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Determination of the elemental composition of molasses and its suitability as carbon source for growth of sulphate-reducing bacteria

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ABSTRACT

Bioremediation of arsenic-contaminated water could be a cost-effective process provided a cheap carbon source is used. In this work molasses was tested as a possible source of carbon for the growth of sulphate-reducing bacteria (SRB). Its elemental composition and the tolerance of SRB toward different arsenic species (As (III) and As (V)) were also investigated. Batch studies were carried out to assess the suitability of 1, 2.5 and 5 g/l molasses concentrations for SRB growth. The results indicated that molasses does support SRB growth, the level of response being dependant on the concentration. The percentage of sulphate reduction with molasses at 1, 2.5 and 5 g/l was not significantly different. However, growth on molasses was not as good as that obtained when lactate was used as carbon source.

Molasses contained the heavy metals Al, As, Cu, Fe, Mn and Zn in concentrations of 0.54, 0.24, 8.7, 0.35, 11.1 and 19.7 μg/g, respectively. Arsenic tolerance, growth response and sulphate-reducing activity of the SRB were investigated using arsenite and arsenate solutions at final concentrations of 1, 5 and 20 mg/l for each species. The results revealed that very little SRB growth occurred at concentrations of 20 mg/l As(III) or As(V). At lower concentrations (1 mg/l) the SRB grew better with As(V) than with As(III). Arsenic pollution in most groundwater sources is below this level (1 mg/l).

1. Introduction

The biological approach to bioremediation of arsenic-contaminated groundwater involves the use of sulphate-reducing bacteria (SRB) that can reduce sulphate to sulphide while oxidising their carbon source. The sulphide so generated can remove arsenic, precipitating it as arsenic sulphide. The effectiveness of SRB in removing arsenic from contaminated groundwater depends on the choice of an appropriate organic carbon source for use by the bacteria. The primary consideration when selecting a carbon source is its effect on the extent of microbial activity (biotreatment efficiency) and economic feasibility [1]. A major problem associated with the treatment of arsenic-contaminated water using SRB could be the lack of an electron donor. The raw organic materials assessed in previous studies (especially in treatment of acid mine drainage (AMD)) cover a wide range of agricultural and food processing by-products [1–4]. However, only a few studies have involved quantifying the biodegradability of the different organic carbon and cellulosic materials [1,2,4–7].

Among the main sources of carbon available to SRB for biological sulphate reduction are volatile fatty acids and short-chain fatty acids. Long-chain fatty acids and certain aromatic compounds (e.g. benzoate [8]) are occasional substrates [9]. The products from fermentation processes such as, methanol [10] and ethanol [11] are additional sources; as are other simple carbon compounds such as butyrate [12]. However, polymers such as cellulose and hemicellulose are not good sources, as cellulose is not known to be degraded by SRB [4,13]. Gibert et al. [1] assessed the degradability of different carbon substrates for SRB and concluded that the lower the lignin contents of a substrate, the greater its degradability. Similarly, proteins, carbohydrates and lipids or even simple sugars are generally not utilisable by SRB [5]. But, other bacteria can metabolise the sugars making fermentation end-products such as lactate and acetate that can be used by SRB [3]. Investigations by Akagi and Jackson [14], Parshina et al. [15] and Kaksonen et al. [16] have shown the utilisation of sugars by SRB. Researches have also shown that using mixtures of natural substrates rather than a single substrate can increase sulphate reduction [2,7,17,18]. An organic substrate for the growth of SRB especially *Desulfovibrio* and *Desulfotomaculum* [19] can be supplied through mushroom compost.

SRB oxidise organic matter to bicarbonate anaerobically using sulphate as a terminal electron acceptor according to the reaction:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

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Table 1
Composition of molasses (after Paturau [25])

% Molasses		
Usual range	Indicative average	Components
17–25	20	Water
30–40	35	Sucrose
4–9	7	Glucose
5–12	9	Fructose
1–5	3	Other reducing substances
2–5	4	Other carbohydrates
7–15	12	Ash
2–6	4.5	Nitrogenous compounds
2–8	5	Non-nitrogenous acids
0.1–1	0.4	Wax, sterols and phospholipids

where CH_2O represents the organic substrate. The hydrogen sulphide generated may form insoluble complexes with many heavy metals [20–23]. The energy substrates for SRB can thus range from hydrogen to aromatic compounds [24].

However, for economic reasons the carbon source for SRB should preferably be cheap and readily available. Molasses is a by-product of sugar processing and can be employed as a relatively cheap carbon source. The composition of molasses can be influenced by a number of factors and Table 1 gives the indicative values often found in many cane-producing countries [25].

