Evaluation of fine organic mixtures for treatment of acid mine drainage in sulfidogenic reactors

N. Pérez, A. Schwarz and J. de Bruijn

ABSTRACT

The performance of passive biochemical reactors in acid mine drainage (AMD) treatment could be enhanced by using fine organic substrates in new reactor designs, such as diffusive exchange reactors. This work evaluated the effect of fine cellulosic components in organic mixtures and of enrichment with inoculum, on sulfate and metals removal in discontinuous cultures for three types of synthetic AMD. The cellulosic substrates evaluated were sawdust, microcrystalline cellulose, and forestry cellulose fibers, supplemented with cow manure and leaf compost. Using microcrystalline cellulose and forestry cellulose fibers with the less concentrated AMD, high sulfate reduction rates (73 mg/L-d and 58.2 mg/L-d, respectively) were achieved. Correspondingly, iron concentrations were reduced by 69% and 86.6%. Based on their higher sulfate reducing capacity, cellulose fibers obtained as fiber boards from a local kraft pulp mill were selected for treating a synthetic AMD with a high copper concentration (273 mg/L) and pH 4.94. In batch culture, low sulfate reducing activity (13.10 mg/L-d) was only observed at the highest substrate/AMD ratio (0.5:10) tested. Results show that the use of forestry cellulose fibers in reactive mixtures supplemented with inoculum could be an alternative for optimization of diffusive exchange reactors for AMD treatment.

Key words | acid mine drainage, diffusive exchange reactor, reactive material, sulfate-reducing bacteria

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INTRODUCTION

Massive mine waste, mainly tailings, waste dumps and heap leach pads, are a potential danger for the environment, especially when the wastes contain minerals which oxidise to produce acid mine drainage (AMD). Among the pollution problems produced by AMD is contamination of water bodies with high concentrations of metals, high sulfate contents (>2,500 mg/L) and low pH (2.0–4.5) (Benner *et al.* 1999).

To avoid its impact on the environment, AMD must be treated through either passive or active systems before being discharged into water bodies. Passive solutions, characterized by low operating and maintenance costs, reduced use of chemicals, and minimal energy consumption, are typically preferred in the closure phase of mining projects, low flux cases, or remote places (Sheoran *et al.*, 2010; Fitch 2015). They promote AMD contact with reactive material, alkaline (e.g. limestone, sea shells, ash) and/or organic (e.g. hay, wood chips, leaf compost, manure), in porous beds with low gravitational flow. Chemical and/or biochemical reactions take place that remove sulfates, immobilize metals and neutralize acidity. Particularly, in biological passive systems, the reactive bed is mainly made of organic particles that promote the biological reduction of sulfate to sulfide, which forms sparingly soluble complexes with most toxic metals (Kijjanapanich *et al.*, 2012; Zhang & Wang 2014). A potential limitation of passive biological systems, however, is their low sulfate reduction rate, which is subject to slow hydrolysis reactions of coarse organic substrates, used in conventional designs. To mitigate this problem, Schwarz & Rittmann (2010) proposed providing preferential routes for AMD transport through the beds in Diffusive Exchange System (DES) reactors

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(using drainage tubes, for example), to allow the use of finer, more bioavailable substrates while maintaining bed permeability.

The organic reactive material normally consists of mixtures of particles in the size range 5–25 mm (McCauley *et al.* 2009), but in some works zero-valent iron (Fe^0) has also been included in the mixture. Both these materials promote sulfate reduction by sulfate-reducing bacteria (Karri *et al.* 2005; Costa *et al.* 2009).

The sulfate-reducing bacteria (SRB) are key to bioremediation of AMD, because they neutralize acidity and simultaneously remove sulfate and metals (Zagury *et al.* 2006; Neculita *et al.* 2007). SRB oxidize the products of fermentation, including organic compounds and hydrogen (electron donors), using sulfate as the final electron acceptor to produce sulfide (Rabus *et al.* 2013), which reacts with the metals to produce insoluble precipitates. The organic reactive material allows growth of a microbial consortium, which hydrolyses and ferments long-chain organic compounds like celluloses and hemicelluloses to electron donors for SRB (like acetic acid, butyric acid and propionic acid).

For the construction of sulfate-reducing bioreactors, affordable substrates are used that will provide organic material in the long term. The composition of the reactive mixture is fundamental for the efficiency of the sulfate-reduction process. Research suggests that the most reactive mixture is one containing various carbon sources (Waybrant *et al.* 1998; Zagury *et al.* 2006) and 10% zero-valent iron (Lindsay *et al.* 2008). Substrates with cellulose have been shown to be more efficient than lignocellulose (Waybrant *et al.* 1998). An optimum mixture should contain cellulosic and organic waste in equal proportions (Neculita *et al.* 2008). Organic wastes (compost and manure) accelerate the activity of sulfate reducers during the start of the operation (McCauley *et al.* 2009).

