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Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria

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ABSTRACT

A mixed culture of sulphate-reducing bacteria was used to study the bioremoval of arsenic species (As(III) or As(V)) from groundwater. During growth of a mixed SRB culture adapted to 0.1 mg/L arsenic species through repeated sub-culturing, 1 mg/L of either As(III) or As(V) was reduced to 0.3 and 0.13 mg/L respectively. Sorption experiments on the precipitate produced by batch cultured sulphate-reducing bacteria (SRB-PP) indicated a removal of about 77 and 55% of As(V) and As(III) respectively under the following conditions: pH 6.9; biomass (2 g/L); 24 h contact time; initial arsenic concentration, 1 mg/L of either species. These results were compared with synthetic iron sulphide as adsorbent. The adsorption data were fitted to Langmuir and Freundlich isotherms. Energy dispersive X-ray analysis showed the SRB-PP contained elements such as sulphur, iron, calcium and phosphorus. Biosorption studies indicated that SRB cell pellets removed about 6.6% of the As(III) and 10.5% of the As(V) from water containing an initial concentration of 1 mg/L of either arsenic species after 24 h contact.

1. Introduction

The presence of elevated concentrations of arsenic and other heavy metals in groundwater and surface waters is creating serious problems for humans as well as other living organisms (Jang et al., 2006). These contaminants may be removed through biological and/or chemical methods. The mechanisms of contaminant removal by microorganisms can include (i) extracellular accumulation/precipitation; (ii) cell-surface adsorption or complexation; and (iii) intracellular accumulation (Muraleadharan et al., 1991). The extracellular polymeric substances (EPSs) surrounding many microorganisms, especially bacteria, comprise, depending on the strain and culture conditions, a mixture of polysaccharides, mucopolysaccharides and proteins (Zinkevich et al., 1996) that play a major role in the biosorption of heavy metals (Beveridge and Doyle, 1989). Cell-sorption occurs with dead or living microorganisms whilst intracellular entrapment requires microbial activity (Igwe and Abia, 2006).

There are several conventional sorbents for arsenic species; some of the most widely studied are iron hydroxides and oxides (such as amorphous hydrous ferric oxide, ferrihydrite and goethite; Fendorf et al., 1997; Raven et al., 1998; Appelo et al., 2002), activated alumina (Singh and Pant, 2004; Kuriakose et al., 2004), iron-modified activated carbon (Chen et al., 2007) and cellulose sponge (Munoz et al., 2002). The removal of arsenite and arsenate by hydrous ferric oxide (HFO) has been studied extensively due to HFO's high isoelectric point (IEP = 8.1) (Dixit and Hering, 2003) and selectivity for arsenic species (Deliyanni et al., 2003). Chen et al. (2007) have shown that combining carbon and iron is effective in arsenic removal as the activated carbon supports the preloading of

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iron. Oxyanions (such as $H_2AsO_4^-$ and $HAsO_4^{2-}$) can react with iron in ligand exchange reactions to form an inner sphere monodentate or bidentate surface complex (Fuller et al., 1993; Grossl et al., 1997).

Microbial sulphate reduction decreases the toxicity of several metals by precipitating the dissolved metals as metal sulphides; by incorporating them into sulphide minerals, or by adsorption onto mineral surfaces (O'Day et al., 1998). Iron sulphide minerals can induce metal retention, mobility and bioavailability that are governed by reactions occurring at the surface of the iron sulphide moiety (Huerta-Diaz and Morse, 1992). Watson et al. (1995) investigated the adsorbent properties of iron sulphide produced by SRB. They found that the SRB-produced adsorbent had a considerably higher specific uptake capacity for different metal ions from solution than other adsorbents, such as activated carbon. Moreover, the advantages of using biogenic sulphide to precipitate metals include lower sludge formation and formation of low solubility products as compared to hydroxide precipitation (Hao, 2000). Increasing knowledge of biosorption during the past few decades has revealed the high adsorption capacities [e.g., biosorption of arsenic by *Lessonia nigrescens* ranged from 45.2 ($pH = 2.5$) to 28.2 mg/L ($pH = 6.5$) (Hansen et al., 2006)], low cost and regenerability of natural biosorption materials (Volesky, 2003; Gavrilescu, 2004).

