Biosorption and bioprecipitation of heavy metals in biological permeable reactive barriers

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A combined chemical/biological treatment in a two-column apparatus was tested for attenuating AMD pollution. Pre-treatment increased pH and allowed the total removal of Fe, Cu and As. Iron and copper were completely removed in the end side of the pre-treatment column where pH conditions were adequate for precipitation of these metals, scarcely soluble as hydroxides and carbonates. Arsenic was removed in the first column by sorption and coprecipitation with iron. Manganese and zinc (partially removed in the first column by sorption onto limestone and iron precipitates) were completely removed in the second column by bioprecipitation (end-side) and biosorption mechanisms. *Lepidium sativum* and *Daphnia magna* were used to evaluate the abatement of toxicity after each treatment. Chemical treatment (raising pH to 5-6 and eliminating copper) generated effluents with reduced toxicity for *L. sativum*, while 100% immobilization was still observed for *D. magna*. Final effluents of biological treatment (raising pH to 8 and eliminating all metals) resulted toxic for both organisms. Dynamic trends of toxicity for the first output denoted a gradual improvement leading to ormesys for *Lepidium* (for gradual release of organics), while a constant residual toxicity remained for *Daphnia* (sulphidric acid release due to sulphate reducing bacteria).

1. Introduction

Acid mine drainage (AMD) is the worst environmental problem associated with mining activities. Polluted waters are generated by the biologically enhanced oxidation of metal sulphides, which results in ore dissolution and release of sulphates, protons and toxic metals in soil solution (Sheoran and Sheoran, 2006). Remediation options involve both abiotic and biological strategies. Active abiotic systems add chemicals (such as limestone) to neutralise AMD pH and to determine iron precipitation:

$$2Fe(HCO_3)_2 + 1/2O_2 + H_2O \rightarrow 2Fe(OH)_3 \downarrow + 4CO_2 \quad (1)$$

$$2Fe_2(SO_4)_3 + 6CaCO_3 + 3H_2SO_4 \rightarrow 2Fe(OH)_3 \downarrow + 6CaSO_4 \downarrow + 6CO_2 \quad (2)$$

Biological remediation can be performed in passive systems (such as permeable reactive barriers, PRB) exploiting sulphur production by sulphate reducing bacteria, SRB (Johnson, 2006). Organic mixtures are generally used in PRB construction as electron donor in the dissimilatory reduction of sulphate to sulphide, which generates alkalinity and promotes metal precipitation:

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2H_2CO_3 \qquad (3)$$

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

Organic components used in PBR are a mix of biological materials chosen on the base of the local availability (mushroom compost, manure of cow, horse and sheep, municipal compost; sawdust, peat, straw, leaf compost) (Waybrant et al., 1998; Gibert et al. 2004). Full scale applications of organic-carbon based sulphate reducing PRB are also characterised by the addition of gravel to improve barrier permeability and limestone to increase pH and favour SRB growth.

Treatment efficiency is generally evaluated in terms of removal of polluting heavy metals. Nevertheless, interesting data can be obtained also from toxicological tests in order to evaluate global impact abatement. Toxic effects of AMD can be mainly related to both low pH and high concentration of heavy metals, such as copper, cadmium, lead, zinc, manganese, and arsenic. AMD toxicity have been addressed by using different test-organism systems such as microrganisms, daphnids, shrimps, fishes and plants (Arambasic et al., 1995; Montvydiene and Marciulioniene, 2004; Yim et al., 2006).

In this work the experimental results of a combined chemical-biological treatment of synthetic samples of AMD in column reactors were reported with particular attention to the effects of each step of treatment on both chemical and toxicological properties of the effluent.

2. Materials and Methods

Synthetic AMD samples were obtained by dissolving reagent grade chemicals ($FeSO_4$, $MnSO_4$, $CuSO_4$, $ZnSO_4$, As_2O_5) in distilled water (feed column in Table 1).

Table 1. Average values of chemical composition and toxicological parameters (IC_{50}: sample dilution giving 50% germination for *L. sativum*; LC_{50}: sample dilution giving 50% immobilization of *D. magna*) for synthetic AMD in the feed, after chemical pre-treatment and after biological treatment (n.d.: not detectable, <0.01 ppm).

