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

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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.

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

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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

biologically consumed, producing lactic and acetic acid as byproducts.

The main chemical elements present in the soluble part of the PCF suspension are listed in Table 1. Even though the vast majority is present in low concentrations, these data may indicate that PCF could be used as a nutrient source for bacterial growth, since essential elements such as calcium, potassium, magnesium, phosphorus and sulfur are present. Relative to the PCF solid part, BET analysis results pointed to a surface area of 0.787 $m^2\ g^{-1}$ and a micropore volume of 0.00037 $cm^3\ g^{-1}$. Such parameters as surface area and micropore volume are important for materials that will be used as adsorbents, given that surface area is usually directly related to its sorption capacity and inversely proportional to the diameter of the material particles. Despite micropore volume and surface area values being lower than those observed for other adsorbent materials, powdered chicken feathers were successfully used as adsorbent for As(III) [4] in abiotic systems.

Sulfate reduction and arsenic removal

Although the initial culture pH was adjusted to 7.0, there was a pH decrease during the first 24 h in almost all the flasks regardless of experimental conditions (Fig. 1).

This effect was even more remarkable when PCF was present and acidification was directly proportional to PCF content. In addition, this phenomenon depends on bacterial activity, because decrease in pH is discrete in the abiotic flasks. This could be attributed to microbial degradation of proteins and sugars from the culture medium (agar and yeast extract), or, even more significantly, from PCF substrate (soluble proteins and glucose), producing fat acids as previously reported and discussed [2,19] and here experimentally observed ("PCF characterization"). Over time, however, pH values increase again to the initial values, most likely as bicarbonate ions are produced [20].

Fig. 1 also depicts Eh values at different growth conditions in the presence of As(III). The measured Eh values were favorable to