Under anaerobic conditions, SRB can transform sulphate to hydrogen sulphide using simple organic substrates that can have importance in bioremediation of pollutants (Chang et al., 2000). The resultant H_2S, in the absence (or limiting presence) of metal species, may dissociate according to the following equilibrium equations (Moosa and Harrison, 2006):

$$H_2S \leftrightarrow HS^- + H^+ \tag{1}$$

$$HS^- \leftrightarrow S^{2-} + H^+ \tag{2}$$

Total hydrogen sulphide concentration can be determined by the following relationship:

$$H_2S_{total} = H_2S_{aq} + HS^- \tag{3}$$

In the pH range 6–8, hydrogen sulphide exists as a mixture of H_2S and HS^-. Below pH 6, the undissociated form (H_2S) dominates. The HS^- dissociates further to S^{2-} near pH 17 (Migdisov et al., 2002). Even though SRB have the highest tolerance to sulphide of all anaerobic microorganisms, their activity is nonetheless inhibited in its presence (O'Flaherty et al., 1997). Two of the hypotheses for the inhibition mechanisms are (1) metal sulphides precipitate so that the SRB are deprived of the essential trace metals required as cofactors for their enzyme systems (Loka Bharathi et al., 1990) and (2) the sulphide is absorbed into the cells of the microorganisms and denatures proteins by acting as a cross-linking agent between the polypeptide chains (Postgate, 1979) thereby interfering with the metabolic coenzymes through sulphide bond formation. However, when present at concentrations below the inhibitory level, H_2S can react with dissolved metals to form insoluble precipitates that are non-toxic to microorganisms (Lyew and Sheppard, 2001). Metals may also be precipitated by bubbling H_2S through a metal-containing solution (Vogel, 1996; Hao, 2000). A study by Newman et al. (1997) showed that *Desulfotomaculum auripigmentum* precipitated arsenic trisulphide (As_2S_3) that resulted from the reduction of As(V) to As(III). The stability of As_2S_3 is highly dependent on pH and sulphide concentration. The following diagram shows the occurrence of arsenite and arsenate at different pHs.

The objectives of the study were to investigate the bioremoval of arsenic species (As(III) and/or As(V)) during the growth of a mixed culture of sulphate-reducing bacteria (SRB); sorption of the arsenic species on the surface components of the bacteria; and, precipitation of the metalloid as a sulphide salt.

2. Materials and methods

2.1. Preparation of arsenic solutions

Stock solutions of As(III) and As(V) were prepared by dissolving respectively solid sodium arsenite ($NaAsO_2$) or sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) in deionised water to a concentration of 1000 mg/L. Working solutions were freshly prepared each time by diluting the stock solutions with appropriate amounts of deionised water. The concentrations of both arsenic species are always given as an elemental arsenic concentration.

2.2. Nutrient medium and source of sulphate-reducing bacteria

The mixed culture of SRB used for the arsenic bioremoval studies was grown on Postgate medium B (PMB) (Postgate, 1979) with the following composition (g/L): KH_2PO_4 (0.5); NH_4Cl (1); $CaSO_4$ (1); $MgSO_4 \cdot 7H_2O$ (2); sodium lactate (3.5); ascorbic acid (0.1); thioglycollic acid (0.1) and $FeSO_4 \cdot 7H_2O$ (0.5). The pH of the medium was maintained between pH 7.0 and pH 7.5 using 2 M NaOH. Some precipitate formed when the pH was adjusted to within the specified pH range. The medium was boiled for a few minutes and flushed with nitrogen gas to drive off the oxygen.

The culture of sulphate-reducing bacteria (SRB) was isolated from anaerobic sediments from the Msunduzi River (Pietermaritzburg, South Africa) and maintained by regular transfer to fresh PMB.

2.3. Bioremoval studies

The cells present in 50 mL of an exponentially growing mixed SRB culture grown in the presence of either iron or arsenic species (either As(III) or As(V) at 0.1 mg/L) were collected by centrifuging late log phase cells at 10 000 rpm ($12\,096 \times g$) (Avanti J-26 XPI high-performance centrifuge, Beckman Coulter) for 20 min for use in the experiments as described below. All batch experiments were performed in duplicate using these cells with appropriate controls in each instance.

2.3.1. Precipitation of arsenic species as the sulphide salt

Cell pellets collected from SRB cultures adapted to grow in either 0.1 mg/L As(III) or As(V) as described in the previous section were used to study the bioremoval of arsenic as