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Design of a bioprocess for metal and sulfate removal from acid mine drainage

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ABSTRACT

The high levels of sulfate and the metals in acid mine drainages generate important environmental problems. This paper describes the synergistic combination of a biosorption process and a new sulfate removal process. The treatment for the elimination of metals by biosorption with a *Bacillus* strain allowed reducing the high metal concentrations that had a toxic effect on the sulfate-reducing bacteria (SRB). On the other hand, the sulfate removal process used a microbial sulfate-reducing halotolerant consortium, which was able to reduce the sulfate concentration using low-cost organic substrates such as spirulina, cellulose and industrial starch. Independent of substrate present in the culture medium, the SRB was the predominant group. The sulfate-reducing consortium was cultured on a bench-scale upflow anaerobic packed bed bioreactor filled with Celite R-635. It was possible to reduce the concentration of sulfate in the culture medium in batch or semi-continuous operation. This integrated process is an inexpensive alternative for the elimination of metals by biosortion and the elimination of sulfate using a sulfate-reducing consortium.

1. Introduction

Sulfate and metals are pollutants present in acid mine drainage (AMD). AMD is the acidic metal rich water formed by the reaction between the water and rock containing sulfur-bearing minerals. The resulting acidic solution acts as a leaching agent containing dissolved metals and sulfuric acid that in contact with water bodies can contaminate those, making them unfit for use (Costa et al., 2017). This is one of the most significant environmental challenges facing the mining industry and environmental management agencies across the globe (Skousen et al., 2017; Tait et al., 2009; Wei et al., 2016).

There are currently some alternatives to remove sulfate and metals from water. The most commonly used method for treating AMD is based on chemical neutralization (e.g., lime addition) resulting in the precipitation of metal hydroxides (de Godoi et al., 2017; Johnson and Hallberg, 2005; Kefeni et al., 2017). However, these alternatives pose important issues such as not reducing the concentration of sulfate to the levels required or being cost-ineffective. In recent years, several studies have evaluated different techniques such as solvent extraction, ultrafiltration, microfiltration, nanofiltration, reverse osmosis, organic and inorganic ion exchange, and adsorption (Oyewo et al., 2018). However, these treatments are too expensive to be applied at large scale. Considering the above issues the need for developing an economical and efficient alternative for the removal of sulfate is required.

The use of sulfate-reducing bacteria (SRB) is an option for the treatment of water with high levels of sulfate (Sousa et al., 2015), but is limited for two reasons: substrates of low molecular weight (i.e. lactate, pyruvate or ethanol) are very expensive and mining effluents contain high concentrations of metals that have a toxic effect on the SRB (Kaksonen and Puhakka, 2007; Utgikar et al., 2003). In this research, the use of microbial consortia capable of hydrolyzing, fermenting more economical complex substrates and producing low molecular weight metabolites as substrates for SRBs is proposed (Boshoff et al., 2004 and Hao et al., 2014).

In this study, a halotolerant consortium was enriched using complex substrates such as Spirulina, cellulose and industrial starch. The halotolerance of microorganisms is an important characteristic for the treatment of mining effluents that contain high levels of salts. For metal removal was, a biosorption process previously developed by our research group used (Cotoras et al., 2009). The combined process was used to treat an AMD water.

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Table 1
Sequences, position in the target rRNA and specificity of the probes used in the fluorescence "in situ" hybridization.

Probe	Sequence (5′–3′) of probe	Specificity	rRNA type	Reference
EUB338	GCTGCCTCCCGTAGGAGT	Most bacteria	16S	Amann et al., 1990
ALFlb	CGTTCGYTCTGAGCCAG	α-Proteobacteria	16S	Manz et al., 1992
BET42a	GCCTTCCCACTTCGTTT	β-Proteobacteria	23S	Manz et al., 1992
GAM42a	GCCTTCCCACATCGTTT	γ-Proteobacteria	23S	Manz et al., 1992
SRB385	CGGCGTCGCTGCGTCAGG	δ-Proteobacteria	16S	Amann et al., 1995
CF319a	TGGTCCGTGTCTCAGTAC	*Cytophaga-Flavobacterium*	16S	Manz et al., 1996
ARCH915	GTGCTCCCCCGCCAATTCCT	Archaea	16S	Amann et al., 1995

2. Materials and methods

2.1. Reagents

N,*N*-Dimethyl-1,4-phenylenediamine oxalate, 4′,6-Diamidino-2-phenylindole (DAPI) and microcrystalline cellulose were purchased from Sigma-Aldrich (USA). NH_4Cl, Na_2SO_4, $CaCl_2{\cdot}6H_2O$, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$, H_3PO_4, $ZnCl_2$, $CuCl_2{\cdot}2H_2O$, $(NH_4)_2HPO_4$, $Na_2HPO_4{\cdot}2H_2O$, acetamide, thioglycolic acid, ethanol, formaldehyde (37%), yeast extract, starch were purchased from Merck (Germany). K_2HPO_4, $BaCl_2{\cdot}2H_2O$, NaCl, Tris, SDS, EDTA, NaOH, formamide and H_2SO_4 were purchased from Winkler (Chile). Dry *Spirulina,* casein hydrolysate and mineral oil for microbiology were supplied by General Nutrition Centers (USA), Difco (USA) and Biomérieux (France), respectively. Industrial starch was kindly provided by Corn Products (Chile).