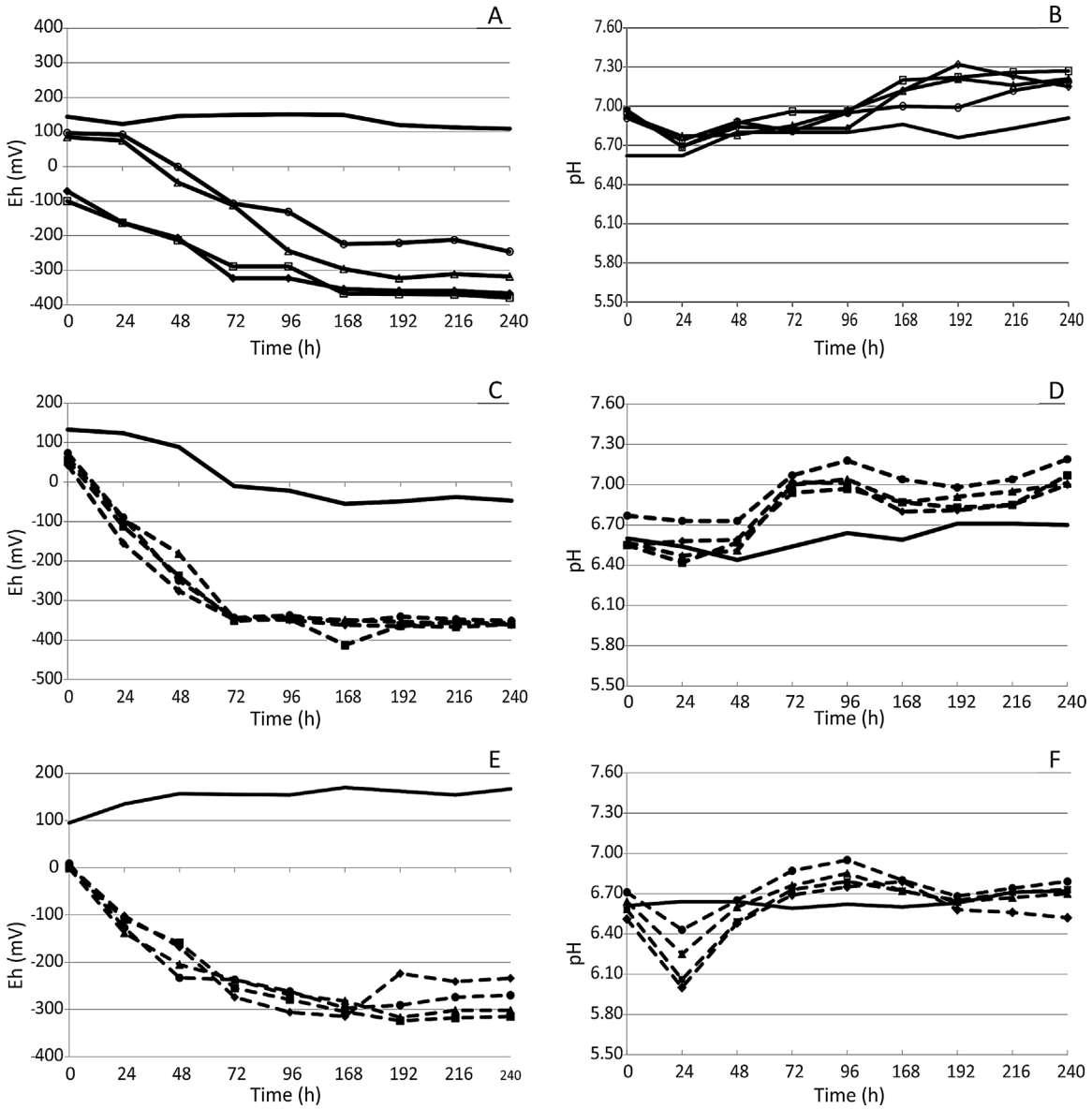

Fig. 1. Effect of COD/sulfate ratios and PCF on Eh and pH. A and B: lactate and As(III) at different COD/sulfate ratios: (○) 0.67; (Δ) 1; (□) 2 and (◇) 3. C and D: lactate, PCF and As(III), E and F: PCF and As(III) at different PCF concentrations: (●) 1%; (▲) 2%; (■) 3%; (◆) 4% and (solid line) abiotic control.

Table 2
Biological removal of sulfate and arsenic by SRB mixed culture using different carbon sources: L, sodium lactate; PCF, powdered chicken feathers; PCFL powdered chicken feathers and sodium lactate.

Experiment	Sulfate consumed ($mg\,L^{-1}$)	SRE (%)	As(III) removed ($mg\,L^{-1}$)	As(III) removed (%)
Carbon source – L				
Negative control	29	1.3	0.147	3.5
$DQO/SO_4^{2-} = 0.67$	44	1.9	1.69	38
$DQO/SO_4^{2-} = 1$	102	4.4	1.90	49.1
$DQO/SO_4^{2-} = 2$	1444	63.7	3.55	87.4
$DQO/SO_4^{2-} = 3$	1911	84.3	3.54	86.2
Carbon source – PCFL ($DQO/SO_4^{2-} = 0.67$)				
Negative control	72	3.2	0.182	5.5
[PCF] = 1%	327	15.9	3.80	91.7
[PCF] = 2%	647	29.4	3.53	87.3
[PCF] = 3%	612	27.2	3.45	80
[PCF] = 4%	559	27.0	3.11	70.3
Carbon source – PCF ($DQO/SO_4^{2-} = 0.67$)				
Negative control	12	0.3	0.184	4.2
[PCF] = 1%	208	8.9	3.21	76.3
[PCF] = 2%	141	6.8	3.15	72.9
[PCF] = 3%	298	10.9	3.34	73.6
[PCF] = 4%	397	13.4	3.55	85

SRB growth and decreased with time for all tested conditions, except for the abiotic controls. The absence of lactate during PCF experiments (Fig. 1, E) had a discrete negative effect on the redox potential. Final Eh values varied between −250 and −300 mV instead of the lower values (−350 to −400 mV) observed in the tests containing lactate (Fig. 1, C), but the values observed were still fairly low and consistent with SRB growth [5].

Table 2 shows that those cultures growing by lactate (L) showed sulfate removal efficiencies (SRE) directly proportional to the COD/sulfate ratio. The highest SRE (84.3%) was achieved using a COD/sulfate ratio equal to 3, in accordance with other authors' results [11,21].