In conventional reactors, the grain size of the organic material used is coarse to allow the contaminated water to flow through the reactive mixture, which limits its reactivity because of reduced specific surface area. One way of improving the performance of biochemical reactors is to use fine organic substrates for increased reactivity in new reactor designs (Pérez *et al.* 2018), such as diffusive exchange reactors, which provide routes by which the AMD is transported through the reactor, and in which the biologically active zones are protected from the toxicity of the metals by chemical gradients (Schwarz & Rittmann 2010; Pérez *et al.* 2017).

Among passive AMD treatments, biological solutions are often rejected because they demand the highest hydraulic retention times, defined by slow sulfate reduction rates. Hence, with the aim of developing smaller and less expensive passive bioreactors, this research evaluates the effect of three cellulosic components (sawdust/microcrystalline cellulose/forestry cellulose fibers) within fine organic mixtures, along with the inoculum supplied, on sulfate reduction and metals removal for three types of AMD of varying acidity and metal content to span a wide range of hydrochemical conditions.

MATERIALS AND METHODS

Source of inoculum, organic substrate and AMD wastewater

The inoculum of SRB was enriched from anaerobic digester content of the local sewage treatment plant by repeated transfers into modified Postgate C culture medium (Benner *et al.* 2000). On the other hand, cellulolytic bacteria were enriched from the sediment of a small lake on the campus of Universidad de Concepción by transfer into culture medium, which provides cellulose as the only carbon source (Atlas 2005).

The organic substrates evaluated were sawdust obtained from a local sawmill, bleached forestry cellulose fibers obtained as fiber boards from a local kraft pulp mill, microcrystalline cellulose (Sigma Aldrich, 20 μm), fresh cow manure, and campus leaf compost.

The experiments were carried out with three types of synthetic AMD wastewater, a, b, and c, with different metal concentrations and acidity (Table 1). The chemical composition of simulated mine water a was based on mines located in Ontario and Manitoba (Lindsay *et al.* 2008), while mine water b was based upon contaminated groundwater from a nickel-copper mine in Ontario

Table 1 | Composition of the AMD prepared for the experiments

Elements	Drainage a (mg/L)[a]	Drainage b (mg/L)[b]	Drainage c (mg/L)
SO_4^{2-}	3,600	3,620	2,710
Fe (II)	750	1,080	105.6
Zn	100	0.81	10.34
Mn	20	–	22
Ni	15	1.15	0.34
Cd	10	–	0.13
Cu (II)	4.76	–	273
pH	2.5	3.5	4.94

[a]modified from Lindsay *et al.* (2008); [b]modified from Waybrant *et al.* (1998).

(Waybrant *et al.* 1998). On the other hand, solution chemistry of mine water c was based on data from a large copper mine in central Chile (Pérez *et al.* 2017). Since mine waters a and b have a positive record of biological treatment, they were selected to evaluate the effect of fine organic mixtures and acid drainage composition on sulfate reduction, whereas mine water c, deemed considerably more toxic, was selected to determine the feasibility of treating AMD containing high copper concentrations.

Preparation and characterization of organic substrates

The sawdust, cow manure and cellulose fibers were ground; the sawdust and manure were selected by sieving at 20 μm. The sawdust was delignified in proportions of 1:10 (w/v) solid/water sodium hydroxide at 8%, shaken constantly for 2 hours at 150 °C. The hydroxide was removed by reiterated washing with distilled water.

All the substrates were dried previously at 60 °C to constant weight. In all the organic substrates, the percentage of organic matter and moisture (Karam 1993), the pH (ASTM 1995), the ORP and the C, N and P contents were measured (Gibert *et al.* 2004). The dissolved organic carbon (DOC) was determined in the leaf compost and the manure (Neculita *et al.* 2011) by mixing 20 grams of the substrates in 200 mL of nanopure water for 2 hours; the extracts were centrifuged at 800 rpm for 10 minutes and subsequently filtered (pore size 0.45 μm) and the carbon content was determined in the filtered liquid with a HACH kit (Total Organic Carbon, Direct Method, High Range Test 'N Tube).

Effect of the organic mixture and the type of acid drainage on sulfate reduction

The delignified sawdust, the microcrystalline cellulose and the forestry fibers were incubated during 48 days in batch reactors with cow manure, leaf compost and zero-valent iron (Table 2). The incubations were carried out in duplicate with two types of drainage, a and b, with different metal concentrations and acidity (Table 1). Mixtures were prepared of 150 grams/L in 500 mL and 2 L flasks in which the anaerobic cultures were developed (Figure 1).

The viable bacteria of the inoculum (SRB and cellulolytic bacteria) were quantified using the LIVE/DEAD® Bac LightTM VIABILITY kit; calculations were made to determine the inoculum volume to be added to reactors ($4-5\times10^4$ viable cells/reactor).

The batch reactors were sealed with rubber plugs. The drainages were incubated in batch reactors as a negative control. All the treatments were incubated at ambient temperature during the summer months (16 ± 6 °C).

Samples of all the treatments were taken weekly (approximately 5 mL, using sterile syringes and shaking the flasks vigorously before sampling) to determine pH, ORP and concentrations of sulfate, sulfide and iron.

