Journal of Environmental Chemical Engineering 2 (2014) 70–75

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Bioremoval of arsenite and sulfate by a mixed culture with sulfate-reducing capacity growing on powdered chicken feathers

Patrícia F. Costa [a], Letícia P. Matos [a], Versiane A. Leão [b], Mônica C. Teixeira [a,*]

[a] Environmental Biotechnology Laboratory, Universidade Federal de Ouro Preto, Rua Costa Sena, 171. Centro, Ouro Preto, MG 35400-000, Brazil
[b] Bio&Hidrometallurgy Laboratory, Universidade Federal de Ouro Preto, Campus Morro do Cruzeiro, Ouro Preto, MG 35400-000, Brazil

ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 5 November 2013
Accepted 26 November 2013

Keywords:
SRB
Protein-rich waste material
Arsenic sulfide
Adsorption

ABSTRACT

A relatively unusual and low-cost waste material was investigated for As(III) and SO_4^{2-} removal by a mixed culture containing sulfate-reducing bacteria (SRB). Powdered chicken feathers (PCF) were tested as an organic nutrient source for SRB growth and also as solid support for As(III) immobilization. PCF's efficiency as a growth substrate was compared with that of sodium lactate, used as a positive control. As(III) removal increased, from 38% (in the presence of sodium lactate only) to 80%, in the presence of PCF and sodium lactate together. The soluble organic part of PCF contained 2302 mg L^{-1} of carbon, suggesting the possibility of using PCF as an electron donor for SRB growth. When PCF was the only carbon source, the achieved sulfate removal was lower (13.4%) than that observed when PCF and lactate were added to the medium (27.0%), but higher than those obtained when only lactate was employed at COD/sulfate ratios of 0.67 or 1. Arsenic removal increased from 38% (lactate, COD/sulfate = 0.67) to 80% in the presence of PCF and lactate. The results suggest an alternative biological route for arsenite removal which does not require the use of a strong oxidizing agent to promote As(III) oxidation to As(V) before its removal.

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Introduction

Conventionally, sulfate and metals are removed from acid mining drainage (AMD) by precipitation with lime or calcium carbonate, a process that produces very large amounts of sludge, which need to be dewatered and disposed of. Alternatively, biological sulfate reduction by sulfate-reducing bacteria (SRB) may be considered as one of the most promising alternatives for treating acid mine drainage (AMD) and other sulfate-rich, metal-containing effluents. By this process, sulfate is biologically reduced to sulfide, reacting with soluble metals and metalloids and so precipitating as sulfides [1,2].

Arsenic is considered very toxic to all living organisms. Arsenic-containing compounds, whether organic or inorganic, are often converted to arsenic trioxide, which reacts very quickly with sulfhydryl groups (–SH), causing enzymatic inhibition and blocking cellular respiration [3,4]. When present in drinking water or wastewater, arsenic is removed mainly by its soluble species being converted into insoluble products [5]. This removal can be biologically obtained through the use of SRB or even by physico-chemical methods, such as adsorption onto iron or aluminium oxi-hydroxides, reverse osmosis, and ion exchange [6]. Another alternative is biosorption that uses low-cost waste materials as adsorbents. Such waste materials include powdered chicken feathers [4], leather industry wastes [7], orange juice residues [8], sugar cane bagasse [9], and rice husks [2]. The search for new adsorbent materials may acquire even greater relevance. Materials formerly considered wastes may become economically interesting for their biotechnological applications. In addition, the combination of physicochemical and biological processes may result in greater removal efficiencies as compared with each process on its own.

This study has aimed to investigate the suitability of a low-price poultry waste material, powdered chicken feathers (PCF), as a solid supporting material and organic substrate for the growth of a mixed sulfate-reducing bacteria culture intended to obtain simultaneous sulfate and arsenic removal. PCF was chosen for its confirmed arsenic adsorption capacity [4] and for its carbon content. Arsenic ions can be adsorbed by PCF, but not by the other materials frequently used as supporting material or as solid carbon sources for SRB growth. Furthermore, PCF is efficient in removing arsenic-reduced species, dismissing the oxidative stages usually required by the other arsenic adsorbents, whether organic or inorganic [4].

Materials

Microbial culture

A mixed SRB culture was obtained and cultured according to Barbosa et al. [11]. Enrichments were achieved by culturing with a

* Corresponding author. Tel.: +55 31 35591628; fax: +55 31 3559 1626.
E-mail addresses: monicacristinateixeira@uol.com.br, teixeira@ef.ufop.br (M.C. Teixeira).

2213-3437/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jece.2013.11.022

Postgate C liquid medium modified by Cheung and Gu [12]. 50 mL glass bottles, containing 5 mL of pond sediment collected at an urban pond, were used for culture enrichment. Initial pH was adjusted to 7.0 with a 0.2 M NaOH solution. Bottles were sealed and incubated at 35 ± 1 °C until bacterial growth was evinced by the presence of biologically produced ferrous sulfide.