Fermentative bacteria can easily change sucrose into carbon dioxide, hydrogen and short-chain fatty acids. SRB can use these fatty acids as a source of carbon for growth.

The activity of SRB can be affected by the presence of heavy metals. Hence, efficient treatment of metal-contaminated waters by SRB depends on their susceptibility to various heavy metals. Metal microbe interactions have been studied in aerobic bacteria [26,27], anaerobic consortia [28] and mesophilic SRB [29]. Studies by Booth and Mercer [30], Saleh et al. [31] and Capone et al. [32] have shown the toxicity of heavy metals to SRB. Utgikar et al. [33] have quantified the toxic and inhibitory impact of Cu and Zn on mixed cultures of SRB.

Metal ion inhibition to SRB activity may occur with elevated dissolved metal concentrations [34]. Heavy metals exert a negative effect on bacterial communities by deactivating enzymes, denaturing proteins, and competing with essential cations [35,36]. The effect of metal ions may be a reduction in numbers and species diversity of a mixed consortium of SRB, or development of strains capable of tolerating high concentrations of metal ions [20,37,38]. Sani et al. [39] observed a negative effect on *Desulfovibrio desulfuricans* of Pb at concentrations greater than 3 mg/l and a Cu(II) concentration of 0.85 mg/l caused a 50% inhibition in maximum specific growth rate. Poulson et al. [23] reported inhibition of sulphate-reducing activity in *D. desulfuricans* by nickel and zinc in excess of 1.6 mg/l. These differences in inhibitory concentrations of heavy metals to SRB are partly due to the differences in the rate of precipitation and adsorption of the solubilised metals [40]. The presence of sulphide can decrease the toxicity effect due to precipitation of metal sulphides.

Microbes protect themselves from the effects of heavy metals by complexation, extra-cellular precipitation, impermeability, or reduced transport of the metals across the cell membrane. Moreover, microorganisms can synthesise metal binding metallothioneins [41]. Biomethylation, volatilisation, biopolymerisation, bioprecipitation, biosorption and intracellular traps can also be employed by microorganism against the effects of heavy metals.

The objectives of this study were to determine the elemental composition of molasses; assess its capacity to sustain SRB activity and to investigate the effect of arsenic species (As(III) and As(V)) on the growth of a mixed culture of SRB in a molasses containing medium

Table 2
Elemental composition of molasses

Element	Concentration (μg/g)	Limit of detection (μg/g)
Al	0.54 ± 0.03	0.004
As	0.24 ± 0.01	0.005
Cu	8.70 ± 0.45	0.004
Fe	0.35 ± 0.02	0.005
Mg	0.15 ± 0.01	0.001
Mn	11.10 ± 0.63	0.003
Zn	19.70 ± 0.84	0.001

2. Materials and methods

2.1. Nutrient medium and source of sulphate-reducing bacteria

The growth medium used was Postgate medium B [42] 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 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 SRB was enriched from anaerobic sediments from the Msunduzi River (Pietermaritzburg, South Africa).

2.2. Source of molasses and its elemental composition

Molasses was obtained from Voermol Feeds (Pty) Ltd., South Africa. The elemental composition of the molasses was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES). Raw molasses was evaporated on a hot plate for about 1 h until a homogenous mixture was formed. About 4 g of the dried molasses was ashed using 40 ml concentrated HNO_3 in a beaker placed on a water bath until the characteristic brown gas stopped evolving [43]. The solution was diluted to 100 ml using distilled water. A blank was also prepared following the same procedures but without the addition of molasses. Table 2 shows the concentration of the metals detected.

Cd, Pb, Hg and Ni were not detected in the molasses used in our experiments. The absence of toxic heavy metals in significant concentrations in molasses can make it a potentially useful carbon source for culturing SRB and other microorganisms.

2.3. Experimental cultures

Experimental cultures were grown with different concentrations of molasses (1, 2.5 and 5 g/l) as carbon source. The growth studies were performed in duplicate using a 20% (v/v) inoculum of log phase cells that had been sub-cultured three times. All cultures were incubated in the dark at room temperature (25 ± 2 °C) for 1–2 weeks. Growth of the SRB was monitored microscopically by direct cell counts and verified by measuring sulphate reduction levels. As a control, the same medium was used but with lactate as carbon sources as most sulphate reducers can grow well with this compound.