Table 2 | Composition (% m/m) of mixtures to evaluate the effect of the source of cellulose and inoculum on sulfate reduction

Material	CD-a/ CD-b	AD-a/ AD-b	FD-a/ FD-b	C	A	F
Cow manure	15%	15%	15%	15%	15%	15%
Leaf compost	30%	30%	30%	30%	30%	30%
Microcrystalline cellulose	45%	–	–	45%	–	–
Sawdust	–	45%	–	–	45%	–
Cellulose fibers	–	–	45%	–	–	45%
Fe0	10%	10%	10%	10%	10%	10%
Anaerobic sludge (ml)	10	10	10	10	10	10
Cellulolytic inoculum (ml)	2	2	2	–	–	–
SRM inoculum (ml)	2	2	2	–	–	–

Treatments: CD-a (cellulose and drainage a); AD-a (sawdust and drainage a); FDa (forest fibers and drainage a); CD-b (cellulose and drainage b); AD-b (sawdust and drainage b); FD-b (forest fibers and drainage b). Columns C (microcrystalline cellulose), A (sawdust) and F (forest fibers) correspond to experiments with sludge as the only source of inoculum and type a drainage.

Effect of microorganisms present in the anaerobic sludge on sulfate reduction

It is important to determine the most affordable source of the inoculum which produces the highest sulfate reduction rates. For this reason, the three sources of cellulosic substrates were incubated in duplicate during 48 days with drainage a, using only anaerobic digester content as the inoculum source, leaving enrichments out (Figure 2).

The reactors were called F, C and A (Table 2). The results were contrasted with the results obtained in the previous experiment (FD-a, AD-a, CD-a) using enriched inoculum.

Feasibility of treating AMD containing copper

Once the best mixture had been determined, batch experiments were carried out during 58 days with forestry fibers (49%), calcium carbonate (3%), leaf compost (31%), cow manure (17%) (Table 3), and AMD characteristic of a copper mine in central Chile (drainage c in Table 1). The purpose of this experiment was to assess

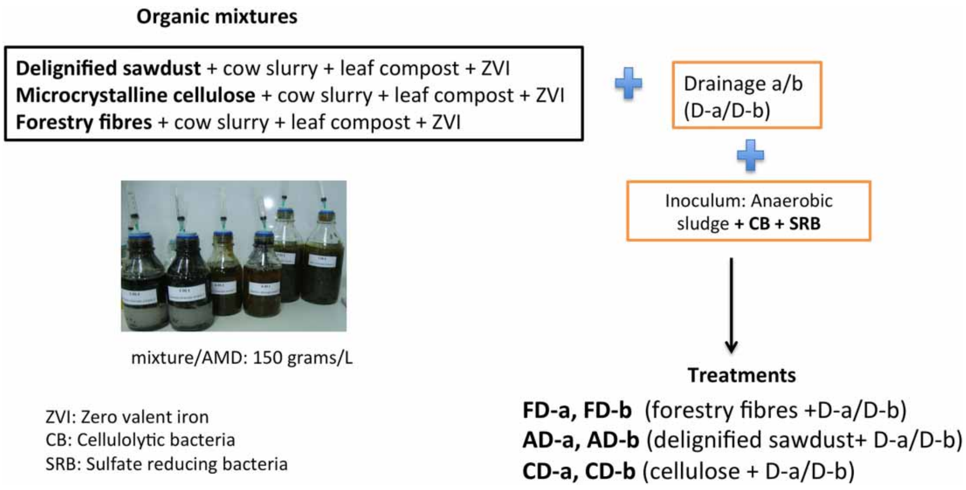

Figure 1 | Evaluation of the effect of the organic mixture and the type of AMD on sulfate reduction.

treatment feasibility of this drainage with the best substrate from previous steps, and to determine the substrate/AMD ratio for which biological activity is possible and that should be used in future substrate evaluation experiments.

The organic matter mixture concentration in AMD treatments varied, working with mixture concentrations X/10, X/8, X/5, X/3 and X/2, where X = 160 g mixture/L (Figure 3). The AMD was incubated alone as negative control and all the treatments were done in duplicate. Samples of all the treatments were taken weekly (approximately 5 mL, using sterile syringes and shaking the flasks vigorously before sampling) to determine pH, ORP and concentrations of sulfate, sulfide and copper.

Chemical analyses

The pH and ORP were measured immediately after sampling. The samples were filtered (pore size 0.45 μm) to remove the interfering solids before analysing the sulfate, sulfide and dissolved metals (iron and copper). The redox potential (ORP) was determined with the Thermo Scientific Orion 9180B electrode and the pH with the HACH HQd electrode.

The sulfate was measured using the 4500-SO_4^{2-}E method (APHA 2005) according to HACH (Sulfaver 4). The sulfides were measured by HACH's 'Methylene Blue' colorimetric method, 4500-S^{2-}D (APHA 2005). Iron was measured using HACH's 'Phenanthroline' method (APHA 2005). Copper was measured with the CuVer kit (HACH).

