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Short Communication

Biological treatment of mining wastewaters by fixed-bed bioreactors at high organic loading

Svetlana Bratkova [a,*], Bogdana Koumanova [b], Venko Beschkov [c]

[a] Department of Engineering Geoecology, University of Mining and Geology, "St. Ivan Rilski", Prof. B. Kamenov Str.,1700 Sofia, Bulgaria
[b] Department of Chemical Engineering, University of Chemical Technology and Metallurgy, 8 Kliment Ohridsky Blvd., 1756 Sofia, Bulgaria
[c] Institute of Chemical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Blvd. 103, 1113 Sofia, Bulgaria

HIGHLIGHTS

- Water contaminated with Fe and Cu was remediated by microbial hydrogen sulfide.
- Sulfate-reducing bacteria were immobilized on saturated zeolite in fixed-bed bioreactor.
- Solution containing ethanol, lactate, citrate and glycerol was used as donor of electrons.
- Influences of SO_4^{2-} volume loading rates, temperature and pH to the rates of the process were studied.
- Efficient removal of COD, H_2S, N and P was achieved in a three-sectional bioreactor.

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ABSTRACT

Acid wastewaters contaminated with Fe – 1000 mg L^{-1} and Cu – 100 mg L^{-1} were remediated by microbial sulfate-reduction at high organic loading (theoretical TOC/SO_4^{2-} ratio 1.1) in a laboratory installation. The installation design includes a fixed-bed anaerobic bioreactor for sulfate-reduction, a chemical reactor, a settler and a three-sectional bioreactor for residual organic compounds and hydrogen sulfide removal. Sulfate-reducing bacteria are immobilized on saturated zeolite in the fixed-bed bioreactor. The source of carbon and energy for bacteria was concentrated solution, containing ethanol, glycerol, lactate and citrate. Heavy metals removal was achieved by produced H_2S at sulfate loading rate 88 mg L^{-1} h^{-1}. The effluent of the anaerobic bioreactor was characterized with high concentrations of acetate and ethanol. The design of the second bioreactor (presence of two aerobic and an anoxic zones) makes possible the occurrence of nitrification and denitrification as well as the efficiently removal of residual organic compounds and H_2S.

1. Introduction

Acid mine drainage is a major environmental hazard that affects the aquatic ecosystems around mines. Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal containing waters. In recent years, the use of sulfate-reducing bacteria (SRB) has been proposed as an alternative to hydroxide precipitation (Kaksonen and Puhakka, 2007). Sulfate-reducing bacteria oxidize simple organic compounds with sulfate under anaerobic conditions. The main products from the process are hydrogen sulfide and bicarbonate ions. The HCO_3^- ions increase the alkalinity of the water. The soluble sulfide reacts with the metals to form insoluble metal sulfides.

Numerous reactor designs for microbial sulfate reduction and a variety of used donors of electrons have been reported (Kaksonen et al., 2004; Bayrakdar et al., 2009; Nevatalo et al., 2010). The preferred configurations for anaerobic wastewater treatment are mainly the upflow anaerobic sludge bed reactor, the fluidized bed reactor and the anaerobic filter. Biological sulfate reducing reactors used for metal precipitation can have either one or more stages, i.e., the sulfate reduction and metal precipitation can occur simultaneously, or in separate process units (Hao et al., 2000). The separation of microbial sulfate reduction and metal precipitation alleviates toxicity on SRB. Various parameters such as temperature, pH, sulfide and metal concentrations in the acid mine drainage affect the growth and activity of sulfate-reducing bacteria. Other important parameter is the total organic carbon SO_4^{2-} ratio. Velasco et al. (2008) reported that the feed chemical oxygen demand (COD)/SO_4^{2-} ratio can be an useful parameter to control the hydrogen sulfide production in the metal precipitation process. Kaksonen et al. (2004) showed that the stoichiometric COD/SO_4^{2-} ratio of 0.67 was adequate to attain around 60% of sulfate reduction with an initial sulfate concentration of 2000 mg L^{-1}.