2.4. Arsenic tolerance study

The influence of different concentrations of arsenic species (As(III) and As(V)) on growth of the SRB consortia was studied. Arsenite and arsenate solutions were prepared from $NaAsO_2$

Table 3
Effects of 14 days growth of SRB in media with different molasses (A) and arsenic species (B)

A					
	Initial		Final		
C-source	pH	Redox (mV)	pH	Redox (mV)	$\%SO_4^{2-}$ reduction
3.5 g/l lactate	6.5 ± 0.1	245 ± 16	7.2 ± 0.2	−269 ± 19	45
1 g/l molasses	6.4 ± 0.1	254 ± 14	6.9 ± 0.2	−179 ± 13	28
2.5 g/l molasses	6.4 ± 0.2	248 ± 18	7.0 ± 0.2	−195 ± 13	31
5 g/l molasses	6.3 ± 0.2	235 ± 20	7.1 ± 0.2	−210 ± 18	33

B[a]						
Arsenic concentrations						
As(III) (mg/l)	$\%SO_4^{2-}$ reduction					
Time (days)	1	3	5	7	10	14
1	1.4	5.8	9.6	10.9	18.1	28.7
5	1.3	1.9	6.8	9.8	12.6	19.4
20	−0.25	1.2	2.8	3.5	4.8	7.1
As(V) (mg/l)						
1	1.9	5.9	12.6	13.1	23.8	37.1
5	1.8	2.1	7.6	10.6	14.8	24.8
20	0.12	1.3	4.3	6.2	8.9	12.1
Control	10.8	19.5	26.9	31.7	35.2	49.0

[a] Prepared using Postgate medium B.

and $Na_2HAsO_4{\cdot}7H_2O$, respectively. These arsenic solutions and the growth medium were sterilised separately and appropriate volumes of either arsenate or arsenite were added to the culture medium from stock solutions to give final concentrations of 1, 5 and 20 mg/l. Controls (lactate as carbon source) comprised the same growth media but without arsenic.

The effect of the two arsenic species on the growth of SRB, that utilise molasses as carbon source, was evaluated according to the sulphate activity assay described in Section 2.5. Molasses (1 g/l) was used as carbon source throughout this experiment. Samples were collected daily to measure bacterial growth, pH, redox potential and sulphate concentrations.

2.5. Sulphate-reducing activity

The sulphate activity assay, which involves sulphate-reduction kinetics and measures maximum sulphate reduction level, was performed in 250 ml serum bottles. SRB cultures were transferred to the bottles containing 200 ml modified (molasses substituted) Postgate medium and incubated at 25 ± 2 °C. Samples for analysis were collected each day for 14 days.

2.6. SRB generated precipitate and bacterial cell characterisation

The precipitate formed during the growth of SRB in the presence of 5 mg/l of either As(III) or As(V), and 2.5 mg/l each of the arsenic species in flasks were characterised. Energy dispersive X-ray analysis (EDX) coupled with both environmental scanning electron microscopy (ESEM) (Philips, FEI XL 30) and transmission electron microscopy (TEM, Philips, CM 120, biotwin) were used to study the mineralogy and morphology of the precipitates and cells collected by membrane filtration (0.22 μm). A small drop of the SRB culture was placed carefully on copper grids (200 holes per 25 mm^2) using a micropipette and TEM analysis carried out at 100 kV. The attached EDX spectrometer (DX4 system, EDAX microanalysis) was used to characterise the internal and surface elemental composition of the cells. A spot size of 300 nm and 100 s live count time were used to collect the spectra.

Also the precipitates and bacterial cells were collected by centrifugation (10 000 rpm) for elemental analysis using ICP-OES following acid treatment (1 ml of conc. HCl was added to the precipitates/cells and diluted to 10 ml using deionised water).

2.7. Analytical methods

Redox potential was measured using a platinum electrode and Ag/AgCl reference electrode. Sulphate was measured photometrically using a SQ 200 photometer and the Spectroquant Sulphate test kit (Merck). The concentration of organic carbon (TOC) of molasses was analysed using a TOC-VCPN analyzer (Shimadzu).

2.8. SRB enumeration

SRB cell counts were performed by direct counting using a Neubauer counting chamber and phase contrast microscopy (Zeiss).

3. Results

The results given in the following figures were summarized in Table 3.

3.1. Growth of SRB on molasses

The suitability of molasses as a carbon source for growth of SRB was investigated at different concentrations. At 1 g/l, cell number increased constantly from approximately $(8.0 \pm 0.2) \times 10^6$ cells/ml to about $(3.7 \pm 0.1) \times 10^7$ cells/ml after 4 days. When the concentration of molasses was increased to 2.5 and 5 g/l, the number of cells/ml at the end of 4 days were $(3.7 \pm 0.1) \times 10^7$ and $(3.8 \pm 0.1) \times 10^7$, respectively. This showed that molasses is a potential carbon source for the growth of SRB. However, with lactate (3.5 g/l) as carbon source, growth was better than that on any of the three molasses concentrations (Fig. 1).