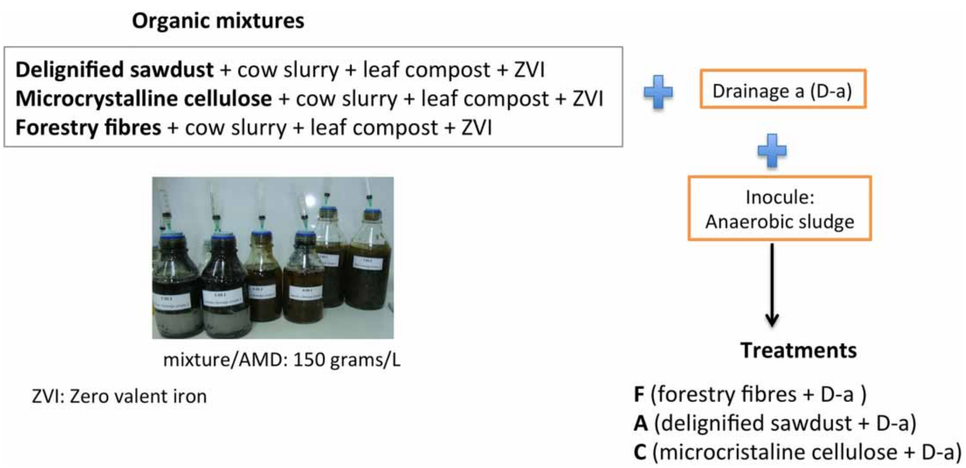

Figure 2 | Evaluation of effect of the inoculum.

Table 3 | Variation in the proportion of organic substrates used in batch reactors and incubated with AMD c

Material	X/2	X/3	X/5	X/8	X/10
Cow manure (17%)	6.4 g	4.3 g	2.6	1.6	1.3
Leaf compost (31%)	11.6	7.8	4.7	2.9	2.3
Forest fibers (49%)	18.4	12.3	7.4	4.6	3.7
$CaCO_3$ (3%)	1.1	0.8	0.5	0.3	0.2
Total mass of mixture (g)	37.5	25	15	9.4	7.5

All the reactions were measured in a HACH spectrophotometer DR 2800.

RESULTS AND DISCUSSION

Characteristics of the substrates

Cow manure and leaf compost contain high concentrations of DOC (Table 4), and provide electron donors (short chains) for immediate sulfate reduction; they also provide nitrogen and phosphorus, elements which are fundamental for the development of the microorganisms. The more complex organic sources (forestry fibers, microcrystalline cellulose and sawdust) provide long-term electron donors and are the organic substrates required for the growth of the consortium responsible for the gradual release of short chains (ITRC 2013).

Although the mixtures contain high C/N ratios of between 20 and 30, because of the short experimental time this factor did not limit bacterial metabolism. Works like Gibert *et al.* (2004) also tested mixtures with C/N ratios between 17 and 26. To obtain high, long-term metal removal, an additional nitrogen source must be included to correct the C/N ratio to values close to 10 (Béchard *et al.* 1994).

Effect of the organic mixture and the type of acid drainage on sulfate reduction

The evolution of the pH and sulfate concentration are shown in Figure 4. The effect of the type of reactive mixture on the initial pH was observed in all the experiments (Figure 4(a)). Although the pH of drainages a and b was low (2.5 and 3.5, respectively), when they came into contact with the mixtures containing sawdust (time 0) their pH increased to 5.7 and 5.9, respectively (increases of 3.2 and 2.4 units). A smaller effect was noted in the mixtures containing microcrystalline cellulose, in which the pH rose by only 1.5; and those with forest fibers, which increased by 1.4 units. This increase is due to the contribution of the components of the mixtures to the generation of alkalinity. The pre-treatment of sawdust (substrate with pH = 10.3, see Table 4) helped to obtain higher initial pH values. Furthermore, the generation of alkalinity in the mixtures with microcrystalline cellulose and fibers is due principally to the presence of manure, which, after sawdust, is the substrate with the greatest capacity for generating alkalinity. At the end of the experiments, all the mixtures showed a tendency to reach a pH close to 6.

Because cultures enriched with SRB were inoculated, the sulfate concentration diminished immediately (Figure 4(b))