Monitored parameter	Feed	After chemical pre-treatment	After biological treatment
pH	3.0 ± 0.1	5.6 ± 0.2	8.1 ± 0.3
Fe (mg l^{-1})	400 ± 50	2 ± 1	n.d.
Cu (mg l^{-1})	50 ± 10	0.2 ± 0.1	n.d.
Zn (mg l^{-1})	50 ± 10	47 ± 5	n.d.
Mn (mg l^{-1})	50 ± 10	42 ± 5	n.d.
As (mg l^{-1})	2.0 ± 0.5	n.d.	n.d.
IC_{50} *L. sativum*	6.0	24.2	46.4
LC_{50} (24h) *D. magna*	2.4	43.5	12.2

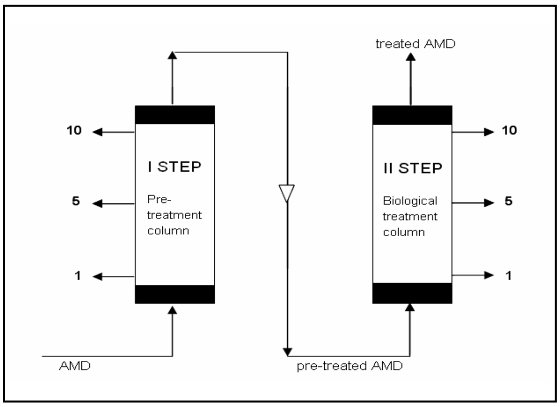

Figure 1. Schematic representation of the two-column apparatus used for the combined treatment of AMD.

AMD was treated in a two-column apparatus by a chemical treatment (first step) and then by a biological treatment (second step) (Figure 1).

Each treatment step was performed in a distinct Plexiglas column (height 1 m; diameter 0.2 m; column volume, v_c=6.65*10^{-3} m^3) with 10 equally distant outputs (0.1 m) along the axial length, numbered from the bottom to the top of the column.

The first column (first step of chemical treatment) was filled with natural limestone. The second column (second step of biological treatment) was filled with a natural solid mixture (80% v/v of compost, 15% v/v of cow manure, 5% v/v of straw and traces of limestone) and inoculated by sulphate reducing bacteria (SRB).

Synthetic AMD was fed through the first column from the bottom to the top with a residence time of 24 h. The effluents of the first column were then fed to the second column with the same residence time. Samples from the different outputs of the first and second columns were collected during time and used for analytical determination (pH and residual metals) and for toxicological tests.

Toxicity of synthetic AMD samples, before and after treatments, was evaluated by monitoring germination and growth of *Lepidium sativum* and immobilization of *Daphnia magna* (Arambasic et al., 1995).

Toxicity indices for *L. sativum* were evaluated considering two end-points: the number of germinated seeds and their root length.

The germination index (G) comprehends simultaneously the toxic effect exerted on both germination and root length:

$$G = \frac{G_S L_S}{G_C L_C} \cdot 100 \qquad (6)$$

where G_S and G_C are the number of the germinated seeds in the sample and the control, and L_C and L_S are the average root length in the control and in the sample.

3. Results and discussion

The efficiency of AMD treatment was monitored during time for pH, ferrous iron and toxic metal concentrations in the different outputs of the columns. The abatements of the different species (ferrous iron and toxic metals) were reported as dimensionless concentrations by dividing the concentration measured in the output stream for the inlet concentration of the synthetic AMD fed. By this way the effect due to the variability of AMD samples prepared during time was eliminated. Dimensionless concentrations were reported as a function of the volume of treated AMD divided by the column volume, v_c.

Chemical pre-treatment of AMD determined the complete removal of ferrous iron (>99%) with residual concentrations ranging from 1 to 10 ppm. Arsenic and copper were also completely removed by the chemical treatment, while zinc and manganese presented residual increasing concentration during the time of this experiment (Table 1).

The profiles of iron concentration and pH along the column (Figure 2) showed opposite trends: pH raised from the bottom to the top, while residual iron concentration decreased along the same direction (residual ferrous concentrations in the first outputs were larger than in the final ones). The profiles of zinc along the column outputs denoted the gradual saturation of the column (Figure 2). Similar profiles were found for manganese, but in this case larger residual concentration were observed even during the early stage of pre-treatment (Figure2).

The abatement of toxic metals in pre-treatment column can be related to different mechanisms and, in particular, precipitation of insoluble species and sorption onto both limestone and precipitated iron oxides. Specific mechanisms of removal for each metal were investigated by considering the profiles of pollutants along the column length, the simulation of metal speciation in the investigated conditions and the digestion of solid sample from different column sections (experimental data not reported here).

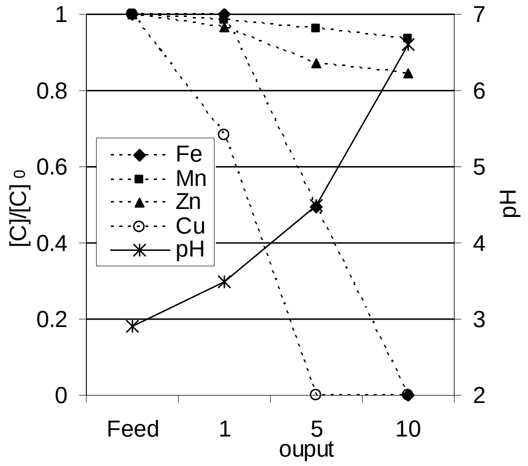

Figure 2 Column test of chemical precipitation: pH, ferrous iron, manganese, zinc, and copper profiles along the column outputs (V=15).