Solid waste material

PCF was kindly provided by a poultry plant located in Minas Gerais, Brazil. According to Scapin et al. [13], it is a mixture of crushed chicken feathers, viscera, and boiled blood. Such material consists basically of insoluble proteins, mainly keratin. Its protein content is around 80%; methionine and cysteine contents are 3.68% and 0.67%, respectively [13]. PCF was sieved, and the portion with particles smaller than 0.71 mm (24 mesh Tyler) was selected for subsequent experiments. PCF total surface area and micropore volume were determined by BET technique [14] (Quantachrome Nova 1000). Easily water-soluble PCF components were submitted to chemical analysis after filtration of a 2% (w/v) aqueous suspension. Before filtration, this suspension was sterilized (20 °C, 20 min), to make its components more soluble, and filtered through a 0.45 μm cellulose membrane (Sartorius). The soluble portion's COD (chemical oxygen demand), BOD (biochemical oxygen demand) [15], TOC (total organic carbon) content (Hiper TOC analyzer equipment, Thermo Scientific) were determined. Glucose and protein contents were also determined by colorimetric methods (Laborlab enzymatic kits), and soluble sulfate was determined by the turbidimetric method [15,16]. Soluble metals were quantified by atomic spectroscopy (Emission Spectrophotometer with Plasma source, Spectro, Ciros CCD with Radial Vision). Before this analysis, 10 mL samples were centrifuged (Thermo Multifuge X1R, rotor Fiberlite F155-8 × 50cy, 10,000 rpm, 15 min), filtered (0.45 μm cellulose membrane, Sartorius), acidified with concentrated nitric acid (100 μL), and stored at 4 °C. PCF was mixed with culture medium (2% w/v) and bacterial *inocula* (5% w/v) in order to make biologically soluble some of the organic material, mainly organic acids. After 240 h of incubation, the soluble portion was filtered through a 0.45 μm filter (Sartorius), and the concentration of the remaining organic acids was determined by ion chromatography (Metrohn, column for short-chain organic acids, eluted with H_2SO_4 0.001 mol L^{-1}).

Culture in the presence of arsenite

In the experiments testing SRB's tolerance to arsenic, a $NaAsO_2$ (Fluka) stock solution containing 1000 mg L^{-1} of As(III) was prepared. The solution was sterilized by autoclaving (120 °C, 20 min) and stored at 4 °C. During bacterial adaptation, varied As(III) stock solution volumes were added to the culture medium to obtain As(III) concentrations ranging from 0.5 to 4.0 mg L^{-1}. Each adaptation step was repeated three times. The As(III)-adapted culture was used for the next experiments testing removal of SO_4^{2-} and arsenic. The organic substrates used were powdered chicken feathers (PCF), powdered chicken feathers and lactate (PCFL), and lactate alone (L).

Sulfate reduction and arsenite removal

Sulfate and As(III) removal experiments were carried out (i) under different PCF concentrations (1%, 2%, 3% and 4% w/v), (ii) in the presence or absence of lactate (1.2 g L^{-1}) as a supplementary source of organic substrate. 600 mL glass bottles filled with 500 mL of culture medium that was amended with 2.1 mL of As(III) stock solution were inoculated with the SRB culture (5% v/v); the final As(III) concentration was 4.2 mg L^{-1}. Bottles were sealed and incubated at 35 °C for 240 h. For a positive control, the experiment was repeated with only lactate as electron donor.

Different COD/sulfate ratios (0.67, 1.0, 2.0, and 3.0) were also tested. To obtain such COD/sulfate ratios, lactate concentrations added to the growth medium were 1.2, 1.8, 3.7 and 5.6 g L^{-1}, while sulfate concentration was kept constant at 2.0 g L^{-1}. Abiotic controls containing lactate (1.8 g L^{-1}), lactate (1.8 g L^{-1}) plus PCF (2%, w/v), or PCF only (2%, w/v) were also prepared. Since direct microscopic cell-counting was rendered impossible by the brownish color of the growth medium caused by the presence of PCF, microbial metabolism was assessed indirectly by monitoring of the culture medium's pH and Eh values (digital meter Digimed), as well as its residual arsenic and sulfate concentrations. All experiments were duplicated and results averaged. All reagents and salts were of analytical grade.

Sulfate consumption was expressed as sulfate removal efficiency (SRE) by mass balance.

Results and discussion

PCF characterization

Results obtained during partial PCF characterization are summarized in Table 1. COD and BOD analysis may indicate PCF biodegradability, since the obtained results are in accordance with theoretical available data [17]. The soluble organic portion of PCF contained 2302 mg L^{-1} of carbon, suggesting that PCF may be used as an electron donor for SRB growth.

Soluble-protein and glucose values were only 23 mg L^{-1} and 6 mg L^{-1}, respectively, insufficient for sustaining SRB growth. Only 84 mg L^{-1} of sulfate were released from the material. Although it could be used as an electron acceptor in the SRB metabolism, its content was considered insufficient to guarantee SRB growth, since culture media generally use much higher sulfate concentrations [18], usually 2–4 mg L^{-1}. Therefore, sulfate amendments are mandatory if PCF is to be used to obtain high SRB growth yields.

Lactic and acetic acids, also at very low concentrations (4.0 and 12.5 mg L^{-1}, respectively), were found after PCF had been incubated biologically for 240 h. The absence of other organic acids, such as butyric, propionic, isovaleric, and isobutyric acids, indicates that (i) there was not a significant solubilization of volatile fatty acids from PCF or (ii) all soluble compounds were

Table 1
Physicochemical characterization of powdered chicken feathers (PCF).

Insoluble fraction		Soluble fraction					
		Chemical elements (mg L^{-1})					
Granulometry	<24 mesh	Sulfate	84	Copper	0.013	Iron	0.47
Density	1.242 g cm^{-3}	BOD	6661	Phosphorus	71.1	Sulfur	340
Surface area	0.787 m^2 g^{-1}	COD	7607	Potassium	163	Silica	3
Micropores volume	0.00037 cm^3 g^{-1}	COD/BOD	1.14	Magnesium	36.65	Zinc	0.94
Micropores area	1.038 m^2 g^{-1}	TOC	2302	Manganese	0.67	Calcium	15.6

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