The changes in pH that occurred during the growth of SRB on the different concentrations of molasses are depicted in Fig. 2. At a concentration of 1 g/l the pH increased slightly from (6.4 ± 0.1) to

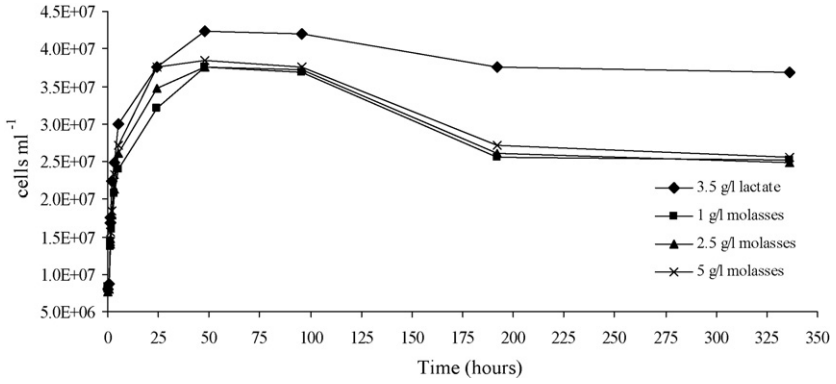

Fig. 1. Cell number as a function of time following growth of SRB in 1, 2.5 and 5 g/l molasses and with 3.5 g/l lactate.

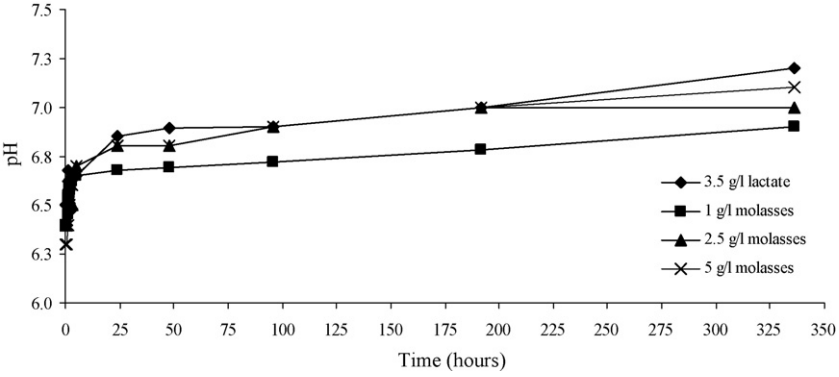

Fig. 2. Changes in pH as a function of time during growth of SRB on 1, 2.5 and 5 g/l molasses and with 3.5 g/l lactate.

(6.9 ± 0.2) over a 14-day period. For the same period, with molasses at 2.5 and 5 g/l the pH increased to (7.0 ± 0.2) and (7.1 ± 0.2), respectively.

In parallel with the pH changes, the redox potential of the medium declined from (254 ± 14) to (−179 ± 13) mV over 14 days for 1 g/l molasses; from (248 ± 18) to (−195 ± 13) mV for 2.5 g/l molasses; and from (235 ± 20) to (−210 ± 18) mV for 5 g/l molasses (Fig. 3). However, the corresponding change when lactate was used as carbon source was from (245 ± 16) to (−269 ± 19) mV (Fig. 3). There was a slight difference in redox potential for the different molasses concentrations at the start of the experiments.

The percentage of sulphate reduced during the 14-day batch culture experiments on SRB growth with different concentrations of molasses as carbon source is shown in Fig. 4. The graphs show that the rate of sulphate reduction was higher in the lactate grown cultures than in molasses grown cultures. The percentage reduction was fairly similar in all the molasses containing media. The detection limit, calculated as the concentration corresponding to three times the standard deviation of the blank, of sulphate analysis was 0.2 mg/l and %RSD in the range of 5–9%.

The slight increase in pH and decrease in redox potential corresponded with the level of sulphate reduction during the same period of time for each of the molasses concentrations.

