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Full Length Research Paper

Bioremoval of arsenic in purpose designed laboratory-scale bioreactors

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Laboratory scale bioreactors were used to investigate the treatment of arsenic species deliberately contaminated groundwater. A mixed culture of sulphate-reducing bacteria (SRB) with molasses as carbon source was immobilised on a polystyrene support matrix. The artificial groundwater contained either As(III) or As(V) at concentrations of 20, 10, 5, 1 or 0.1 mg/l as well as 0.1 mg/l of a mixture with As(III) accounting for a total of 20, 30, 40, 60 and 80%. More than 90 and 60% of the As(V) and As(III), respectively, were removed by the end of a 14-day experiment. Total arsenic had been reduced to below the WHO acceptable level of 10 µg/l when the proportion of As(III) was 20 and 30%, while at 40% As(III), this level was reached only after 21 days treatment. The efficiency of As(III) removal was increased by first oxidising it to As(V) using MnO_2.

Key words: Arsenite, arsenate, bioreactor, polystyrene, sulphate-reducing bacteria.

INTRODUCTION

Arsenic contamination of groundwater is a worldwide problem, especially in Bangladesh where 30 - 40 million people (Roberts et al., 2004) are estimated to be consuming water with arsenic concentrations greater than 50 µg/l. Due to its acute and chronic toxicity to human beings, arsenic has been widely studied (Chen et al., 2004) and technologies for its removal has become increasingly important (Choong et al., 2007). The World Health Organisation (WHO) has set the maximum contaminant level (MCL) of arsenic in drinking water at 10 µg/l (WHO, 1996). Factors controlling the distribution and speciation of arsenic in the environment can be identified using geochemical modelling (Cullen and Reimer, 1989). Redox potential (Eh-measure of electrochemical potential within a system) and pH are the most important factors controlling arsenic speciation (Smedley and Kinniburgh, 2002). At low pH (<6.9) and under oxidizing conditions, $H_2AsO_4^-$ is dominant, whereas at higher pH, $HAsO_4^{2-}$ dominates. Under extremely acidic (pH $<$ 2) and alkaline (pH $>$ 12) conditions, H_3AsO_4 and AsO_4^{3-} may be present, respectively. Under reducing conditions and low pH, arsenic (III) acid becomes stable, mainly as H_3AsO_3 (Cullen and Reimer, 1989).

Several strategies exist for the treatment of arsenic contaminated groundwater. The main categories are: *ex-situ* technologies such as “pump-and-treat” systems; and *in-situ* technologies such as “permeable reactive barriers” (PRBs) (Zouboulis and Katsoyiannis, 2005). Coagulation/filtration (Zouboulis and Katsoyiannis, 2002), solar-driven membrane distillation (Manna et al., 2010), adsorption on iron oxides or activated alumina (Jeong et al., 2007) and reverse osmosis (Geucke et al., 2009) have been used to treat groundwater contaminated with arsenic. For efficient removal of As(III), an oxidation step may be performed by the addition of chemical reagents, such as potassium permanganate, chlorine, ozone, hydrogen peroxide or manganese oxide prior to the application of the above mentioned processes (Kim and Nriagu, 2000).

Biological treatment is currently receiving attention for the removal of arsenic species from contaminated waters (Wang and Zhao, 2009). Advantages of biological treatment over physicochemical treatment methods are that it uses microorganisms instead of chemicals to reduce/

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Abbreviations: SRB, Sulphate-reducing bacteria; **CPD,** critical point drying; **Eh,** redox potential; **WHO,** world health organization; **ESEM,** environmental scanning electron microscopy.

oxidise or remove contaminants; incurs lower costs; is more efficient when metal ion concentrations are below 1 mg/l; shows selectivity in removal of the desired metals (Brierley, 1990). In contrast, chemical treatment methods has high operational and maintenance costs and produce large amounts of sludge that requires disposal (Zouboulis and Katsoyiannis, 2005). Biological treatment can be used alone or in combination with adsorption, filtration and other physico-chemical procedures.

A variety of microorganisms may be involved in biological treatment of contaminated waters. An important group of microorganisms in this regard is the sulphate-reducing bacteria (SRB) that have been used for the treatment of acidic and sulphate contaminated waters (Jong and Parry, 2003). Sulphate-reducing bacteria oxidizes simple organic compounds by utilising sulphate as electron acceptor (Lièvremont et al., 2009). Sulphide and alkalinity is then generated and metals are removed as insoluble sulphides (Luptakova and Kusnierova, 2005). Kirk et al. (2004) showed that the presence of sulphate-reducing bacteria in groundwater could reduce the level of arsenic. In the favoured microenvironment of sulphate-reducing bacteria, the combination of neutral pH, low Eh and high sulphide concentration makes the availability of soluble metals extremely low (Utgikar et al., 2002). This causes the growth of sulphate-reducing bacteria in environments containing high levels of toxic elements. Hence, there is a great interest in the use of SRB for bioprecipiation of toxic metals from contaminated environments (Gadd, 2009).

This study was undertaken to investigate the bioremoval of arsenic species from groundwater using purpose made bioreactors containing SRB, growing on molasses as carbon source, sulphate as electron acceptor and polystyrene as bacterial support matrix in the presence of 20, 10, 5, 1 and 0.1 mg/l As(III) or As(V) alone or in a mixture with As(III) accounting for a total of 20, 30, 40, 60 and 80% (0.1 mg/l). Growth of the bacteria on the surfaces of the polystyrene surface was investigated using environmental scanning electron microscopy (ESEM), and dry-ashed polystyrene samples were analysed for arsenic and iron using ICP-OES. Chemical oxidising agents were used in combination with the biological process to asses the efficiency of the removal of the arsenic, particularly As(III).

MATERIALS AND METHODS

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 ($Na_2HAsO_4.7H_2O$) in deionised water to a concentration of 1000 mg/l. Working solutions were freshly prepared for each experiment by diluting these stock solutions with appropriate amounts of deionised water.

The arsenic-contaminated synthetic groundwater used in this study was prepared by spiking tap water with As(III) and/or As(V). The concentrations used for both forms of arsenic were 20, 10, 5, 1 and 0.1 mg/l or a mixture with As(III) accounting for 20, 30, 40, 60 and 80% as the total when 0.1 mg/l was used. Arsenic concentration of 10 mg/l has been reported by Kempster et al. (2009) in a study to monitor the contaminant in South African water resources.

Nutrient medium and source of sulphate reducing bacteria

The culture of SRB used in these studies was grown on postgate medium B (Postgate, 1979) with the following composition (g/l): KH_2PO_4 (0.5); NH_4Cl (1); $CaSO_4$ (1); $MgSO_4.7H_2O$ (2); sodium lactate (3.5); ascorbic acid (0.1); thioglycollic acid (0.1) and $FeSO_4.7H_2O$ (0.5). The pH of the medium was maintained between 7.0 and 7.5 using 2 M NaOH. Some precipitate formed when the pH of the medium was adjusted to 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). The presence of SRB was ascertained by the formation of a black precipitate (ferrous iron) that appeared a few days after the inoculation of the culture. Blackening of lead acetate impregnated filter paper, indicated the release of hydrogen sulphide and verified SRB activity.