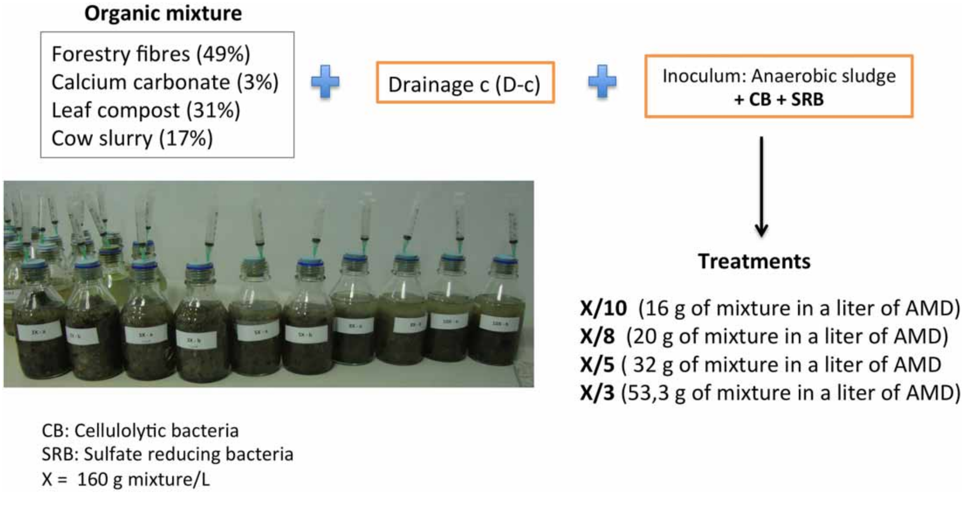

Figure 3 | Treatment of AMD containing copper.

Table 4 | Chemical characterization of the organic substrates evaluated

	Cow manure	Leaf compost	Delignified sawdust (A)	Fibers of forest origin (F)	Microcrystalline cellulose (C)
pH	9.21	7.17	10.3	5.2	
% Organic matter	83.58	43.5	97.0	99.9	100
% Humidity	83.2	55.6	49.8	3.0	2.0
DOC	2,646	3,906			
Total P (%)	0.7	0.14	0.01	0.01	0.08
Total N (%)	3.7	2.07	0.15	0.01	0.01
Total C (%)	49.7	21.83	54.85	51.1	51.1
C/N ratio	13.4	10.5	365.7	5,110	5,110

without occurrence of the typical acclimatisation phase (Zagury *et al.* 2006; Lindsay *et al.* 2008; Neculita *et al.* 2011). After 48 days, in mixtures with microcrystalline cellulose the sulfate concentration diminished to 50 mg/L (CD-b) and 250 mg/L (CD-a); while in the mixtures with forest fibers the lowest values recorded were 450 mg/L (FD-b) and 950 mg/L (FD-a). Iron and sulfate concentrations (Figures 4(b) and 4(c)) behaved similarly, because a key iron removal mechanism must have been precipitation with biogenic sulfide (Zagury *et al.* 2006; Neculita *et al.* 2007).