During the tests that assessed the influence of PCF on sulfate removal, in the presence of lactate and PCF (PCFL tests), 1.2 $g\,L^{-1}$ of lactate were used by SRB, resulting in a COD sulfate ratio of 0.67 minimum (considering only sodium lactate); thus, a discrete sulfate consumption was expected if PCF was not present. When the results obtained at L (COD/sulfate ratio of 0.67) condition and PCFL were compared, however, it was observed that sulfate consumption was improved by PCF. SRE increased from 1.9% (L tests) to 15.9%, 24.9%, 27.2%, and 27% in the presence of 1%, 2%, 3%, and 4% (w/v) PCF, respectively, after 240 h (Table 2). In this way, PCF's capacity to provide organic compounds to support bacterial growth was confirmed.

When PCF was used without any supplementary carbon source (lactate), the achieved sulfate removal was lower (13.4%) than that observed during PCFL tests (27.0%), but higher than those obtained when only lactate (L) was employed at COD/sulfate ratios of 0.67 or 1. It was demonstrated that PCF could be used as an organic substrate for SRB growth, but its use can be more interesting if combined with that of other organic compounds, such as sodium lactate or ethanol. PCF combined with other electron donors may have economic benefits, since it may reduce some of the costs of the industrial process, such as that employed in AMD and treatment of sulfate-rich industrial wastewater.

As for As(III) removal, the arsenic concentration applied in this study, namely 4 $mg\,L^{-1}$, does not inhibit the mixed-culture activity or hinder the reduction of sulfate to sulfide. This mixed culture may therefore be considered arsenic-tolerant. During growing experiments using only lactate (L), the greater the sulfate removal was, the larger the sulfide concentration, and the higher the removal of arsenic, possibly by precipitation as arsenic sulfide. SRB oxidizes simple organic compounds by using sulfate as an electron acceptor and generating sulfide, S_2, and alkalinity. Biogenic S_2 can react with dissolved metals and metalloids such as arsenic to form metal sulfides, which are slightly soluble. The solubilities of most toxic metal sulfides are generally very low. For example, the log Ksp for As_2S_3, ZnS, and CuS are −11.9, −28.39, and −40.94, respectively [10]. While sulfate is biologically reduced to sulfide, the solubility of arsenic is diminished by its precipitation as arsenic sulfides and/or its biosorption onto the PCF surface.

In the presence of PCF, as shown in Table 2, arsenic removal increased from 38% during L tests (COD/sulfate = 0.67) to 80% during PCFL. During PCFL and PCF tests it was possible to observe that, in addition to not affecting sulfate reduction significantly, PCF contributed extensively to arsenic immobilization (Table 2).

Fig. 2 depicts As(III) removal as a function of sulfate consumption. Only results obtained for the following tests—L ($COD/SO_4^{2-} = 3$), PCFL ($COD/SO_4^{2-} = 0.67$ and 4% PCF), and PCF ($COD/SO_4^{2-} = 0.67$ and 4% PCF)—were shown. Observing the results of L tests (Fig. 2A), one may note that the arsenic content diminishes as sulfate concentration is reduced; therefore, these processes are directly related. Given the stoichiometry of arsenic precipitation as arsenic sulfide (As_2S_3), and also that only the sulfate ions were added to the synthetic liquid medium, regardless of a sulfate reduction of only 10%, sulfide concentration would be 25 times greater than As(III) concentration, thus guaranteeing the formation of arsenic sulfide.

The lowest residual arsenic concentration was observed during the final growth stage, when the smallest residual sulfate concentration was also observed. Arsenic removal in the L test was gradual, but the same feature was not observed in the other conditions (PCFL and PCF). These results indicate that the arsenic removal mechanisms may change, depending on the growth conditions and the composition of the culture medium. During PCFL and PCF tests (Fig. 2B and C), the concentration of sulfate barely changed over time, while arsenic concentrations diminished drastically, particularly in the first 48 h of cultivation. It can be inferred that arsenic could be immobilized by another mechanism, such as sorption onto the PCF surface, rather than being only precipitated as arsenic sulfide. Alternatively, PCF could also be used as a solid substrate for indirect nucleation of arsenic sulfides. In the presence of PCF (PCFL and PCF tests), the obtained As(III) removal was approximately 1.75 $mg\,g^{-1}$, the same figure obtained by Teclu et al. [1] using biogenic-produced sulfide for As(V) adsorption at pH 6.5. When this biogenic-produced sulfide was tested for As(III) adsorption, sorption capacity decreased to 0.20 $mg\,g^{-1}$. In this same study, the adsorptive capacity of that