Bioreactor configuration and experimental set-up

The bioreactors used in this study (Figure 1) were constructed from plastic containers in the Department's workshop. Each bioreactor had a capacity of 12 L. The inner containers, with mesh at the bottom and top to disperse the upwards flow of the medium, were filled with polystyrene (cut into small pieces approximately 10 - 15 mm x 12 - 16 mm x 9 - 12 mm) as support matrix. The bioreactors were inoculated with a mixed SRB culture containing ~3 x 10^4 cells/ml (20% v/v). The void volume in the inner containers when filled to capacity with polystyrene was approximately 4.2 L.

Molasses served as carbon source and sulphate as electron acceptor. Water artificially contaminated with either As(III) or As(V) at concentrations of 20, 10, 5, 1 and 0.1 mg/l or in the case of the 0.1 mg/l concentration as combination of As(III) as As(V) as discussed in the preparation of arsenic solutions was fed into the bioreactors with a calibrated peristaltic pump (Watson Marlow model 504U, England). The bioreactors were operated batch-wise, with regular monitoring of SRB activity. The parameters measured were SRB growth, pH, Eh and concentrations of SO_4^{2-} and arsenic species. The effect of the support matrix on the performance of the bioreactors in terms of SO_4^{2-} reduction and arsenic removal was assessed. Matrix-free bioreactors served as a positive control and in each case, an appropriate negative control without SRB was used. The configuration of an operational bioreactor is shown in Figure 2. The main components of the synthetic groundwater are shown in Table 1.

Oxidation of arsenite

Batch experiments were set-up to study the oxidation of arsenite to arsenate using pumped air, atmospheric air and MnO_2. Mixtures of As(III) and As(V) in: 80:20; 70:30; 60:40; 40:60; 30:70 and 20:80 ratios (total arsenic concentration, 100 µg/l) were exposed for 24 h to 0.1, 1 and 2 g/l MnO_2 at 25 ± 2°C, pH 6.9. The air treatments were of similar duration.

Analytical determinations

The parameters monitored over the experimental period were: SRB

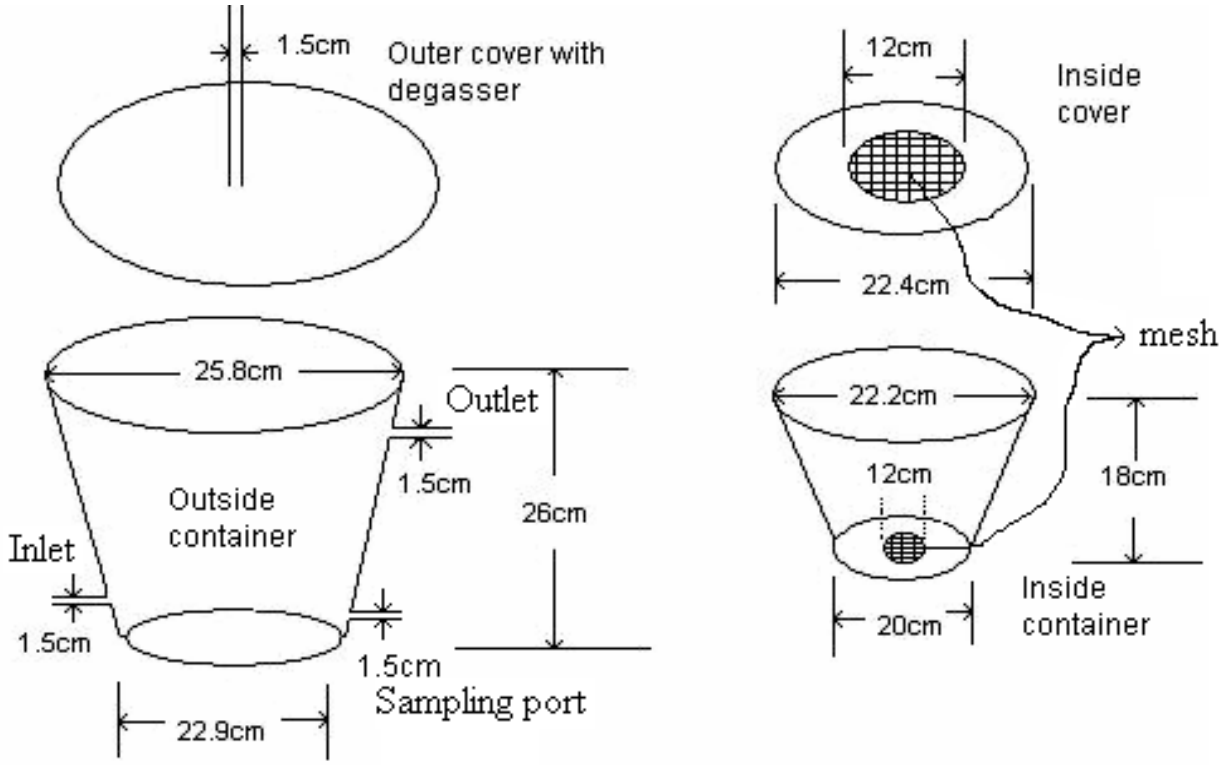

Figure 1. Sketch of a plastic bioreactor. The inner container which fits inside the outer one, is packed with polystyrene blocks as support matrices.

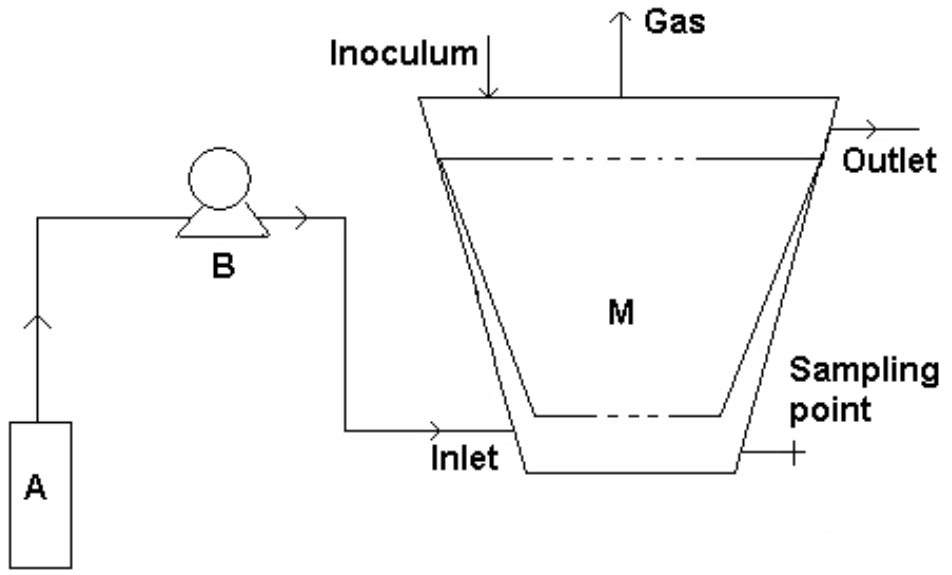

Figure 2. Bioreactor configuration: A – arsenic-contaminated water reservoir; B – peristaltic pump; M – support matrix within the inner channels.

populations (cells/ml); pH; redox potential; SO_4^{2-}, S^{2-} and arsenic species concentrations. All the pH and redox potential measurements were made using a Crison combination pH electrode (platinum electrode paired with an Ag/AgCl reference electrode) coupled to a Crison 2000 pH meter. Total arsenic and arsenite (As(III)) were analysed using hydride generation (HG) coupled to an ICP detection system according to the modified method developed by Müller (1999). Other metals were analysed using ICP-OES.