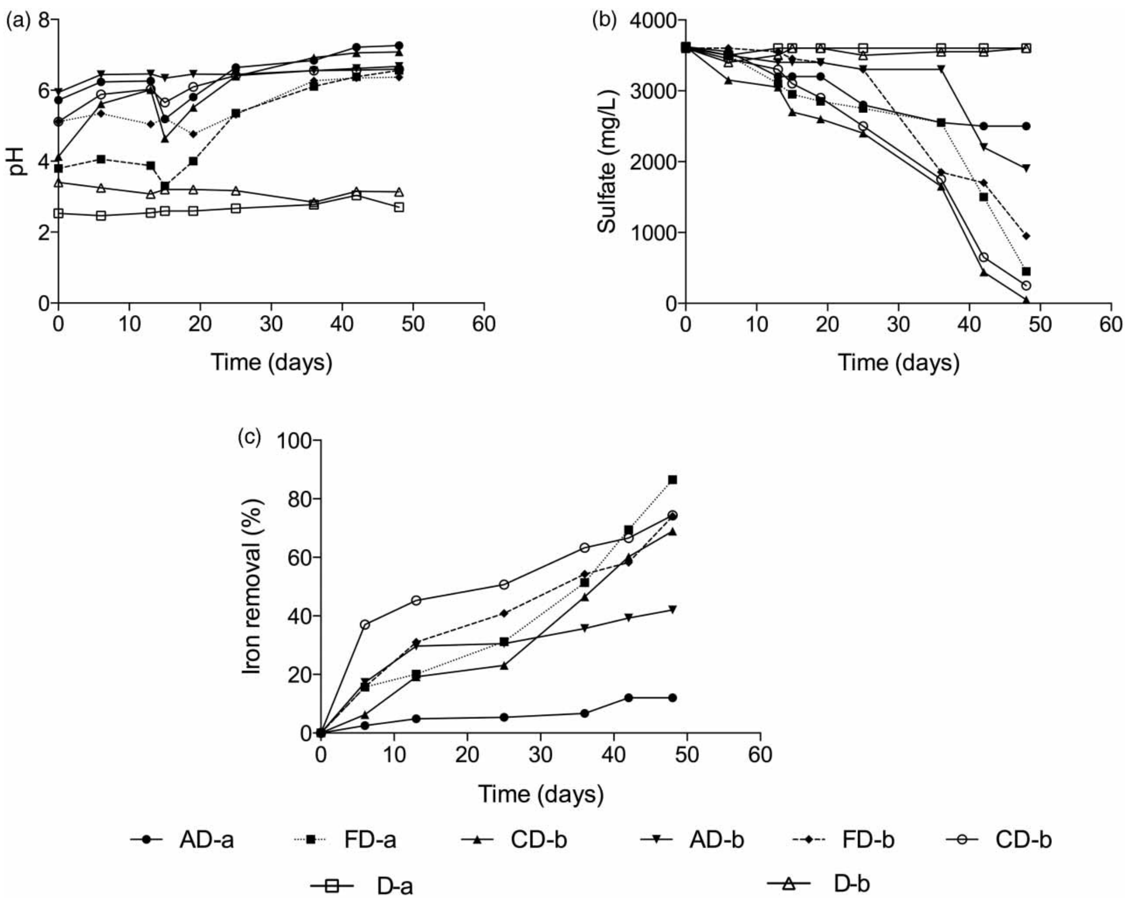

Figure 4 | Changes in pH (a), sulphate concentration (b) and iron removal (c) obtained during evaluation of the effect of the sources of cellulose and drainage on sulfate reduction. Treatments: CD-a (microcrystalline cellulose and drainage a); AD-a (sawdust and drainage a); FD-a (forest fibers and drainage a); CD-b (microcrystalline cellulose and drainage b); AD-b (sawdust and drainage b); FD-b (forest fibers and drainage b).

Iron removal was lowest in sawdust treatments, with a minimum of 12 and 42% (AD-a and AD-b, respectively), and highest in forestry fibers treatments, reaching a maximum of 86.6 and 74% (FD-a and FD-b, respectively).

The sulfate reduction rates, which were calculated using the least squares regression (Neculita *et al.* 2008), showed significant differences between mixtures. However, no effect of the type of acid drainage was observed on the sulfate reduction rate. Microcrystalline cellulose presented higher rates (Table 5). This substrate was included to quantify the sulfate reduction potential with a pure, more bioavailable cellulose; however, due to its high cost, it cannot be used in passive systems. Based on the total volume occupied by the acid drainage and the reactive mixtures in the flasks, the mixtures with fibers presented sulfate consumptions of 58.2 mg/L-d, much higher than was found for the sawdust mixtures (36 and 22 mg SO_4^{2-}/L-d). The reduction rates obtained for the fibers are higher than those reported by Gibert *et al.* (2004) (Table 6) in batch assays.

The redox potential in all the mixtures diminished with time (data not shown), indicative of anaerobic activity. At the end of the experiments, the ORP varied between −180 mV and −250 mV, reaching optimum values for sulfate reduction; for this metabolic process, potentials lower than −150 mV have been reported to be optimum values (Gibson 1990). In terms of sulfide production, the reactors presented final concentrations between 3.6 mg/L and 67.65 mg/L. The sulfide concentrations obtained were higher than reported by Lindsay *et al.* (2008) who obtained 0.2 mg/L.

Table 5 | Sulfate reduction rates obtained during the evaluation of the effect of the source of cellulose and inoculum on sulfate reduction

Treatment	Sulfate consumption (mg*L/day)
FD-a	58.2
AD-a	32.0
CD-a	73.0
FD-b	58.2
AD-b	26.1
CD-b	72.8
F	43.9
A	26.6
C	45.3
FD-c	13.10

Treatments: CD-a (cellulose and drainage a); AD-a (sawdust and drainage a); FD-a (forest fibers and drainage a); CD-b (cellulose and drainage b); AD-b (sawdust and drainage b); FD-b (forest fibers and drainage b). Rows C (microcrystalline cellulose), A (sawdust) and F (forest fibers) correspond to experiments with sludge as the only source of inoculum. Treatments F, A and C were incubated with Db. Treatment FD-c was incubated with D-c (drainage c).

Viggi *et al.* (2009) report batch assays with rates of 590 mg SO_4^{2-}/L-d, working with mixtures with a high limestone content (22%), which releases calcium when it dissolves, forming $CaSO_4$ precipitates. The mixtures used in this study contain no limestone, so the sulfate reduction is assumed to result mainly from biological activity. Nevertheless, when treating very AMD, limestone could be included in reactive mixtures for added neutralization potential, such as in reactive zones of sulfidic diffusive exchange systems (Pérez *et al.* 2017).

Another important factor affecting the sulfate reduction rate in batch systems is the concentration of the mixture used. Lindsay *et al.* (2008) carried out their batch experiments with mixtures of 150 g/L solids; Pereyra *et al.* (2008) used mixtures of 100 g/L and ITRC (2013) recommends mixtures of 520 g/L. These variations in the concentration of organic matter influence the rates obtained, and therefore the volumetric rates based on the volume of substrate saturated are the best way of expressing the results. For the design of passive treatment systems, ITRC (2013) recommends using a sulfate reduction rate between 0.1 and 0.3 mmol/m^3/d as a design factor. Thus, based on the saturated volume occupied by the reactive mixtures in the flasks, the sulfate reduction rates obtained for microcrystalline cellulose, fibers and sawdust are 1.6 mol/m^3/d, 1.2 mol/m^3/d and 0.6 mol/m^3/d, respectively. Due to the low volume occupied by fibers and the high rates obtained, it is expected that a reactor constructed with this substrate will present the highest sulfate reduction per m^3.