3.2. Effect of arsenic species on the growth of SRB

Arsenite and arsenate had a negative effect on the SRB, the growth rate being slower as the concentrations of the arsenic species increased from 1 to 20 mg/l. The duration of the lag phase also increased with increasing concentrations of each arsenic

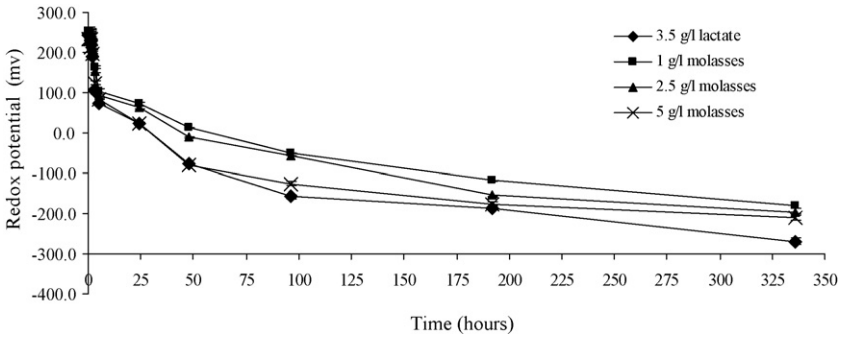

Fig. 3. Changes in redox potential as a function of time during the growth of SRB on 1, 2.5 and 5 g/l molasses and 3.5 g/l lactate.

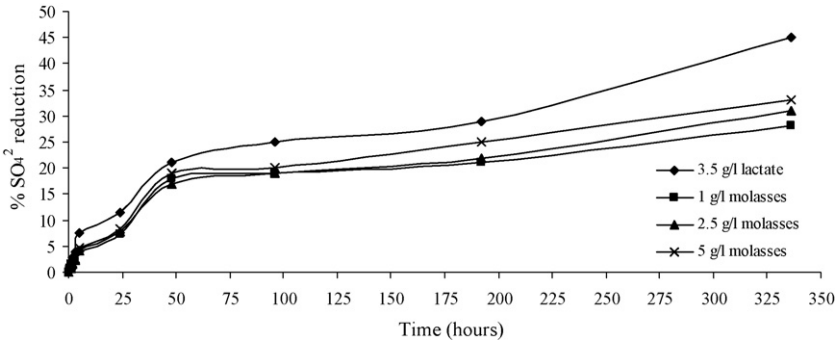

Fig. 4. Percentage SO_4^{2-} reduction as a function of time during growth of SRB on 1, 2.5 and 5 g/l molasses and 3.5 g/l lactate.

species, indicating that at high concentrations of arsenite and arsenate the growth of SRB was inhibited to some extent. Fig. 5 shows the growth of SRB in the presence of increasing concentrations of arsenic species at the end of 14-day of batch culture.

Figs. 6 and 7 show the percentage sulphate reduction occurring in the presence of different concentrations of As(III) and As(V), respectively. For the arsenic species at 1 mg/l the reduction of sulphate reached 5% on day 3 and thereafter increased at a roughly uniform rate. At 5 mg/l of either arsenic species the 5% reduction level was reached only on day 5. At 20 mg/l As(III) only 7.1% of the available sulphate was reduced by the end of the 14-day experiment, whereas for As(V) 12.1% was reduced after 14 days. This indicated that at high concentrations of either arsenic species the ability of SRB to reduce sulphate to sulphide was greatly decreased.

Regardless of the initial concentration of arsenic, sulphate reduction was better in the presence of As(V) than in the presence of As(III).

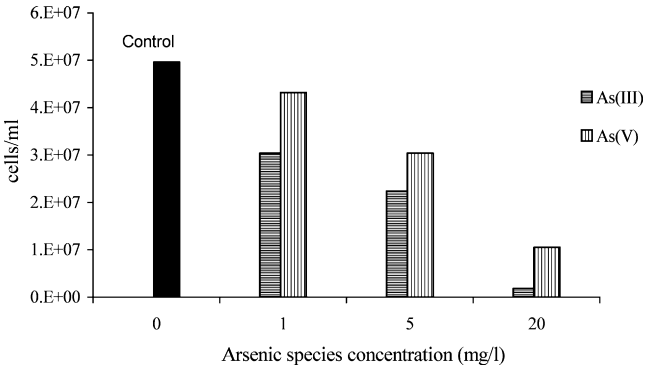

Fig. 5. Cell number as a function of arsenic species (As(III) and As(V)) concentrations (14 days).

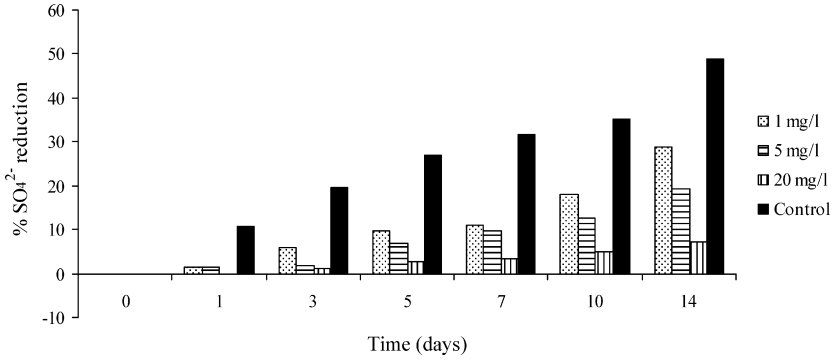

Fig. 6. Percentage SO_4^{2-} reduction as a function of time during the growth of SRB in the presence of different As(III) concentrations.