Table 1. Composition and operational conditions of the synthetic groundwater.

Parameter	Value
pH	6.9
Redox potential, mv	227 ± 6
Temperature (°C)	25 ± 3
SO_4^{2-} (mg/l)	175 ± 5
NO_3^- (mg/l)	6.29 ± 0.31
Ca (mg/l)	112 ± 6
Mg (mg/l)	64.4 ± 1.8
Na (mg/l)	102 ± 6
Fe (total) (mg/l)	3.2 ± 0.09
As (µg/l)	<2
Conductivity (µS cm^{-1} at 25°C)	1120

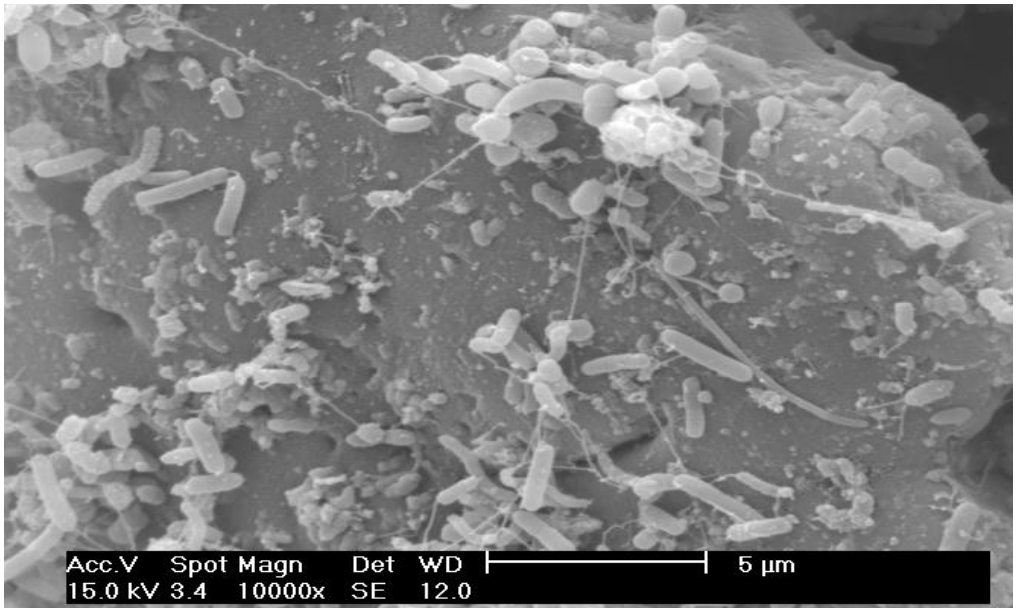

Figure 3. ESEM micrographs of SRB colonising a polystyrene surface.

Sulphate and sulphide were analysed using the modified turbidimetric method of Kolmer et al. (2000) and the methylene blue method, respectively. Environmental scanning electron microscopy (ESEM) was used to study the biofilms and the surface characteristics of the polystyrene support matrix. Samples of the polystyrene support matrices colonised by bacteria were fixed in 3% (v/v) glutaraldehyde, washed twice in 0.05 M cacodylate buffer (pH 7.1) for 10 min and dehydrated in an alcohol series (10 min each in 30, 50, 70, 80, 90% and 3 × 10 min in 100%) in a fume cupboard. The specimens were then transferred into critical point drier baskets under 100% alcohol and placed in a pre-cooled Hitachi HCP-2 critical point drier. Following critical point drying (CPD) and gold palladium sputter coating (Polaron Equipment Limited SEM, coating unit E5100), the samples were viewed in the ESEM (Philips, FEI XL 30) at an accelerating voltage of 15 keV.

To determine arsenic and iron associated with the polystyrene, it was digested with 20% $Mg(NO_3)_2.6H_2O$ and then examined using ICP-OES. Operating conditions of ICP-OES used were: gas, argon; power, 0.90 Kw; plasma flow, 15 l/min; auxiliary flow, 1.50 l/min and nebuliser flow, 0.75 l/min.

RESULTS AND DISCUSSION

Immobilisation of SRB on polystyrene

ESEM photomicrographs of the biofilms from the bioreactors showed that the SRB colonised on the polystyrene support matrix (Figure 3) when grown with molasses as carbon source. The SRB count in the bioreactors with 0.1 mgl^{-1} of either As(III) or As(V) ranged from 3 x 10^6 to 5 x 10^7 cells/ml from an initial population of about 4 x 10^4 cells/ml. This showed that when essential nutrients are available, biological process can

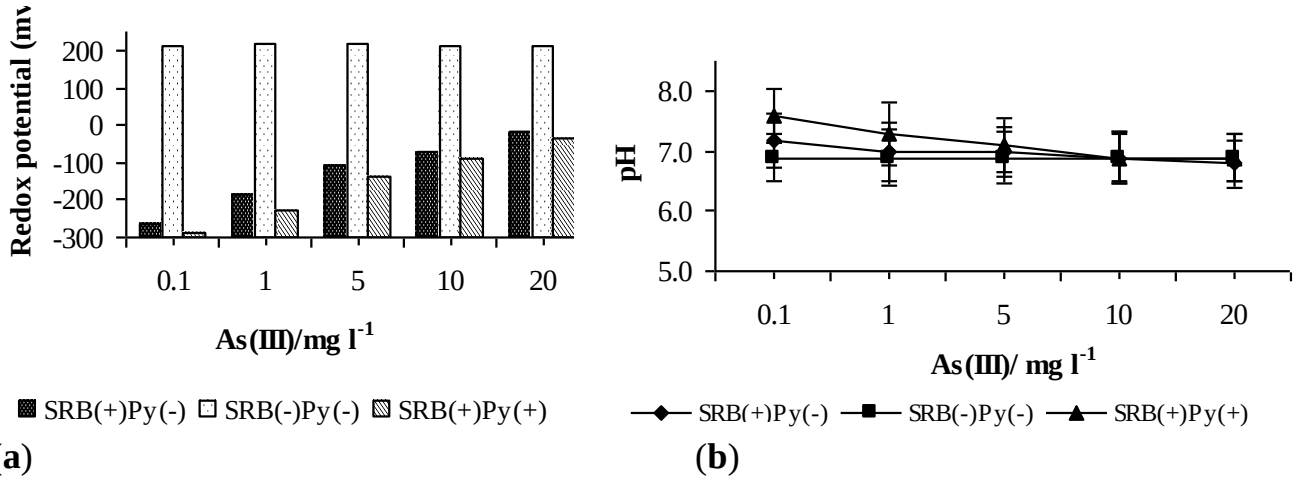

Figure 4 Changes in (a) redox potential and (b) pH as a function of As(III) concentration in the presence of SRB with polystyrene as support matrix (SRB(+) Py(+)), in the absence of SRB and polystyrene (SRB(-) Py(-)) and in the presence of planktonic SRB (SRB(+) Py(-)).