* Corresponding author. Tel.: +359 2 8060 498.
E-mail address: s_bratkova@yahoo.com (S. Bratkova).

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This work reports the experimental results from the treatment of synthetic acid mine water, containing Fe and Cu in a laboratory-scale installation. Microbial sulfate-reduction was realized in a fixed-bed bioreactor with saturated zeolite. The effect of SO_4^{2-} volume loading rates, temperature and pH on rate of sulfate-reduction at high organic loading were determined. Further, the removal of residual concentrations of organic compounds, sulfide, N and P were demonstrated by performance of the second bioreactor.

2. Methods

2.1. Reactors

2.1.1. Fixed-bed reactor for microbial sulfate-reduction

The geometric volume of the anaerobic fixed-bed reactor is 1.2 dm^3. The carrier was produced by saturation of natural occurred zeolite, clinoptilolite of 2.5–5.0 mm size fraction, with the following composition, %: SiO_2 – 67.96, Al_2O_3 – 11.23, Fe_2O_3 – 0.83, K_2O – 2.85, Na_2O – 0.74, CaO – 3.01, MgO – 0.06, TiO_2 – 0.90. The whole 6 L solution containing NH_4Cl – 10 g L^{-1}, K_2HPO_4 – 5 g L^{-1}, $MgSO_4{\cdot}7H_2O$ – 4 g L^{-1} was used for saturation of 1.20 kg zeolite. The reactor is filled with 1.10 kg zeolite and 0.70 dm^3 modified Postgate medium, containing lactate and theoretical TOC/SO_4^{2-} ratio of 1.1. It is inoculated with 70 ml enriched microbial culture of SRB belonging to genera *Desulfotomacilum*, *Desulfomicrobium*, *Desulfovibrio* and *Desulfobacterium*. The adherence of biofilm of SRB onto the saturated zeolite is carried out for a period of 3 months through repeated periodic replacement of 50% of the liquid phase of the bioreactor with fresh medium.

2.1.2. Three-sectional bioreactor

The construction of the three-sectional bioreactor is inserted into each other of the three cylinders with increasing diameters. The total volume of bioreactor is 2.8 dm^3. The outermost and innermost zones of the reactor are aerated. The gravel with a fraction size of 5–9 mm was used as biomass carrier in the aerobic zones of the bioreactor. The water is fed at the bottom of innermost cylinder. Water overflow enter in the middle digester anaerobic zone. In the perforated bottom of the second cylinder, water enters the outermost zone of the aerobic bioreactor in which the motion is upstream. The bioreactor was inoculated with 80 ml activated sludge taken from a wastewater treatment plant.

2.2. Influences of SO_4^{2-} volume loading rates, temperature and pH on the rates of the microbial sulfate-reduction in anaerobic fixed-bed reactor

The continuous cultivation was started up after the formation of active biofilm of SRB. The new medium is mixture of two solutions. First of them is a highly concentrated solution, containing organic compounds (TOC – 200 g L^{-1}). It contained per liter of distilled water: ethanol, 96% – 333 ml, lactic acid, 80% – 36 ml, sodium citrate – 20 g, glycerol – 23.4 g, yeast extract – 5.0 g, NH_4Cl – 10.0 g and K_2HPO_4 – 5.0 g, pH 7.2–7.5. The second solution contains Na_2SO_4 – 2 g L^{-1}, $Mg_2SO_4{\cdot}7H_2O$ – 4 g L^{-1} and conc. H_2SO_4 – 0.1 ml (pH 2.8–3.0). Adjustable flow peristaltic pumps support the maintenance of the theoretical TOC/SO_4^{2-} ratio of 1.1 and concentration of sulfates 3.0 g L^{-1}. The influence of volume loading rate with sulfates on the rate of the process is examined through seven different HRT: 13.8, 17.3, 23.0, 34.5, 46.1, 69.3 and 86.4 h for a period of 3 months. The effluents from anaerobic bioreactor were sampled 5–7 times after reaching a dynamic equilibrium for every mode. The influence of temperature and pH on the rate of the process were evaluated at SO_4^{2-} volume loading rate 0.176 g L^{-1} h^{-1} (HRT 17.3 h). The effect of temperature (21, 27 and 37 °C) was determined for a period of 10 days per each temperature as the samples were collected every 2 days. The concentration of sulfates, organic acids and alcohols and pH were analyzed. The effect of pH in fixed-bed bioreactor on the rate of the process was determined in the range 5.45–7.25 for period of 2 months. For this purpose, the pH of the solution containing sulfates was additionally acidified by adding HCl from pH 3.5–1.7. In this procedure, the following intervals with decreasing values of pH are studied: 7.2–7.3, 6.7–6.9, 6.2–6.4, 5.8–5.9, 5.6–5.7, 5.5–5.6, 5.4–5.5. 3–4 Samples for every mode were collected when bioreactor achieved a steady state.