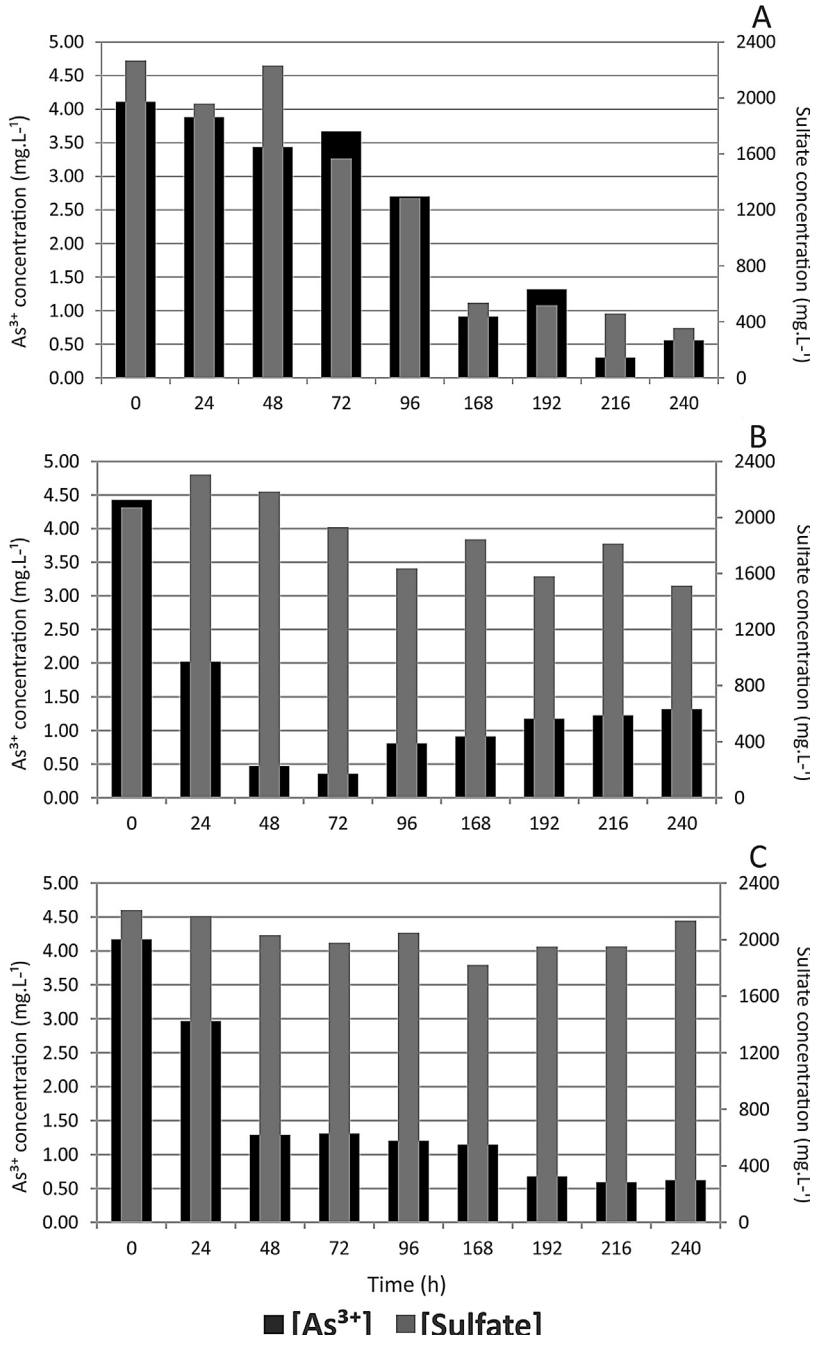

Fig. 2. Arsenic removal and sulfate consumption. A: lactate (COD/sulfate ratio, 3) and As(III); B: lactate (1.2 g L^{-1}), PCF (4%) and As(III); C: PCF (4%) and As(III).

biogenic-produced sulfide was compared with that of other organic and inorganic materials, and, as usually described, all the adsorbent materials tested proved to be more effective in adsorbing the oxidized arsenic species.

Arsenic removal was insignificant in the abiotic control despite the culture media composition (Table 2), and thus was confirmed the role of microorganisms during arsenic immobilization process. Fig. 2 clearly shows, however, that arsenic removal process is not compulsory, being directly related neither to sulfate removal nor to sulfide production. The obtained results indicate that, in the presence of SRB and PCF, As(III) could be removed from solution by chemical precipitation, probably by reacting with biogenic sulfide and/or by adsorption on the PCF surface.