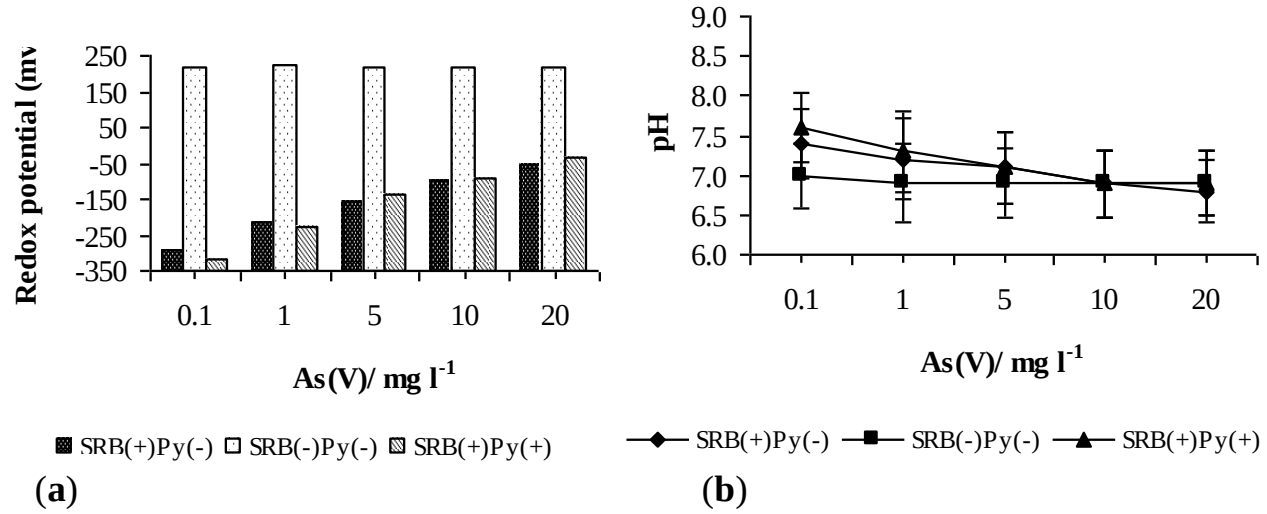

Figure 5. Changes in (a) redox potential and (b) pH as a function of As(V) concentration in the presence of SRB with polystyrene as support matrix (SRB(+) Py(+)), in the absence of SRB and polystyrene (SRB(-) Py(-)) and in the presence of planktonic SRB (SRB(+) Py(-)).

be applied for the bioremediation of contaminated waters with arsenic even at fairly high concentrations, provided wash-out of the SRB is prevented by immobilising the cells on a solid support. Similar results have been reported by Glombitza (2001).

Redox potential and pH

Changes in redox potential and pH within the bioreactors in the presence of different arsenic species were monitored over a period of 14 days. Initially, the pH was about 6.9 and the redox potential was around 215 mv in all the bioreactors. Figures 4, 5 and 6 give the final redox potential and pH in SRB cultures comprising either immobilised or free-living cells growing in the presence of different levels of As(III), As(V) and in various ratios of As(III): As(V) while keeping the total initial arsenic content at 0.1 mg/l. In an earlier flask study, it was found that 20 mg/l of either As (III) or As(V) inhibited the growth of SRB and the data given in Figure 4 supports this finding.

Figures 4, 5 and 6 provide evidence that in the presence of either As(III) or As(V), the redox potential becomes more reducing (more negative) as the arsenic concentration decreases from 20 to 0.1 mg/l. In every instance, the pH also increased in those bioreactors inoculated with SRB and in which polystyrene was present as support matrix. In the positive control (inoculated

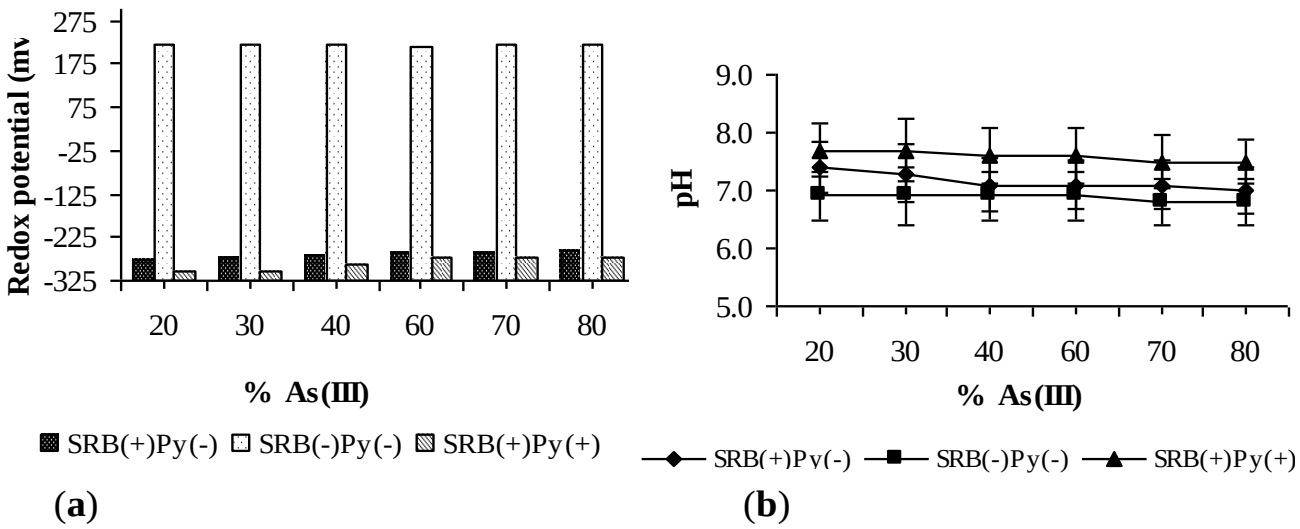

Figure 6 Changes in (a) redox potential and (b) pH as a function of initial percentage of As(III) in a total arsenic concentration of 0.1 mg/l in the presence of SRB with polystyrene as support matrix (SRB(+) Py(+)), in the absence of SRB and polystyrene (SRB(-) Py(-)) and in the presence of planktonic SRB (SRB(+) Py(-)).

with SRB in the absence of polystyrene), there was a decrease in redox potential and an increase in pH but the changes were smaller than those in the bioreactors containing polystyrene as support matrix. In all bioreactors containing neither SRB nor polystyrene, there was no change in either redox potential or pH. With changing As(III): As(V) ratios, viz 20:80, 30:70, 40:60, 60:40, 70:30 and 80:20 in which the total initial arsenic concentration was always 0.1 mg/l, only small differences in pH and redox potential were observed with increasing As(III) concentration, resulting in less reducing conditions and a smaller decrease in pH.

Polystyrene appeared to contribute more significantly to the lowering of the redox potential as the As(III) concentration increased (Figure 4a). This could be a reflection of the greater toxicity of As(III) than As(V). As the As(V) concentration increased, the polystyrene contribution to redox appeared to get less and the pH was affected alittle by the various treatments toward the end of the experiment. Care must be taken in interpreting these results, since the initial pHs were somewhat different (Figure 5).

