of their carbon and nitrogen content, as well as their easily available substances content (EAS) (Zagury et al., 2006). At day 350, the treated

water was analyzed for pH and oxydo-reduction potential (ORP) using a HACH Model sensION1 portable pH/mV/temperature meter with a gel-filled pH electrode and a combination Ag/AgCl redox potential electrode. Sulphates, sulfides, alkalinity and ferrous iron were analyzed using an HACH UV-VIS DR/2010 spectrophotometer according to standard methods. Metal content (Na, K, Ca, Mg, Fe, Mn, Cd, Ni, and Zn) was determined using inductively coupled plasma atomic emission spectrometry ICP-AES (*Perkin Elmer Optima 3100*).

Results from physicochemical analyses of treated water at 0, 5, and 12 days after the beginning of the experiments were used for geochemical modeling with VMINTEQ (version 2.14).

In mineralogical analyses, solid samples prepared as described above were used for qualitative non-destructive analysis using SEM-BSE and compositional mapping (*Hitachi S-3500N* equipped with a microanalysis detector *Oxford Link-Isis*) and for quantitative destructive analyses using inductively coupled plasma atomic emission spectrometry ICP-AES (*Perkin Elmer Optima 3100*) after acid digestion.

### 3. RESULTS

#### 3.1 Physicochemical Analyses

Initial chemical characterization revealed that the total organic carbon of six organic carbon sources varied from 11.5% (leaf compost) to 51% (maple wood chips), while dissolved organic carbon varied from 0.05% (conifer compost) to 6.13% (poultry manure) (see Zagury et al., 2006 for more details). In terms of biodegradability, expressed as EAS, poultry manure had the highest content (63.09%), whereas maple wood chips had the lowest content (28.34%). Furthermore, a C/N ratio of 3.3 suggested poultry manure would be the most suitable organic substrate for SRB. However, results showed that reactors 8 and 8D (mixture of carbon sources) were the most efficient for sulphate reduction and metal removal after 70 days.

Physicochemical analyses of treated water in batch bioreactors at day 350 are presented in Table 1. Results showed that five bioreactors (5D, 8, 8D, 9, and 9D) were efficient for AMD long-term treatment. These bioreactors were filled with reactive mixtures as follows: poultry manure (only one replicate), both replicates of the three organic carbon mixtures (maple wood chips, poultry manure, and leaf compost), and those of the same mixture and formaldehyde. Efficiency was indicated by neutral to slightly alkaline pH (7.68-7.74) and very low concentrations of sulfates (200-800 mg/L) and ferrous iron (9-16 mg/L). In these efficient bioreactors, a reduced geochemical environment about -300 mV or less was maintained throughout the experiments. Heavy metals (Mn, Cd, Ni, and Zn) from initial synthetic AMD were all below analytical detection limits. Moreover, bioreactors filled with the mixtures of organic carbon sources produced the highest alkalinity (36-40 g/l CaCO<sub>3</sub>). Other organic substrates performed less satisfactory, such as bioreactors 6 & 6D, which produced 91 to 107 mg/L CaCO<sub>3</sub> alkalinity because of toxicity due to phenol compounds contained by conifer sawdust (Neculita et al., 2006) or 10 to 62 mg/L CaCO<sub>3</sub> alkalinity because of acetate accumulation, as in the ethanol containing bioreactors 7 & 7D. Therefore, as results indicated (Table 1), batches 8 & 8D were the most efficient in the long-term 350 day batch experiment. This was the reason for using the solids from bioreactor 8D for mineralogical analysis.

Table 1. Physicochemical characteristics of treated water during batch sampling after 350 days

Reactor	Composition	Parameter								
		pH	ORP (mV)	Fe <sup>2+</sup>	SO <sub>4</sub> <sup>2-</sup>	Sulfides	TOC	DOC	Alkalinity	NH <sub>4</sub> <sup>+</sup>
		.....mg/L .....								
1	Maple wood chips	7.30	+45.4	25	3167	0.83	5682	3994	125	3000
1D		7.30	-59.2	5.0	833	1.70	4160	4316	565	2700
2	Sphagnum peat moss	No liquid								
2D										
3	Leaf compost	8.13	+40.4	183	3500	4.30	3744	1123	665	2400
3D		7.84	+54.6	272	2500	0.30	4368	1196	715	2600
4	Conifer compost	7.80	+55.1	25	2500	0.05	2635	530	153	2300
4D		7.69	+24.6	8.3	3167	0.10	2642	478	101	2300
5	Poultry manure	7.36	-301.9	22	4200	0.10	4181	208	795	7800
5D		7.74	-419.6	9	400	0.20	9610	333	1390	6800
6	Conifer sawdust	7.86	-14.7	18	3600	0.60	5366	343	107	2700
6D		8.26	+16.3	2	3300	0.50	4992	135	91	2700
7	Ethanol	7.97	-315.8	7	5100	0.10	6490	62	200	1700
7D		7.80	-17.5	5	5500	0.70	4555	10	109	1600
8	Mixture*	7.72	-292.9	16	200	0.20	5054	894	36000	4000
8D		7.73	-343.2	17	300	0.60	2434	978	39500	3800
9	Mixture and FA**	7.70	-399.1	27	800	0.30	5554	1030	36000	3700
9D		7.68	-310.6	27	300	0.40	6739	1113	40000	4300