2.3. Treatment of waters polluted with heavy metals in the laboratory installation

2.3.1. Scheme of laboratory installation

The fixed-bed bioreactor (1) was fed with concentrated nutrient medium through a peristaltic pump (5) with adjustable flow rate (Fig. 1). Homogenization with upward flow in the reactor is performed by a recirculating pump (7). The microbial produced H_2S contacts with the solution of heavy metals in a chemical reactor (2). The solution of heavy metals was fed into the chemical reactor through a peristaltic pump (6). The geometric volume of the chemical reactor is 0.5 dm^3.The insoluble sulfides precipitated in a vertical-flow settler (3) with a volume of 0.85 dm^3. The greatest part of the effluents of settler was pumped into anaerobic fixed-bed bioreactor by a recirculating pump (8). The other part of effluents was entered into three-sectional bioreactor (4).

2.3.2. Treatment of waters polluted with heavy metals

The acid solution of heavy metals contains heavy metals Cu and Fe, added as $FeSO_4{\cdot}7H_2O$ – 4.98 g L^{-1} and $CuSO_4{\cdot}5H_2O$ – 0.393 g L^{-1}. The concentration of sulfates was adjusted to 3.0 g L^{-1} by addition of Na_2SO_4 and $MgSO_4{\cdot}7H_2O$. This solution was treated in fixed bed anaerobic bioreactor at two different SO_4^{2-} volume loading rates – 0.176 and 0.088 g L^{-1} h^{-1} at 21 °C and TOC/SO_4^{2-} ratio 1.1 for a period of 2 months. The efficiency of removal of heavy metals was found by sampling two times per week. Each sample was analyzed for pH, Eh, sulfate, sulfide, COD and soluble Fe and Cu. The effluents from anaerobic bioreactor at mode 0.088 g L^{-1} h^{-1} were treated in the second bioreactor. The concentrations of ammonium, nitrate and phosphate were also analyzed. The change in exchangeable cations and the amount of organic carbon of natural zeolite after saturation and after formation of biofilm of sulfate-reducing bacteria were also determined.

2.4. Analytical methods

The sulfate concentration was determined using spectrophotometric method by BaCl2. The total sulfide concentration was measured immediately after sampling using Nanocolor test 1-88/05.09. The phosphate concentration was determined by the molybdenum-blue ascorbic acid method. The ammonium concentration was measured by the Nessler method. The nitrate concentration was measured by sodium salicylate method. Organic substrate utilization was estimated by measuring the chemical oxygen demand (COD). The dissolved metal concentrations were determined by ICP spectrophotometry. Organic acids and alcohols were analyzed by high-performance liquid-chromatography. An Aminex HPX-87H column from Bio-Rad coupled to a RI detector (LC-25RI) was used for HPLC; sulfuric acid (0.01 N) was used as an eluent. Exchangeable cations (Na, K, Ca and Mg) were extracted from zeolite using an extracting solution (ammonium acetate) at pH 7.0. Na and K were determined by Flame photometer and Ca and Mg by EDTA complexometric titration. Standard statistical parameters including mean and standard deviation (for more than duplicate data points) were used. The experimental data were also analyzed by one-way ANOVA (95% confidence interval) using STATGRAPHICS Centurion XV software.