Sulphate reduction and sulphide production

The activities of the SRB within the bioreactors were assayed by their ability to reduce sulphate and generate sulphide. Figures 7 to 9 show that the levels of sulphate reduction and sulphide production increased during the experimental period in the presence of SRB with or without the polystyrene support matrix.

Sulphate reduction in bioreactors with immobilised microorganisms was higher than in those containing suspended SRB (Figures 7a, 8a and 9a). The change in sulphate reduction levels in the presence of different proportions of arsenic species (total initial concentration, 0.1 mg/l) was small and the level of sulphate reduction increased with proportional increase in As(V) concentration. Due to the complexity of the reactions involved in sulphate reduction by anaerobic bacteria, different parameters will affect this reduction process including: availability and type of electron donor, pH, temperature, sulphate concentration as well as inhibitory effects of sulphide and any heavy metals present (Gonzalez-Silva et al., 2009; Jong and Parry, 2006; Leitão et al., 2006; Sahinkaya, 2009; Zhao et al., 2010). Temperature has an effect on the magnitude of sulphate reduction with increase in temperature (from 9 to 35°C) resulting in increased reduction levels (Nevatalo et al., 2010). The concentration of sulphate has been shown to affect the activity of SRB (Hwang et al., 2009). Moosa et al. (2002) have studied the effect of sulphate concentration and its volumetric loading on the kinetics of bacterial growth and bioreduction of sulphate. They found that the increase in initial concentrations of sulphate in the range of 1.0 - 10.0 kg/m enhanced the reaction rate from 0.007-0.17 $kg/m^3/h$. In the present investigation, the initial sulphate concentration in the synthetic groundwater was 175 mg/l. The following equation shows the reduction of sulphate by SRB, where CH_2O represents a carbohydrate (Herlihy et al., 1987):

$$2CH_2O + SO_4^{2-} \rightarrow S^{2-} + 2CO_2 + 2H_2O$$

Arsenic species removal

The efficiencies of arsenic species removal within the bioreactors during the growth of polystyrene-immobilised and free-living SRB are indicated in Figures 10 to 12 which

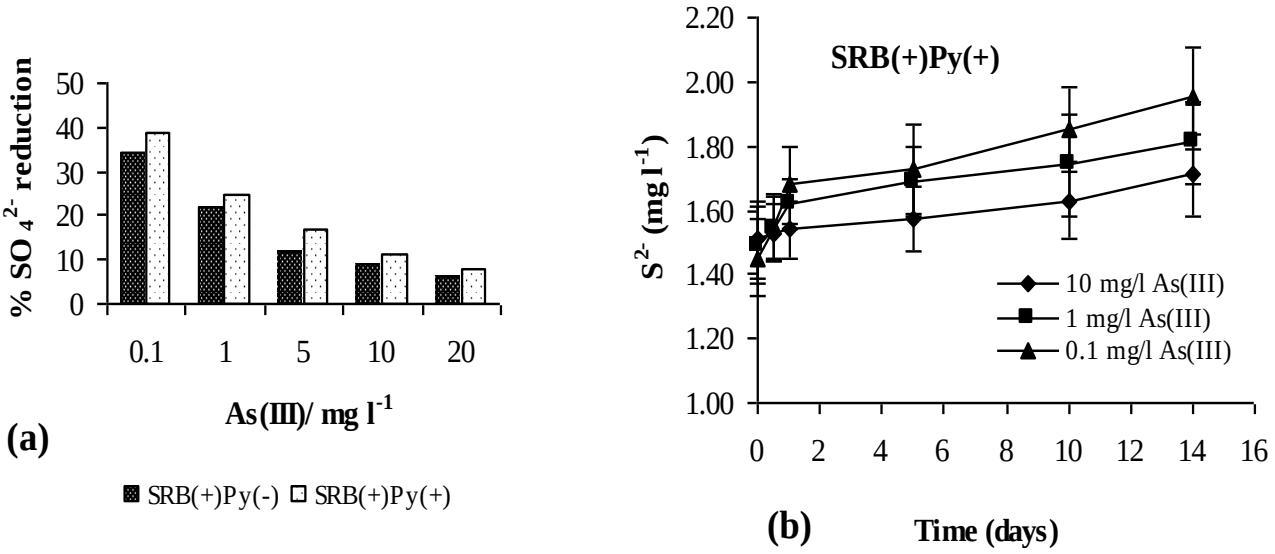

Figure 7. (a) Percentage of SO_4^{2-} reduction as a function of As(III) concentration in the presence of polystyrene immobilised SRB (SRB(+) Py(+)) and in the presence of planktonic SRB (SRB(+) Py(-)); **(b)** Changes in S^{2-} concentration as a function of time in the presence of polystyrene-immobilised SRB (SRB(+) Py(+)) growing in the presence of different As(III) levels.

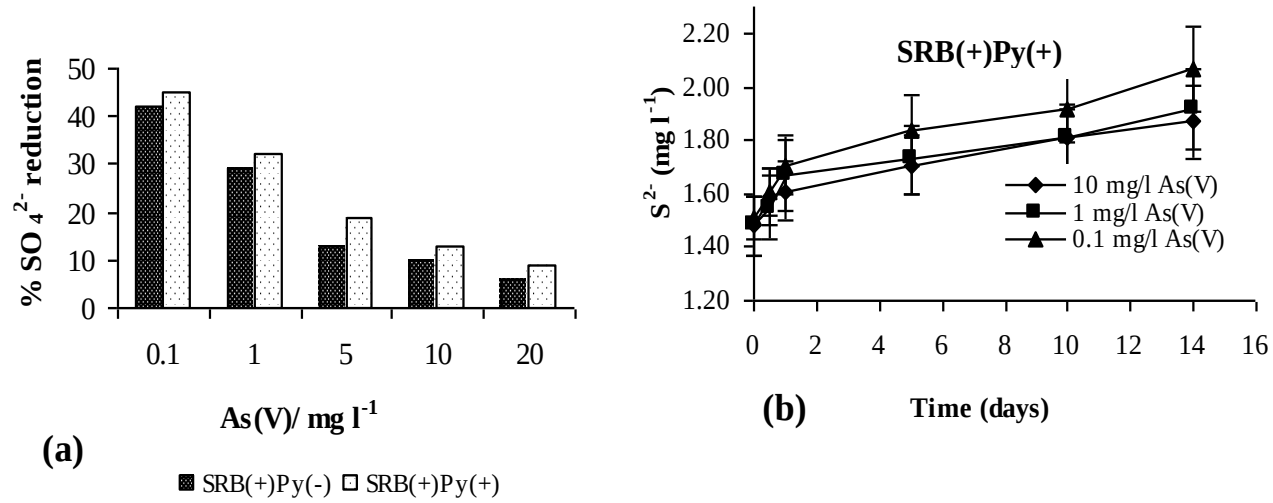

Figure 8. (a) Percentage of SO_4^{2-} reduction as a function of As(V) concentration in the presence of polystyrene immobilised SRB (SRB(+) Py(+)) and in the presence of planktonic SRB (SRB(+) Py(-)). **(b)** Changes in S^{2-} concentration as a function of time in the presence of polystyrene-immobilised SRB (SRB(+) Py(+)) growing in the presence of different As(V) levels.