\* Reactive mixture from bioreactors 8&8D contained (% w/w) maple wood chips (2%), leaf compost (30%), poultry manure (18%), urea (3%), sand (30%), creek sediment (15%), and calcium carbonate (2%).

\*\* Reactive mixture from bioreactors 9&9D contained (% w/w) maple wood chips (2%), leaf compost (30%), poultry manure (18%), urea (3%), sand (30%), creek sediment (15%), and calcium carbonate (2%) and formaldehyde (0.015%w/v) to inhibit bacterial activity.

Table 2. Physicochemical characteristics of treated water and solid phase at the beginning (day 0) and at the end (day 350) of batch experiments (reactor 8D)

Parameter	Liquid		Parameter	Solid	
	Initial (synthetic AMD)	Final (treated water)		Initial (reactive mixture)	Final (waste)
pH	3.9 – 4.2	7.73	pH	-	-
Alkalinity (mg/L CaCO <sub>3</sub> )	130	39 500	Alkalinity (mg/L CaCO <sub>3</sub> )	-	-
Ca <sup>2+</sup> (mg/l)	330 ± 20	93	Ca <sup>2+</sup> (%)	5.72	9.47
Cd <sup>2+</sup> (mg/l)	8.3 ± 2.0	< 0.4	Cd <sup>2+</sup> (%)	-	0.002
Fe <sup>2+</sup> (mg/l)	1683 ± 35	17	Fe <sup>2+</sup> (%)	0.08	4.94
K <sup>+</sup> (mg/l)	55 ± 1	1100	K <sup>+</sup> (%)	-	-
Mg <sup>2+</sup> (mg/l)	99 ± 1	6	Mg <sup>2+</sup> (%)	-	0.831
Mn <sup>2+</sup> (mg/l)	14 ± 1	< 0.4	Mn <sup>2+</sup> (%)	0.0008	0.046
Na <sup>+</sup> (mg/l)	220 ± 30	350	Na <sup>+</sup> (%)	-	0.562
Ni <sup>2+</sup> (mg/l)	15 ± 1	< 0.4	Ni <sup>2+</sup> (%)	-	0.025
Zn <sup>2+</sup> (mg/l)	15 ± 3	< 0.8	Zn <sup>2+</sup> (%)	0.0004	0.036
S total (mg/L)	1415 ± 80	100	S total (%)	0.149	0.342
S sulfides (mg/L)	-	-	S sulfides (%)	0.076	0.200
SO <sub>4</sub> <sup>2-</sup> (mg/L)	4244 ± 239	300	S sulfates (%)	0.073	0.142

Table 2 presents the main parameters of the treated water and wastes from reactor 8D. Results showed that wastes contained a total sulphur concentration of 3240 mg/kg, which represents 1240 mg/kg more sulphur than the initial sulphur concentration, both as sulfates and as sulfides. Sulfides increased from 760 mg/kg in the fresh reactive mixture to 2000 mg/kg in generated wastes. Heavy metals were also found in the wastes. Unfortunately, metal concentrations in the fresh reactive mixture were not analyzed.

### **3.1 Geochemical Modeling**

Results indicated that metal removal in the early stage of treatment (up to day 12) could be attributed to metal precipitation as hydroxides minerals, such as goethite ( $\text{FeOOH}$ ), hematite ( $\text{Fe}_2\text{O}_3$ ), magnetite ( $\text{Fe}_3\text{O}_4$ ), and lepidocrocite ( $\text{FeOOH}$ ) and as carbonates minerals such as otavite ( $\text{CdCO}_3$ ),  $\text{NiCO}_3$ , siderite ( $\text{FeCO}_3$ ), and smithsonite ( $\text{ZnCO}_3$ ).

