

## Arsenic remediation using anaerobically digested sewage sludge

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### ABSTRACT

Anaerobically digested sewage sludge, which is a mixed culture, was used as the inoculum for the volatilization of inorganic arsenic. The sludge was initially activated by feeding with a nutrient solution containing molasses. Activity of the culture was monitored by measuring the gas production from the bioreactor. Inorganic arsenic at different concentrations was spiked into the bioreactor and its effect on gas production was monitored. It was found that arsenic was distributed in solid, liquid and gaseous forms. The results indicated that arsenic concentration up to 34ppm was not toxic to the microorganisms. The present study confirms that microorganisms in anaerobically digested sewage sludge volatilizes inorganic arsenic. © 2005 SDU. All rights reserved.

Keywords: Arsenic; Anaerobic digestion; Environmental; Sewage sludge

### 1. INTRODUCTION

Arsenic in underground rock and soil works its way into ground water and enters food chain through either drinking water or edible plants or cereals that have absorbed the mineral. Arsenic contamination of drinking water has been reported from many parts of the world (Johnston and Heijnen, 2001). This arsenic is present in its highly toxic forms arsenate [As (V)] and arsenite [As (III)]. In marine and estuarine environments arsenic is a common contaminant (Fourqurean and Cai, 2001). It is carried to the marine food chain through primary producers. Sanders *et al.* (1994) reviewed the potential of arsenic contamination in severely altering the marine food webs. Arsenate which is the dominant form of arsenic in marine and estuarine surface waters is taken up by the phosphate systems of plants due to the chemical similarity of arsenate to phosphate (Dixon, 1997). Most of the organic volatile derivatives of metals (Bi, Hg and Sn) and metalloids (As, Sb, Se and Te) exhibit higher toxicity than their inorganic forms, since the organic derivatives are lipophilic and are thus more biologically active (Thayer, 1984, 1995). However, Cullen and Reimer (1989) reported that in the case of arsenic and selenium, the inorganic nonmethylated forms are more toxic than the methylated forms.

Coagulation and flocculation with iron salts and alum, lime softening, ion exchange resins, activated alumina, membrane methods and *in situ* immobilization are the common methods used for arsenic removal. However, flocculation, lime softening and ion exchange resins generate arsenic rich sludges, which by themselves pose major problem in disposing these highly toxic arsenic rich materials. The use of activated alumina and membrane methods have been demonstrated as expensive for arsenic removal from drinking water. *In situ* immobilization is not yet well documented and it has the disadvantage of the possibility of aquifer clogging. Bacteria can catalytically convert arsenic to easily disposable gaseous forms. However, relatively little is known about the potential for biological removal of arsenic from water (Johnston and Heijnen, 2001).

Microorganisms can render inorganic contaminants present at high concentration in the environment non toxic presumably as a defence mechanism and these processes could be used to remediate inorganic contaminants. The production of volatile arsenic compounds by biological action has been known for long, and it was concluded that the garlic odour like gas produced from arsenic containing potato mash culture was diethylarsine. Both bacteria and fungi have been reported to methylate arsenic. The ability to reduce and methylate arsenic varies greatly among species of fungi and bacteria (Cox, 1975; Vidal and Vidal, 1980; Shariatpanahi *et al.*, 1981). The ability of *S. brevicaulis* to methylate arsenic, and organic and inorganic forms of arsenic acid was reported earlier (Challenger, 1945). McBride and Wolfe (1971)

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discovered the production of dimethylarsine from arsenite using *Methanobacterium*. Three fungal species; *C. humicola*, *G. roseum* and *Penicillium* sp. were shown to be capable of converting monomethylarsonic acid and dimethylarsinic acid to trimethylarsine (Cox and Alexander, 1973). Huysmans and Frankenberger (1991) reported *Penicillium* sp. as capable of producing trimethylarsonic acid and dimethylarsinic acid. Tamaki and Frankenberger (1992) established that fungi are capable of transforming inorganic and organic arsenic compounds into volatile methyl arsines. Continuous cultures of *Methanobacterium thermoautotrophicum* fed with arsenate reduced it mainly to arsine and small amounts of dimethylarsine (Bachofen *et al.*, 1995). Pure cultures of microorganisms, which are representatives of anaerobic sewage sludge microflora, namely methanogenic *Archaea* and sulphate reducing bacteria produced arsine when spiked with arsenic compounds (Michalke *et al.*, 2000).