show the changes in concentration of As(III), As(V) and in mixtures of As(III) and As(V), respectively. Both As(III) and As(V) were removed by the mixed culture of SRB either in the presence or absence of the support matrix. Irrespective of the initial concentration, the removal efficiency of As(III) was always inferior to that of As(V). Also, immobilised SRB were superior to freely suspended SRB in removing both arsenic species. Percentage removal of As(III) improved from about 10 to 47% when the concentration was reduced from 20 to 1 mg/l (Figure 10), whereas the corresponding improvement for As(V) was from 39 to 92% removal (Figure 11) during the 14-day experiment in the immobilised system. In the free-living cell systems, the percentage removals after the same period were 43, 33, 12 and 12% for initial As(III) concentrations of 1, 5, 10 and 20 mg/l, respectively, while for As(V), the corresponding removal values were 88, 76, 69 and 34%. The biomass hold-up in an immobilised cell bioreactor and any freely suspended cell present in a system are important in influencing the rate of sulphate reduction (Webb and Dervakos, 1996). The contribution by freely suspended cells is significant at low volumetric loading rates but not at high volumetric loading because wash out of the cells can occur (Baskaran and Nemati,

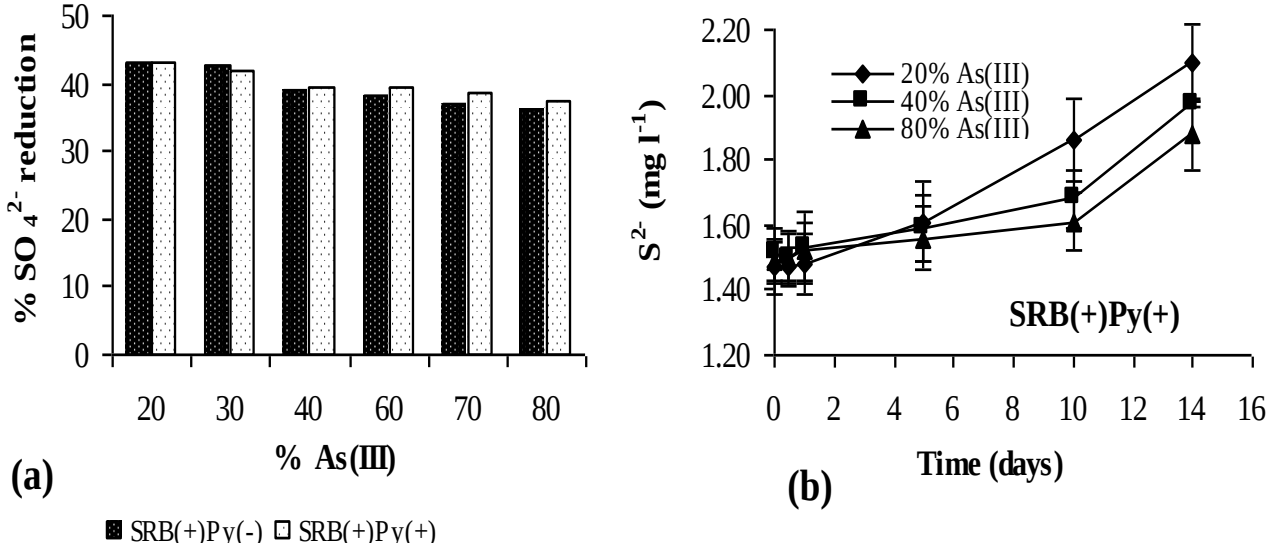

Figure 9. **(a)** Percentage of SO_4^{2-} reduction as a function of the percentage As(III) in a mixture of As(III) and As(V) (total arsenic = 0.1 mg/l) in the presence of polystyrene immobilised SRB (SRB(+) Py(+)) and in the presence of planktonic SRB (SRB(+) Py(-)). **(b)** Changes in S^{2-} concentration as a function of time in the presence of polystyrene-immobilised SRB (SRB(+) Py(+)) growing in the presence of different As(III) and As(V) combinations.

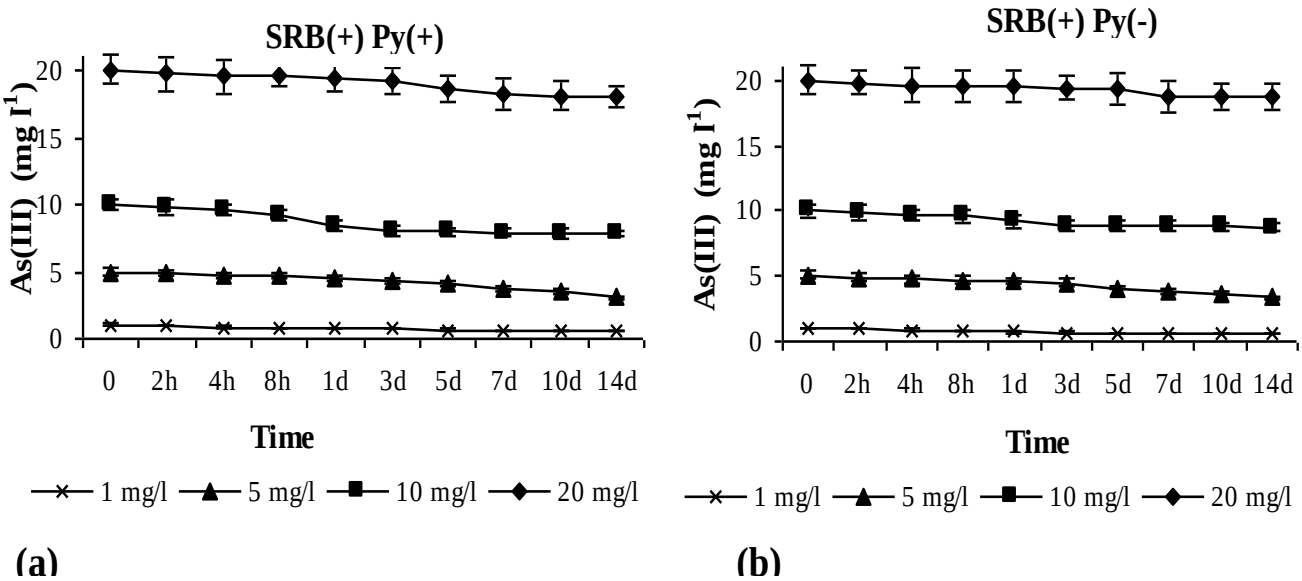

Figure 10. Changes in As(III) concentration as a function of time in the presence of: **(a)** polystyrene immobilised SRB (SRB(+) Py(+)) and **(b)** in the presence of planktonic SRB (SRB(+) Py(-)).

2006). Compared to planktonic cells, immobilised cells usually show more tolerance to environmental stresses such as high levels of toxic substances (Costerton et al., 1994) by the combined actions of chemical, physical and physiological phenomenon that are linked to the phenotypic variations among the constituent biofilm cells (Harrison et al., 2007) and positively influence the sorption, transportation and decomposition of pollutants (Schorer and Eisele, 1997; White and Gadd, 1998).