### **3.2 Mineralogical Analyses**

Figure 1 presents images obtained during SEM-BSE microanalysis and compositional mapping for various elements. As mentioned before, the bright spots indicate elements of high atomic weight through the mechanism of atomic number contrast. Qualitative analysis by EDS conducted on several selected bright spots helped to determine the chemical composition of the bright spots. Compositional mapping (or X-ray elemental map), very representative in the case of highly heterogeneous solids, was conducted on samples to map the distribution of selected elements (Fe, S, Ca, Mg, Si, and O). First, iron and sulphur which had accumulated in the reactive mixture of batch bioreactors was detected. Then, the study of iron and sulphur spots in relation to the weight and the atomic percentage indicated an atomic Fe/S ratio close to 2. Therefore, the bright spots turned out to be pyrite ( $\text{Fe}_2\text{S}$ ). Other heavy metals removed from artificial AMD during batch treatment were not detected in the selected samples.



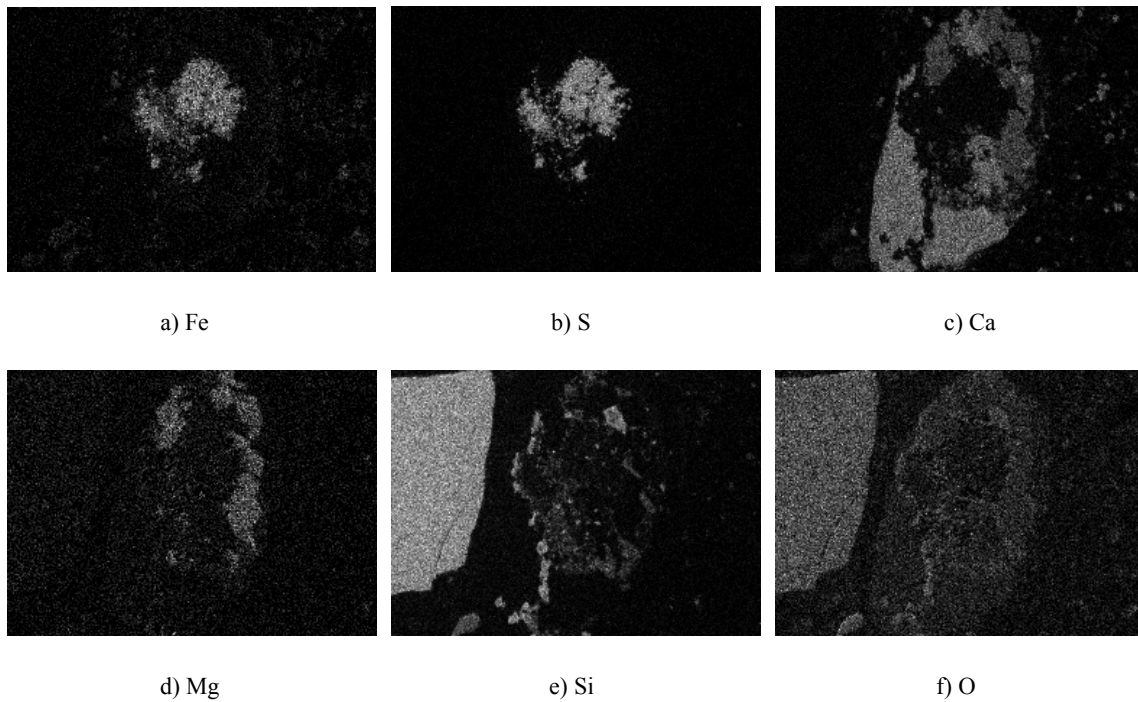
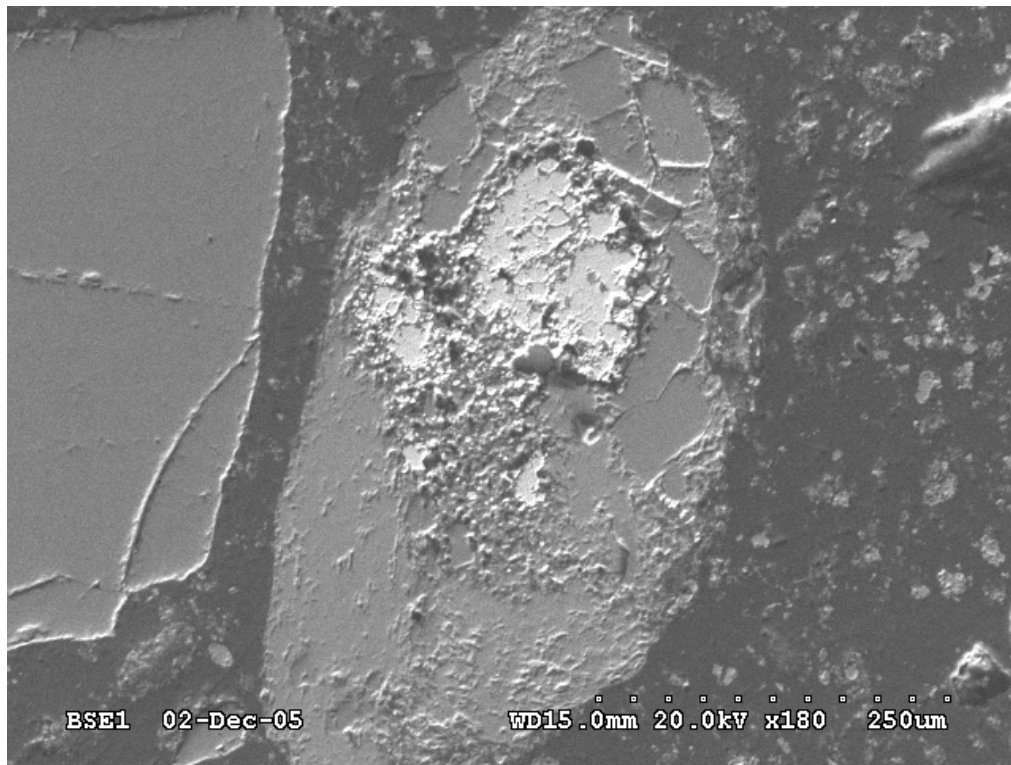