The combination of such results suggested the following main conclusions:

- iron was completely removed by precipitation in the pre-treatment column, whose active zone was only that with adequate pH conditions as evidenced by acid digestion of column sections;
- copper was completely precipitated as iron in the first treatment column
- manganese and zinc cannot be removed only for pH rise: dynamic trends of axial profiles showed a shifting front of saturation along the column and suggested that marginal removals in the first column were due only to sorption onto limestone and iron precipitates; both metals were completely removed in the second column of biological treatment by biosorption and bioprecipitation mechanisms;
- arsenic was removed in the first column both by sorption and copreciptation with iron being concentrated in the solid from the column section active for iron precipitation.

Manganese and zinc were completely removed and cannot be detected in the outputs of the SRB column (<0.01 ppm). Averaged values from acid digestions of column filling material denoted that both zinc and manganese were mainly concentrated in the solid material taken from the upper part of the column (exit section). This finding can be due to the concentration of the gaseous products of SRB metabolism in the upper part of the column and, consequently, the localisation in such compartment of the precipitation of metal as sulphides, while sorption phenomena would lead to an opposite distribution of metal in solid phase along the column axis. Conversely to limestone treatment, sulphate reducing bacteria metabolism and biosorption onto solid substrates seemed to be able to completely remove this metal from AMD.

Synthetic samples of AMD before treatment resulted toxic for both *L. sativum* (G lower than 10%) and *D. magna* (100% immobilization).

Chemical treatment generated effluents with reduced toxicity for *L. sativum* (G=33%); 100% immobilization was observed for *D. magna* even though dosage-effect diagrams denoted an increase of LC_{50} with respect to untreated samples (Table 1).

Effluents from the final output of the biotreatment column denoted high toxicity for both organism-test systems. Nevertheless, an evolution of toxic effects can be observed for the different outputs of the second column: first and fifth column effluents denoted a gradual improvement in *Lepidium* tests (Figure 3), while a constant residual toxicity was observed for *D. magna* (experimental data not reported here).

Chemical composition of AMD samples before and after each treatment allowed isolating the main contributes in AMD toxicity and the effect of each treatment in pollution and toxicity abatement.

In particular, according to previous studies reported in the literature, the synthetic AMD used in this study presented three main contributors to toxicity: pH, Cu and Zn. Chemical pre-treatment (raising pH to 5-6 and eliminating copper) generated effluents whose toxicity was mainly due to Zn for *Lepidium* and also to pH for *D. magna*. Biological treatment (raising pH to 8 and eliminating all metals) was characterised but initial elevated concentration of organics (toxic for *Lepidium*) and sulphide gas especially in the top section (toxic for *Daphnia*).

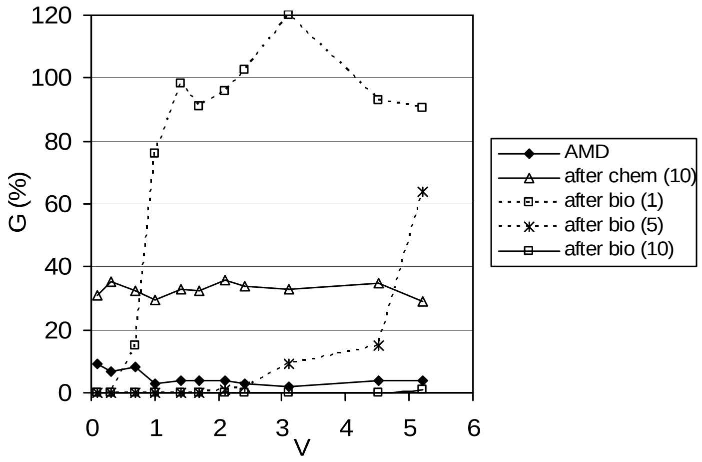

Figure 3 Germination index obtained by toxicity tests on *L. sativum* using synthetic AMD, AMD after chemical treatment (tenth output) and AMD after biological treatment (first, fifth and tenth outputs) for increasing values of dimensionless treated volumes (V).

4. Conclusions

A combined chemical-biological approach succeeded in attenuating AMD pollution:

- pre-treatment in the first column (natural limestone) increased the pH allowing the complete removal of metals with low solubility as hydroxides and carbonates (Fe and Cu);
- refining treatment in the second column (made up of natural organic matter and inoculated by SRB) allowed the removal of the metals which can not be removed by increasing pH (Mn and Zn).

Toxicity tests on column effluents (as simulation of full scale permeable reactive barriers) gave preliminary information about the potential impact of treated effluents in the environment using different organism-test systems. Experimental data here reported showed that, after an initial transient, treated effluents can be suitable for vegetable growth while residual toxicity was observed for daphnids.

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