When the total arsenic concentration (As(III) + As(V) in different proportions) was 0.1 mg/l (100 µg/l), the removal efficiencies were improved for both As(III) and As(V). Percentage removals were 52, 73 and 96% at the end of the 14 day experiment when As(III) of comprised 100, 60 and 0% of the total arsenic content, respectively (Figure 12). When the residence time was increased to 21 days, the solutions containing 40% As(III) or less (40 µg/l As(III) or less in a total arsenic concentration of 100 µg/l) were

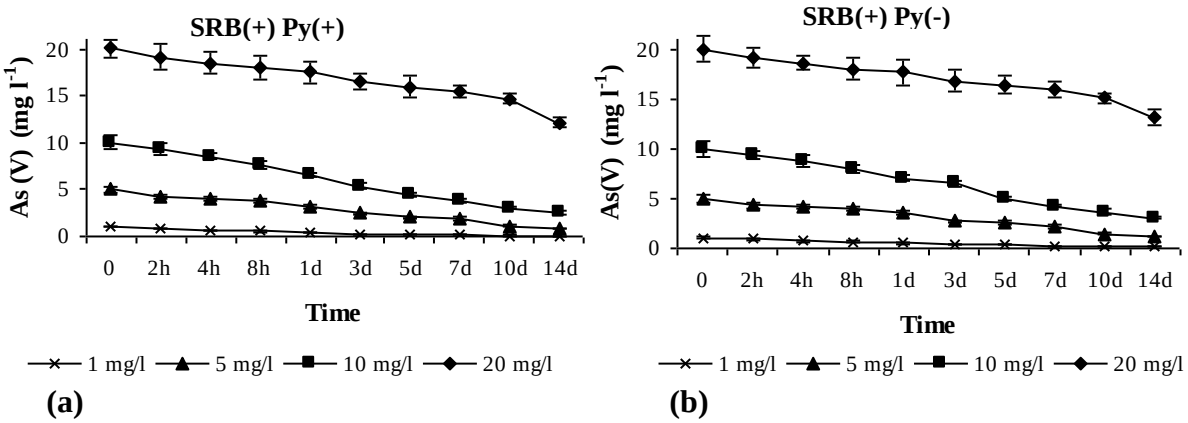

Figure 11. Changes in As(V) concentration as a function of time in the presence of: (a) polystyrene immobilised SRB (SRB(+) Py(+)) and (b) in the presence of planktonic SRB (SRB(+) Py(-)).

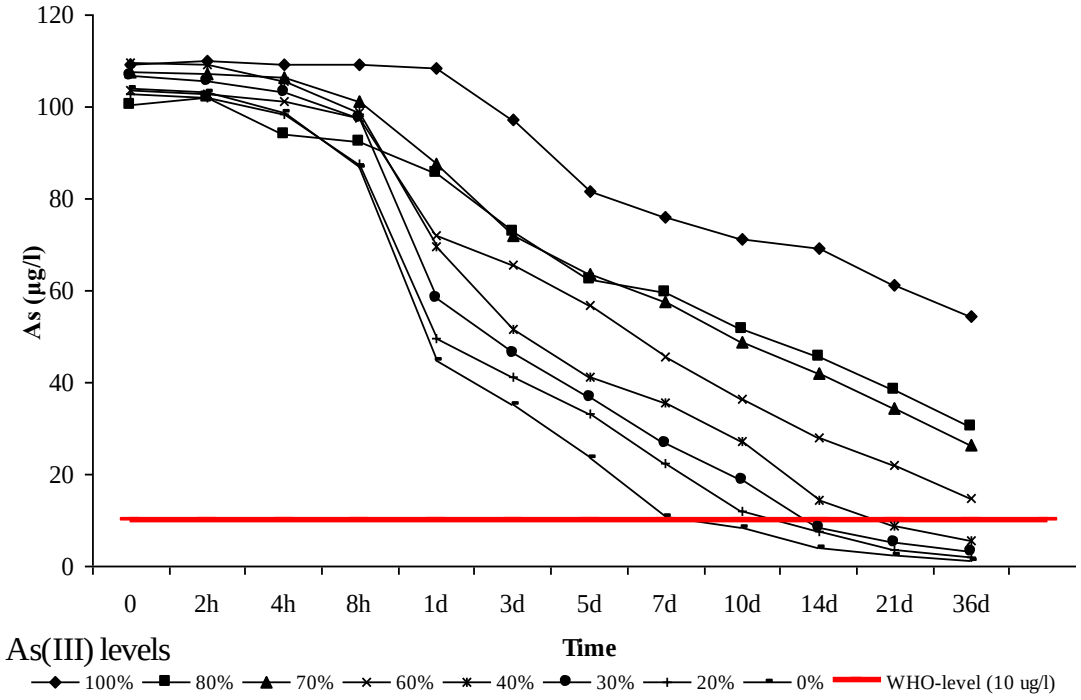

Figure 12. Changes in total arsenic concentration in solutions with different ratios of As(III):As(V) as a function of time in the presence of polystyrene-immobilised SRB.

efficiently bioremediated to below the WHO acceptance limit of 10 µg/l (Figure 12).

Elemental analysis of SRB-inoculated and control polystyrene

Polystyrene samples were taken at the end of the experiments from bioreactors inoculated with SRB (SRB(+) Py(+)) and from control (uninoculated) bioreactors (SRB(-) Py(+)) and dry-ashed to quantify arsenic and iron content. The results are given in Table 2.

The concentration of arsenic (either As(III) or As(V)) associated with the SRB-colonised polystyrene samples was higher than that associated with this material in the bioreactors lacking biofilms. Digestion of the former showed that the concentration of As(V) was higher than that of As(III) and this might be due to the charged nature of As(V) in the pH range used in this study, whereas As(III) would exist mainly as a neutral compound under these conditions. The concentration of arsenic species correlates positively with the concentration of iron in the polystyrene samples. Previously, it had been shown that

Table 2. Arsenic and iron content of dry ashed polystyrene samples from SRB-inoculated [SRB(+) Py(+)] and uninoculated [SRB(-) Py(+)] bioreactors.

Sample	As concentration (mg/g)	Fe concentration (mg/g)
As(III)		
SRB(+) Py(+)	1.79 ± 0.03	2.52 ± 0.01
SRB(-) Py(+)	0.23 ± 0.01	1.86 ± 0.01
As(V)		
SRB(+) Py(+)	2.43 ± 0.03	3.01 ± 0.04
SRB(-) Py(+)	2.07 ± 0.02	2.94 ± 0.03

the surface of microorganisms covered by iron oxides could provide a favourable environment for arsenic to be adsorbed and thus removed from aqueous streams (Katsoyiannis and Zouboulis, 2004). The iron in the influent water could act as the source for the formation adsorbents that subsequently remove arsenic species.

Oxidation of arsenite

Clearly, As(V) was removed more efficiently than As(III). Hence, pre-oxidation of As(III) to As(V) using air (atmospheric and pumped) and MnO_2 was investigated. MnO_2 was the oxidising agent preferred by Ghurye and Clifford (2001) for the treatment of drinking water prior to the removal of arsenic. Synthetic groundwater still containing about 69 µg/l As(III) on day-14 (Figure 12) was withdrawn from the appropriate bioreactors and exposed to atmospheric air, pumped air and MnO_2 (0.1, 1, and 2 g/l) for 24 h at pH ~ 7.0. Atmospheric and pumped air did not cause significant oxidation of As(III), whereas MnO_2 did, with the oxidation rate increasing with increasing concentration of MnO_2. This oxidising compound was further tested at a total arsenic concentration of 0.1 mg l^{-1} with As(III) comprising 80, 70, 60, 40, 30 and 20% of an As(III)-As(V) mixture. The results are given in Figure 13.