Figure 1. SEM-BSE image and compositional mapping for a) Fe; b) S; c) Ca; d) Mg; e) Si, and f) O

#### 4. DISCUSSION

The highest content of EAS and of dissolved organic carbon and the lowest C/N ratio suggested poultry manure would be the best substrate. Unfortunately, results did not confirm this supposition. Water sampled from bioreactors 5 & 5D filled with poultry manure as a single substrate showed

quality depreciation during the first 70 days of the experiments, with increased sulfates and some heavy metal (e.g. Ni) concentrations. However, the same poultry manure which did not give good results as a single substrate worked very efficiently when integrated in a mixture. Therefore, single substrates might be less efficient than mixture of a few substrates, regardless of the amount of “easily available substances” when used in short-term experiments (Zagury et al., 2006). In long-term tests up to 350 days, one of two bioreactors filled with poultry manure showed a higher efficiency for pH neutralization and metal and sulfate removal. Nevertheless, the best performance in short-term and in long-term batch experiments was obtained with the mixture of three organic substrates, which is in concordance with results of other studies (Waybrant et al., 1998). Substrate characterization provided insight on organic carbon availability but did not elucidate their ability to promote sulphate-reduction and metal removal.

Mineralogical analyses suggested that the precipitates which formed in the media containing SRB were amorphous or poorly crystalline iron sulfides. The EDS analysis of the sulfide precipitates in solid samples from the long-term batch experiments (350 days) indicated an atomic Fe/S ratio close to 2, which was interpreted as an indication of the presence of pyrite. Chemical analysis using ICP-AES showed that the wastes contained metals removed from synthetic AMD but SEM-BSE failed to detect these metals. However, the detection limit of SEM is 0.2% which might be too high for concentrations as low as those of Mn, Cd, Ni, and Zn used in this study. Nevertheless, the presence of iron sulfides confirmed that the wastes obtained in this long-term 350 day study are more stable compared to sludge generated during AMD chemical treatment.

## 5. CONCLUSIONS

Passive biological treatment of synthetic AMD in batch bioreactors was successful for up to 350 days. The most effective reactive mixture for pH neutralization, metal removal, and sulfate removal contained a mixture of three organic carbon sources (maple wood chips, poultry manure, and leaf compost). Geochemical modeling of metal speciation suggested that at the beginning of the treatment (0-12 days), metals precipitated in the form of (oxy)hydroxides and carbonates. Chemical analysis of the final wastes showed higher concentrations of sulfides compared with initial concentrations found in components used for the reactive mixture preparation. Moreover, mineralogical analyses by SEM-BSE and X-ray microanalysis confirmed the presence of pyrite, a good indication of waste stability. In summary, physicochemical analyses of treated water coupled with chemical and mineralogical analyses of solid wastes are effective tools for confirming successful treatment of AMD in passive bioreactors. Further research is needed to accurately predict long-term carbon availability and to assess the metal precipitates in spent reactive mixtures.

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