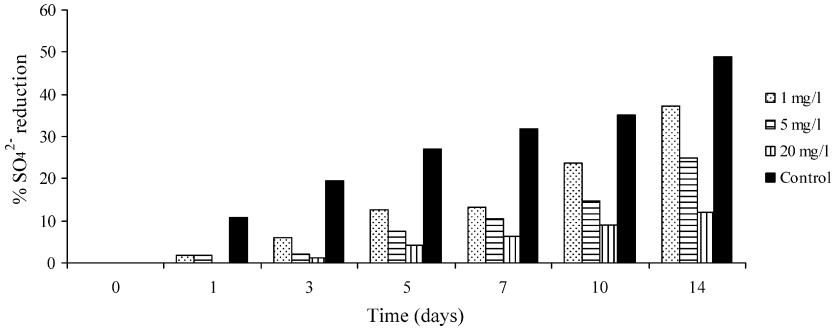

Fig. 7. Percentage SO_4^{2-} reduction as a function of time during the growth of SRB in the presence of different As(V) concentrations.

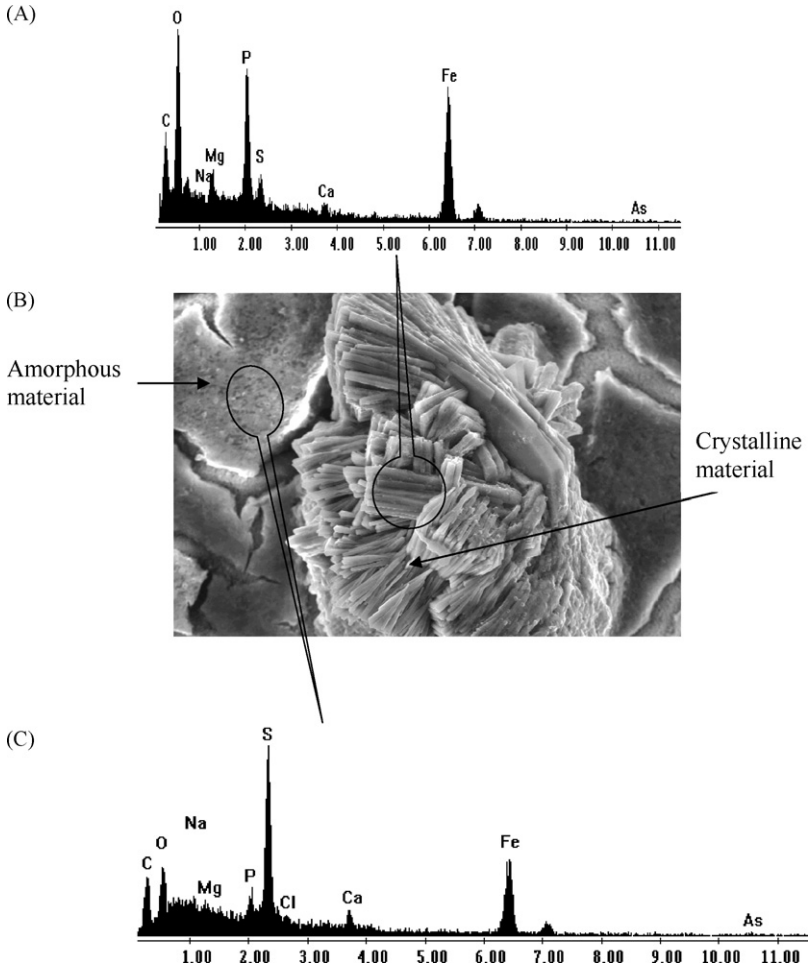

Fig. 8. ESEM micrograph of the precipitate generated in the presence of 5 mg/l As(III). Note the presence of amorphous and crystalline material and the difference in elemental spectra of the two components.

Following adaptation to grow in the presence of arsenic species, higher sulphate reduction activities by SRB were recorded in the presence of both As(III) and As(V) as compared to the initial unadapted cultures (data not shown).

It is evident from these experiments that at lower arsenic concentrations, culture growth and sulphate reduction were not inhibited. Fig. 1 reveals that the maximum population size occurred between 24 and 96 h irrespective of the medium composition. Depletion of nutrients (including carbon source) and/or the accumulation of toxic by-products could be the reason for the significant decrease in the number of bacteria after 96 h incubation.