Effect of the inoculum

In the experiments with forest fibers (F) and microcrystalline cellulose (C) in which the only inoculum was sludge, the sulfate reduction rates obtained were 43.9 mg/L-d and 45.3 mg/L-d, respectively. These rates are lower than those recorded with enriched inoculum (Table 5; 58.2 (FD-b) and 72.8 (CD-b) mg/L-d). Inoculation with enriched cultures and anaerobic sludge accelerated the appearance of sulfate reduction in FD-b and CD-b. Enriched inocula can be used in cases where the high metal contents require rapid, efficient precipitation.

The acclimatisation of the SRB may vary according to the type of drainage. It has been described that the initial pH and the concentration of multiple metals have a synergic toxic effect on the microorganisms (Zhou *et al.* 2013; Zhang & Wang 2014); moreover, due to the effect of the pH on the

Table 6 | Reactive mixtures, changes in pH and sulfate reducing rates in batch studies for treatment of AMD

Mixture	pH Start	pH End	Sulfate reduction rate (mg/L*d)	AMD (mg/L)	Reference
Sheep manure 15% Creek sediment (SRM inocule) 15% Calcite 30% Quartz 40% Solid:liquid ratio (in volume) was 1:10	6	6–7.5	42.2	Without metals SO_4^{2-}: 1,018 pH: 2.4	Gibert *et al.* (2004)
Maple wood chips 2% Leaf compost 30% Poultry manure 18% Urea 3% Creek sediment (SRM inocule) 15% Calcium carbonate 2% Sand 30% Solid:liquid ratio (in volume) was 1:10	6	8–8.5	13 (mixture R8)	SO_4^{2-}: 4,244 ± 239 Cd^{2+}: 8.3 ± 2.0 Fe^{3+}: 1,683 ± 35 Mn^{2+}: 14 ± 1 Ni^{2+}: 15 ± 1 Zn^{2+}: 15 ± 3 pH: 3.9–4.2	Zagury *et al.* (2006)
Maple wood chips 10% Maple sawdust 20% Leaf compost 20% Composted poultry manure 10% Urea 3% Creek sediment (SRM inocule) 15% Calcium carbonate 2% Sand 20% Solid:liquid ratio was 1:4	6.3	8.2–8.4	80–86 (mixture R3)	SO_4^{2-}: 5,500 ± 250 Cd^{2+}: 12.6 ± 0.9 Fe^{2+}: 1,670 ± 66 Mn^{2+}: 13.5 ± 1.2 Ni^{2+}: 16.8 ±1.8 Zn^{2+}: 18.9 ± 1.1 pH: 5.45–5.51	Neculita & Zagury (2008)
Chitin 100% Sodium lactate 100%	4 – 5	7	Chitin: 17.8 Sodium lactate: 24.8	SO_4^{2-}: 690 Al^{3+}: 10 Fe^{2+}: 10 Mn^{2+}: 15 pH: 2.9	Robinson-Lora & Brennan (2008)
Cow manure (15%) Leaf compost (30%) Forest fibers (45%) ZVI (10%) Solid:liquid ratio (in volume) was 1.5:10	4	6.5	58.2 (anaerobic sludge, SRM and CB inocule) 43.9 (only anaerobic sludge)	SO_4^{2-}: 3,600 Cd^{2+}: 10 Fe^{2+}: 750 Mn^{2+}: 20 Ni^{2+}: 15 Zn^{2+}: 100 Cu^{2+}: 4.76 pH: 2.5	This study
Cow manure (17%) Leaf compost (31%) Forest fibers (49%) $CaCO_3$ (3%) Solid:liquid ratio (in volume) was 0.5:10	6	7.5	13.10 (anaerobic sludge, SRM and CB inocule)	SO_4^{2-}: 2,710 Cd^{2+}: 0.13 Fe^{2+}: 105.6 Mn^{2+}: 22 Ni^{2+}: 0.34 Zn^{2+}: 10.34 Cu^{2+}: 273 pH: 4.94	This study

generation time of the sulfate-reducing microorganisms (Sánchez-Andrea *et al.* 2014), studies need to be done to allow the use of specific inocula for each type of drainage. To improve the efficiency of the sulfate-reducing reactors, optimised inocula need to be developed based on the pH and the metal contents.

Treatment of drainage containing copper

The ORP measurements in the different proportions of mixture (X/2 to X/10) and the concentrations of sulfate and copper of mixture X/2 are shown in Figure 5. Only mixture X/2 presented biological activity (diminution of the redox