The percentage oxidation of the initial amounts of As(III) to As(V) by 0.1, 1 and 2 g/l MnO_2 were not significantly different (Figure 13). However, the amount of oxidising agent required for efficient conversion is dependent on the initial As(III) concentration. Thus, for 80% As(III), it would be appropriate to use 2 g l^{-1} MnO_2, whereas for 20% As(III), 0.1 g/l MnO_2 would suffice. Removal of arsenic species from groundwaters using bioreactors inoculated with SRB would be simplified if such water contained much less As(III) than As(V). The total dissolved arsenic concentrations remained fairly constant (data not shown) indicating that the decrease in As(III) was solely the result of its oxidation to As(V) as there was very little adsorption of either As(III) or As(V). Similar results were reported by Scott and Morgan (1995). The adsorption of As(V) onto MnO_2 minerals has been previously reported (Chiu and Hering, 2000; Manning et al., 2002; Ouvrard et al., 2002). However, in the present investigation, very low MnO_2 concentrations were used, so few surface sites were available for arsenic sorption. A study by Radu et al. (2008) using MnO_2 as adsorbent for As(V) found that the adsorption kinetics were very fast, with the concentration of sorbed arsenic remaining constant after about 2 min, whereas As(III) continued to be oxidised for a long time (Driehaus et al., 1995; Tournassat et al., 2002) and its sorption on MnO_2 has not been observed (Amirbahman et al., 2006). Radu et al. (2008) hypothesised that MnO_2 consists of oxidative sites and non-oxidative sorption sites. The oxidative sites are renewable and they rapidly oxidise As(III) and release As(V) to the solution through the mechanism postulated by Scott and Morgan (1995). The mechanism involves a multi-step reaction model, where the first step is the formation of an inner spherical surface complex where As(III) diffuses into oxidative sites and displaces surface-bound OH^- and H_2O via ligand sub-stitution and binds to the oxide metal ion. The second step is the transfer of two electrons from As(III) to the surface. In the third and fourth steps, the surface-bound oxidised As(V) and the reduced metal Mn(II) are released into the solution. In the above process, the total number of reactive surface sites will remain constant as a result of the formation of a new site when the reduced Mn(II) is released and the near-surface Mn–O group is protonated (Scott and Morgan, 1995). The capability of Mn(IV) in oxidising As(III) is represented by the following equation (Driehaus et al., 1995):

$$MnO_2 + H_3AsO_3 \leftrightarrow Mn^{2+} + HAsO_4^{2-} + H_2O$$

Mechanism of arsenic removal

Different mechanisms such as bioprecipitation of arsenic as sulphides and subsequent adsorption on biogenic sulphide precipitates can be postulated for lowering the concentration of arsenic species in the bioreactors. In addition to these microbiologically induced mechanisms, we investigated the possible adsorption of As(III) or As(V) on the walls of the bioreactors and on the polystyrene

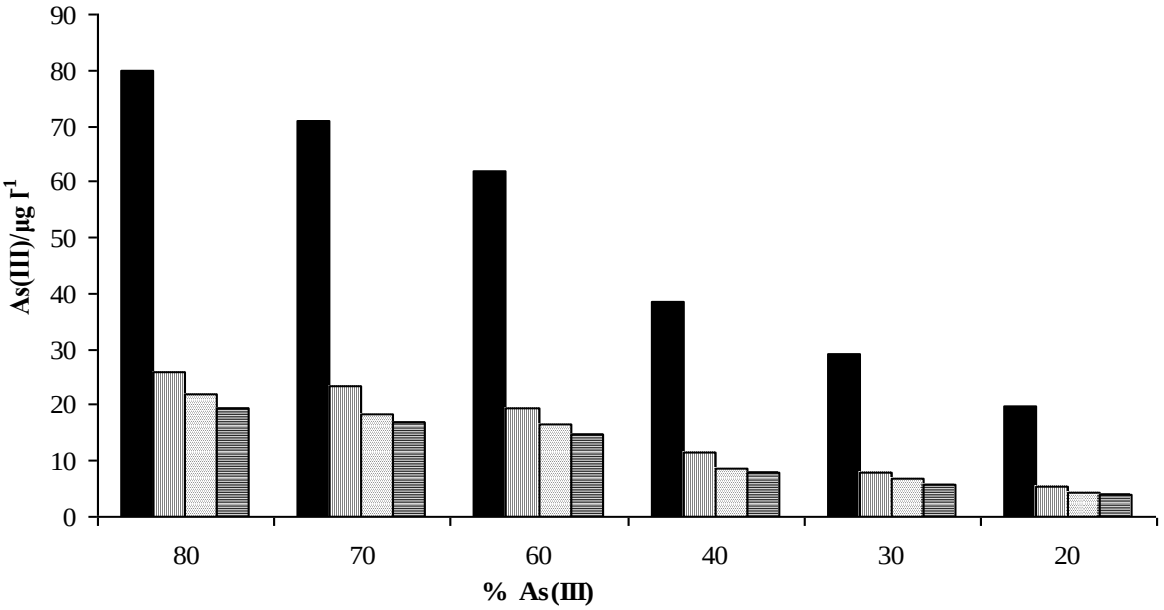

Figure 13. Changes in As(III) concentration when solutions containing various initial concentrations of this arsenic species in an As(III)-As(V) mixture were in contact with 0.1, 1 and 2 g/l MnO_2 for 24 h at pH 6.9.

support matrix. It was found that adsorption of both As(III) and As(V) onto these surfaces was negligible (>10 µg/l).

Conclusions

The bioreactors containing polystyrene-immobilised SRB showed a decrease in redox potential and an increase in pH during the removal of both As(III) and As(V) at initial concentrations of 20, 10, 5, 1 and 0.1 mg/l; however, these changes were markedly greater in solutions containing lower concentrations of the metalloid. Similarly, sulphate reduction and generation of sulphide were observed throughout the duration of the study. Arsenite removal from bioreactors supporting a culture of SRB immobilised on polystyrene was only about 10% when the initial concentration was 20 mg/l; the result for the same initial concentration of As(V) was 39%. Planktonic SRB cultures removed less As(III) and As(V) than their immobilised counterparts. Where the total arsenic concentration of 0.1 mg l^{-1} comprised solely of As(V) and the percentage As(III) in the same total weight of arsenic was 2 and 30%, a reduction to below the WHO's permissible level (10 µg/l) was achieved after 14 days. When the residence time was extended to 21 days, the solution containing 40% As(III) in a total arsenic concentration of 0.1 mg/l was also bioremediated to below this level. Planktonic SRB removed both arsenic species with lower efficiency than their immobilised counterparts. The presence of SRB was essential to the arsenic removal function of the system. The efficiency of As(III) removal was enhanced by oxidising it to the less toxic As(V) using MnO_2.

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