Fig. 8 shows the EDX results of a sample of the precipitate obtained in the ESEM in the presence of 5 mg/l As(III). It contains amorphous and crystalline structures with relatively high percentages of phosphorous (Fig. 8B) and sulphur (Fig. 8C), respectively.

The rod-shaped SRB (Fig. 9A) were exposed to TEM–EDX analysis and the result is shown in Fig. 9B.

Fig. 9B indicates that the metals (iron and arsenic) were not actively adsorbed on the cell surface or absorbed into the cytoplasm. This could be due to the low concentration of the elements present in the samples and the moderately high detection limit of the TEM–EDX technique. Hence, samples of the precipitates formed and of the bacterial cells collected by centrifugation were acid digested and then analysed by ICP-OES. The results are shown in Table 4 (during the growth of SRB in the presence of 5 mg/l As(III)).

4. Discussion

The increase in pH observed when either molasses or lactate were used as carbon source for growth of SRB reflects the oxidation of the organic carbon (electron donor) source into bicarbonate thereby increasing the alkalinity. Concomittantly, sulphate (the final electron acceptor) is reduced to hydrogen sulphide which combines with the metals present to form insoluble metal sulphides [44]. The increase in pH and accompanying decrease of redox potential during bacterial growth indicate the establishment of anaerobic reducing conditions which are conducive to the growth of SRB. Sulphate reduction by SRB occurs when the redox potential is below −100 mV [42].

A satisfactory level of sulphate reduction by SRB using molasses as electron donor has been reported previously [45]. Our study showed that molasses at concentrations of 1, 2.5 and 5 g/l supported the growth of our SRB culture. The use of high concentrations of molasses can introduce additional, non-degradable materials (including products of caramelisation) that can have a deleterious effect on the growth of the bacteria [46]. Moreover, the presence of large amounts of volatile fatty acids when high concentrations of molasses are used can have a negative impact on the growth of SRB [47]. At concentration of 2.5 and 5 g/l molasses imparted a brownish colour to the medium and this would have an aesthetically unacceptable effect on the visual quality of any water treated. Therefore, use of the lowest concentration of molasses that can

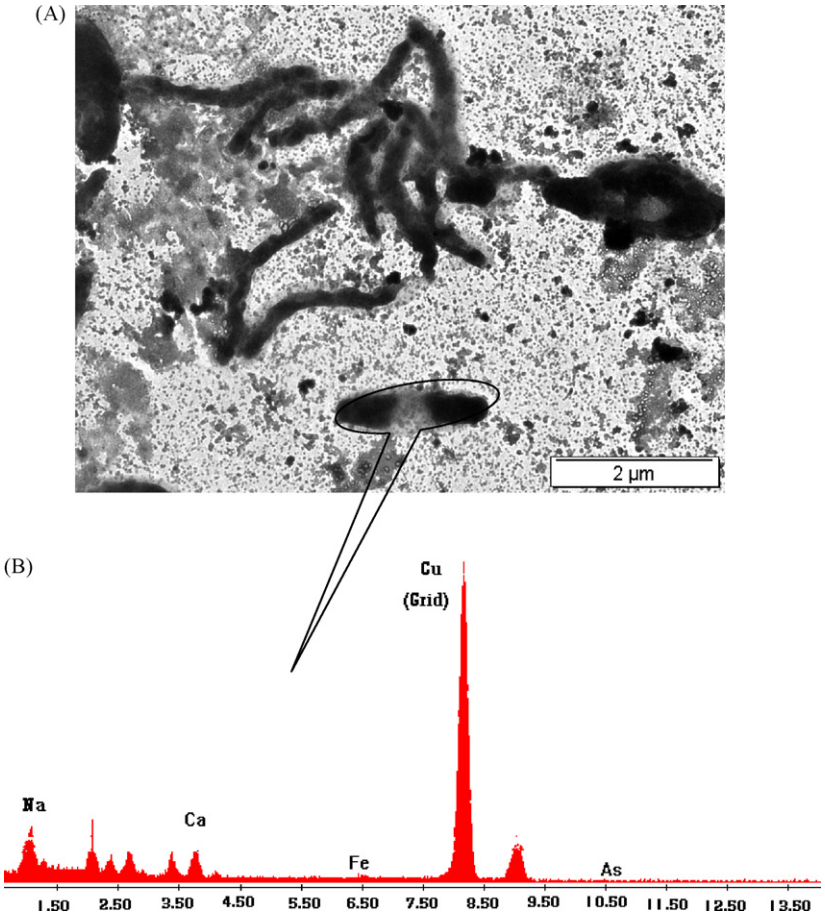

Fig. 9. TEM–EDX spectrum of a single SRB cell.

support the growth of SRB is recommended for the treatment of arsenic-contaminated waters.