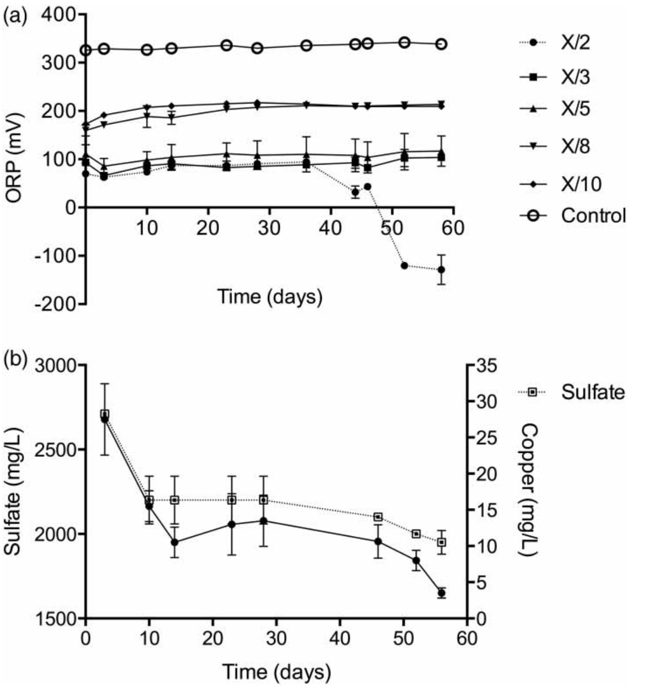

Figure 5 | (a) ORP vs. time of the mixtures with different proportions of reactive mixture during incubation with AMD from a copper mine; (b) changes in the concentrations of sulfate and copper of mixture X/2.

potential to -128.7 ± 30 mV). The biological activity was limited by the concentration of dissolved copper. This metal proved to be much more toxic than iron and the combination of iron, zinc, cadmium and nickel (experiments with drainages a and b). At the start of the experiment, the dissolved copper reacted with the components of the reactive mixture, diminishing its concentration in treatment X/2 from 273 mg/L to 27.5 ± 4.9 mg/L. Copper continued to be removed by biological activity until day 58, when it was measured as 3.5 ± 0.5 mg/L. The dissolved copper concentrations in mixtures X/3, X/5, X/8 and X/10 exceeded the inhibition concentrations for sulfate reduction of 30 mg/L (Kaksonen & Puhakka 2007). The copper concentration affected the sulfate reduction rate of the consortium present in X/2, as low sulfate consumption was obtained (13.10 mg/L-d). Concentrations of other toxic metals in mine water c, including Ni, Cd, and Zn, are below known inhibitory levels to SRB (Kaksonen & Puhakka 2007). For this experiment, the high copper concentration had negative effects on the growth of the sulfate-reducing microorganisms and their metabolic capability. For this reason, in the case of very toxic drainages, when evaluating the composition of the reactive mixtures for biochemical reactors it is first necessary to determine the minimum quantity of substrate to avoid inhibiting sulfate reduction. The variations in the sulfate reduction rates will be explained by the different compositions of the mixtures, not by the inhibition produced by the drainage. The implication for the design of biochemical reactors is that the biological treatment processes can only begin once the toxicity of the acid drainage has been diminished sufficiently by reaction with the substrate. A stratified bed favouring first chemical and then biological reactions could therefore be more efficient for treating AMD with high copper concentrations.

The Chilean economy is based on copper mining. The acid drainage from copper mining is characterised by its high metal content, above the levels that cause inhibition of microorganisms. It is therefore necessary to seek alternatives that will allow these AMDs to be treated with biological systems. In the first place, new reactor designs could be used, such as diffusive exchange systems, which provide the bacterial consortia with zones of protection from the toxicity (Schwarz & Rittmann 2010). Chemical pre-treatment could also be applied to diminish the concentrations of toxic metals and thus ensure the viability of the microorganisms throughout the useful life of the biological reactor. Metal-resistant bacteria can also be incorporated into the treatment systems; sulfate-reducers have been isolated that present activity with 67 and 100 mg/l of copper (Kaksonen & Puhakka 2007). To avoid operating problems in the reactors in the long term, it is necessary to vary the quality and flow of AMD on a seasonal basis. Increasing the proportion of alkalinity-generating substances (limestone, chitin, etc) to between 10% and 30% of the mixture (ITRC 2013; Fitch 2015) will diminish the initial concentration of dissolved metals, resulting in better sulfate reduction rates.

CONCLUSIONS

The laboratory results indicate that the addition of cellulose fibers of forest origin to the reactive mixtures of biochemical reactors could potentially increase the yield of these reactors in the treatment of acid drainage. For forest cellulose fiber we obtained a volumetric sulfate reduction rate of 1.2 mol $SO_4^{2-}/m^3/d$, four times higher than the 0.3 mol $SO_4^{2-}/m^3/d$ recommended for the design of this type of reactor. Furthermore, the mixtures with forest fibers and microcrystalline cellulose presented higher sulfate reduction rates (rates increased by 32% and 61%, respectively) in the presence of the inocula enriched with SRB and cellulolytic populations. Thus the addition of enriched inocula to the reactive mixtures is another possible strategy for increasing the capacity of biochemical reactors, in cases in which the high metal content requires their rapid, efficient precipitation to prevent toxic effects on the bacterial consortia.

Low sulfate reduction rates were obtained during treatment of AMD containing copper (13.10 mg/L-d). A high metal concentration, particularly of dissolved copper, affected biological activity, since at concentrations higher than 42 mg Cu^{2+}/L no sulfate-reducing activity was detected. This shows that treating toxic drainages with biological processes requires strategies to diminish their toxicity, such as including a higher proportion of sources of alkalinity in the mixture, developing consortia of resistant microorganisms, or using reactor designs that provide protection, such as stratified beds or diffusive exchange systems.

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