The waste material used for this study had previously been tested [4] as a biosorbent for As(III). That previous study's authors had proved, for the first time, that powdered chicken feathers were able to specifically adsorb trivalent arsenic species in acidic or neutral-pH conditions, and the maximum arsenic-loading capacity obtained was 170 μmol of arsenic per gram of biomass. To such ends, powdered chicken feathers were pre-treated with sodium thioglycolate to guarantee the interactions between As(III) and the reduced sulfhydryl groups present at the material surface [4].

During the study here described, the biomass pre-treatment was dismissed, but As removal was not impaired, probably because the bacterial metabolism produces large amounts of reducing agents (H_2S), thus maintaining the active groups of the biomass in their reduced state. The low As(III) removal observed in abiotic control can be related to the relatively high redox potential values measured under those conditions. The arsenic immobilization obtained in the presence of SRB mixed culture and PCF is higher than in the abiotic tests (data not shown), so it is possible to assume that arsenic was adsorbed onto the PCF surface in

consequence of SRB metabolism. According to [5], the influence of sulfur-reducing bacteria on arsenic sulfide precipitation is not completely understood, but adsorption and co-precipitation of arsenic compounds could be simultaneously observed under reducing conditions.

Given that, as previously described [4], sulfhydryl groups from cysteine disulfide bonds on the PCF surface are the active sites for As(III) adsorption, and also that those disulfide bonds should be reduced to expose the SH groups from cysteine residues and so guarantee the adsorption of the reduced arsenic species, the PCF surface ought to be chemically or biologically modified for the adsorptive phenomenon to take place. In this present study, because of the microbial metabolism, a decrease of the redox potential was observed. Those negative measured potentials are low enough to reduce the sulfhydryl groups onto the PCF surface without the need of any reductive agent.

Another finding that reinforces the hypothesis that arsenic adsorbs onto the PCF surface is that As was removed faster than in the experiment performed only with lactate, a result that is consistent with adsorptive phenomena in general. This feature was observed for both PCF and PCFL tests (Fig. 2). It is also necessary to consider, too, that PCF may also act as a solid core for arsenic sulfide heterogeneous nucleation. PCF's roughness and surface area, as well as its affinity for arsenite ions, may facilitate crystal growth on its surface.

The literature points out that the best As(III) removals occur at low pH values, usually around 2.0–6.0 [4,5,22]. These pH values, however, are not ideal for SRB growth, and the majority of the described SRB genera could suffer from metabolic impairment if cultured under very low pH values [22,23]. The present study used an initial pH value of 7.0 while aiming to enable the metabolic activity of SRB. Even though experimental conditions were not ideal for arsenic removal, it was possible to obtain satisfactory As removals. Adaptation of SRB cultures to moderately acidic pH values (5.5–6.5) could make for even better arsenic immobilization. If sulfate reduction is not the primary target, however, and instead the main objective to immobilize As(III), it should be preferable to use a slightly acidic pH value.

According to the data here discussed, As(III) seems to be removed from the liquid medium by three different mechanisms: (i) precipitation after reaction with biogenic sulfide sulfide, (ii) adsorption onto a biologically modified PCF surface, at low redox potential, or (iii) precipitation onto a PCF surface.

Conclusions

PCF combined with other electron donors can be used as a nutrient source for SRB, thus providing an alternative material for arsenic (III) removal. PCF degradation increases medium COD, thus stimulating SRB growth. Through microbial growth, sulfate and As(III) are removed from the medium.

Arsenic removal is related to sulfate reduction when PCF is absent in the system, but if PCF is also present, arsenic (III) removal is not directly related to sulfate reduction: there is arsenic removal even at low sulfate reduction yields, probably because As(III) is adsorbed or precipitated onto the PCF surface.

The microbial growth causes sulfide production as sulfate consumption leads to a sharp decrease of redox potential. Biogenic sulfide may react with As(III), thus diminishing its solubility. Furthermore, at low-redox conditions, sulfhydryl groups from the PCF surface will remain reduced, thus permitting As(III)-specific adsorption.

Conflict of interest

None declared.

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