2.2. Acid mine drainage

A sample of AMD was collected from a copper mine located in the Andean Mountains of Central Chile. This AMD had a pH value of 2.95, a sulfate concentration of 3602 mg/L and following concentrations of heavy metals (in mg/L): copper, 1400; iron, 27.9; zinc, 20.4 and nickel 0.60.

2.3. Sulfate-reducing microbial consortium and culture medium

The microbial consortium used in this study was enriched from black anaerobic sediment of a saline lagoon (Atacama Salt Flat, Chile). All growth experiments were performed at 28 °C in modified Postgate C culture medium (Barton and Tomei, 1995) with NaCl 60 g/L and a complex electron donor, using mineral oil overlay (10 mL medium overlaid with 2 mL mineral oil in 20 mL test tubes). Microcrystalline cellulose, starch, *Spirulina* and industrial starch were tested as complex electron donors.

2.4. Bioreactor set-up and experimental design for sulfate reduction

A PTFE bioreactor with a useful volume of 412 cm^3 (dimensions: 49 cm high x 3.3 cm wide). It was packed with Celite R-635 (Celite Corp. Lompoc, CA, USA), a thermally and chemically stable synthesized diatomaceous earth pellets (dimensions 6.35 mm diam × 12.7 mm ht., pore diameter approx. 20 μm and BET surface area of 0.27 m^2/g). This support material has been used successfully in biofiltration processes and in packed bed sulfate-reducing bioreactor to recover dissolved sulfide products (McMahon and Daugulis, 2008; Mezgebe et al., 2017). The upflow anaerobic packed bed bioreactor was incubated at 28 °C (0.1 g/L of thioglycolic acid was used). Postgate C modified culture medium and 2 g/L industrial starch, as substrate, were used. The bioreactor was inoculated with a culture of the sulfate-reducing microbial consortium. Feeding and recirculation were performed using a Masterflex peristaltic pump with Tygon tubing (Cole-Parmer Inc., USA). The bioreactor filled with Celite R-635, was handled batch wise for 97 days. After this time, the bioreactor was fed daily in a semi-continuous manner.

2.5. Bacterial strain and culture medium for metal biosorption

The bacterial strain *Bacillus* sp. NRRL-B-30881 was used in this study (Cotoras and Viedma, 2011). All growth experiments were performed in the following culture medium (in grams per liter of distilled water): D-glucose, 10; Na_2HPO_4, 1.0; KH_2PO_4, 0.3; K_2SO_4, 0.1; NaCl, 0.1; $MgSO_4{\cdot}7H_2O$, 0.02; $CaCl_2$, 0.01; $FeSO_4{\cdot}7H_2O$, 0.001; yeast extract, 1.0 and casein hydrolysate, 1.0 (Cotoras and Viedma, 2011).

2.6. Metals biosorption of a pretreated acid mine drainage

An amount of lime necessary to reach a pH equal to 6.3 to pre-treat the AMD was used. *Bacillus* sp., NRRL-B-30881 was cultured at 28 °C for 16 h in a fermenter (Multigen F-1000, New Brunswick Scientific, USA) with a capacity of 2 L, with aeration (0.75 vvm) and stirring (200 rpm). The obtained aggregate biomass was left to decant and the supernatant was discarded. This biomass was used for the biosorption of the metals present in the AMD. 2 l of AMD were contacted with the biomass in the bioreactor for 1 h with stirring (75 rpm). After the biosorption stage, the biomass was separated by decantation and a solution with low metal concentration was obtained.

2.7. Fluorescence in situ hybridization (FISH)

The characterization of the microbial populations enriched in the different complex organic compounds was done using the in situ hybridization technique. The specific oligonucleotide sequences of CY3-labeled probes and the hybridization conditions are summarized in the Table 1.

To perform the microbial consortium in situ hybridization, 100 μL of culture was taken and placed in 900 μL PBS and centrifuged for 5 min at 4724 x g. Once the centrifuging was done the supernatant was discarded and the pellet was re-suspended in 900 μL PBS, then centrifuged for 3 min at 112 x g. A sample of 50 μL was placed on a glass slide and was fixed with heat. Once fixed, 20 μL 37% formaldehyde was added on each sample for 20 min. Then, 50 μL of the hybridization solution was added to each sample (see Table 2) containing 20 mg of a CY3-labeled probe on each of the samples. They were incubated at 46 °C for 90 min in an equilibrated chamber. Probes BET42a and GAM42a were used with competitor oligonucleotides as described previously (Manz et al., 1992). Then the glass slides were incubated in a prewarmed washing solution (see Table 3) at 48 °C for 30 min.

Table 2
Hybridization solution composition for the fluorescence "in situ" hybridization. The composition of this solution depends on the probe used. The hybridization solution 1 was used for probes ALF lb. and EUB 338, while the hybridization solution 2 was used for probes BET42a, GAM42a, CF319a and SRB385.

Compound	Hybridization solution 1	Hybridization solution 2
Formamide	20%	35%
NaCl	0.9 M	0.9 M
Tris/HCI pH 7.2	20 mM	20 mM
SDS	0.01%	0.01%