In all our batch experiments, lactate was superior to molasses as a carbon source for the SRB. However, due to the high cost of lactate the running expenses of a large-scale operation would be prohibitive.

Molasses may also have trace amounts of toxic heavy metals that can inhibit the growth of SRB. Heavy metals, even at concentrations as low as 5–10 mg/l can adversely influence microorganisms by affecting their growth, morphology or biochemical activities. The impact of the metals on microbial activity could be due to: (1) a decrease in viable cell numbers resulting from death of the less tolerant species due to toxicity; and (2) the metals could decrease the metabolic activity of the survivors in the population [35]. The absence of the potentially toxic metals Cd, Pb and Ni and presence of only very small amounts of Al, As, Cu, Fe, Mg, Mn and Zn in the molasses used in our experiments could be beneficial since, in addition to serving as carbon source it would also supply many of the essential trace elements required by the bacteria for balanced growth.

There are no reports in the literature concerning the maximum concentrations of arsenic species that can be tolerated by growing cultures of SRB. Arsenic at concentrations of 1 and 5 mg/l for both species, (As(III) and As(V)), did not affect the reduction of sulphate by our SRB culture. However, when the concentration was increased to 20 mg/l the level of sulphate reduction was greatly reduced. It is possible that the reduction of the toxic effects of arsenic species on SRB can be due to precipitation and/or complexation of the arsenicals with chemicals present in the growth media. Utgikar et al. [48] reported that the effect of heavy metals on the growth of sulphate-reducing bacteria can be stimulatory at lower concentrations and toxic/inhibitory at higher concentrations. Our experimental culture comprised of a mixture of SRB so the quantification of its heavy metal tolerance could be difficult. Additional complications might be the effects of metal hydroxides and sulphide precipitation, biosorption, and complexation with the constituents of the growth media [49]. Hence, it is important to characterise the dissolved heavy metals in the water to be treated since this could influence the design and operation of any bioremediation processes involving SRB.

Table 4
ICP-OES analysis of acid treated SRB generated precipitates and bacterial cells

	Precipitate (mg/l)	Cells (mg/l)	Dissolved arsenic (mg/l)	Total arsenic accounted for (mg/l)[a]	%Error[b]
As(III)	2.12 ± 0.15	0.06 ± 0.01	2.43 ± 0.21	4.61 ± 0.39	7.8

[a] *Note*: sum of precipitate, bacterial cells and arsenic in the liquid phase.
[b] *Note*: %error calculated using ((initial arsenic (5 mg/l) − total arsenic accounted)/initial) × 100.

The sulphide produced by the metabolic activity of SRB may react with the dissolved metals to form a sulphidic precipitate that can lead to a decrease in the availability of the dissolved elements. Watson et al. [50] investigated the adsorbent properties of iron sulphide produced by SRB. They found that the SRB produced an adsorbent that had a very high specific uptake of different metal ions from solution compared to other adsorbents, such as activated carbon. In our study we found that comparatively higher amounts of arsenic and iron were associated with the precipitates (that might be produced by either chemical or biological activities, or a combination thereof) rather than solely by the SRB cells. *Postgate* medium B initially contained some precipitate in the absence of bacteria due to the pH adjustment. However, in the presence of SRB the amounts of sulphur at the two forms of precipitate were considerably greater than when no bacteria were present, suggesting that the SRB were contributors of these substances. The nature of surface charge of the SRB cells and pH plays a role in biosorption of arsenic species. Hence, the produced precipitates could contribute to the removal of arsenic from contaminated waters.

5. Conclusions

Molasses at concentrations of 1, 2.5 and 5 g/l supports the growth of SRB. With increased molasses concentration the growth also increased to some extent. This is accompanied by a slight increase in pH and decrease in redox potential. However, since at high molasses concentration the water turns brownish, lower levels (i.e. 1 g/l) are recommended for the SRB bioremediation of arsenic-contaminated waters.

The heavy metals occurring in molasses are not present in high enough concentrations to inhibit the growth of SRB.

At 20 mg/l both arsenic species, but particularly As(III), were shown to reduce the growth of SRB. Likewise sulphate reduction was diminished to less than 8% when this concentration of either As(III) or As(V) was present. At much lower concentration of both arsenic species, the growth of SRB is much better but a prolonged lag phase (adaptation to growth conditions) is evident.

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