Biomethylation was reported to detoxify toxic arsenic forms by forming monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and also trimethylarsine oxide (TMAO) (Panstar-Kallio and Korpela, 2000). Cullen and Reimer (1989) reported that arsenate and arsenite could be volatilized to arsine ( $\text{AsH}_3$ ), monomethylarsine ( $\text{CH}_3\text{AsH}_2$ ), or dimethylarsine [ $(\text{CH}_3)_2\text{AsH}$ ] and TMAO to trimethylarsine [ $(\text{CH}_3)_3\text{As}$ ]. Trimethylarsine was reported to be dominant species formed by biomethylation (Cullen and Reimer, 1989).

The microbial population, redox potential, other compounds and elements, organic matter content and pH affect the formation and stability of arsenic species (Gao and Burau, 1997). But the major difference between bacteria and fungi is that the reduction of methylarsenic to arsines is a more common response by bacteria (Cullen and Reimer, 1989).

Although there are several published reports in the literature on the volatilization of arsenic using microorganisms, there are no reports on the volatilization of arsenic using a mixed cultures of anaerobically digested sewage sludge. Therefore, the work reported in this paper focuses on the efficacy of using these microorganisms for volatilization of inorganic arsenic as well as establishing the tolerance level of arsenic for the microbial consortia.

## 2. EXPERIMENTAL

### 2.1. Inoculum

Anaerobically digested sewage sludge, collected from Water Corporation's Woodman Point Sewage Treatment Plant located in Kwinana, Perth WA, was used as the inoculum in all experiments.

### 2.2. Feed

The composition of the feed used was; molasses (40g/l),  $\text{KH}_2\text{PO}_4$  (0.96g/l),  $\text{NH}_4\text{Cl}$  (1.64g/l),  $\text{NaHCO}_3$  (10g/l), yeast extract (6g/l) and trace metal solution (TMS) (6ml/l). Composition of TMS solution was;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (5g/l),  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (1g/l),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (1g/l),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (1g/l),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (0.3g/l),  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  (0.2g/l),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.1g/l),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1g/l) and  $\text{H}_2\text{BO}_3$  (0.1g/l).

### 2.3. Arsenic stock solution

One litre of 1000ppm stock solution of arsenic was prepared by dissolving 0.320g of  $\text{As}_2\text{O}_3$  in NaOH (2g) solution and neutralising the resulting solution by using 1M HCl to pH 6.5 to 7.  $\text{NaHCO}_3$  was added to the above solution to adjust the pH between 7 and 8. This stock solution was diluted as required and used for spiking arsenic in the digester.

### 2.4. Apparatus

A 4.5L cylindrical shaped airtight plexiglass digester was designed and fabricated by the Murdoch University workshop. This was equipped with an internal stirrer. The vessel had two ports at the top and three ports on the side at different heights. One port at the top was used to introduce the feed and the other port for venting the gas. Sample collection and the removal of digester liquid were carried out using the outlets on the sides of the vessel. The digester was maintained at constant temperature ( $39 \pm 1^\circ\text{C}$ ) by keeping it in a water bath equipped with a thermostat control and stirring facility. A schematic diagram of the apparatus is shown in Figure 1.

### 2.5. Gas volume measurement

A positive displacement gas meter was used to measure gas production rate from the digester. The primary component of this device was a U-tube containing silicone oil (Dow Corning Pty. Ltd.), a three way solenoid valve, float switch and a timer. The gas produced accumulated on one side of the U-tube and

displaced the liquid so that the liquid level on the other side increased. When the liquid reached a certain point, the float switch was activated. This triggered three events simultaneously: a signal was sent to the counter to record the number of clicks and display it; accumulated gas was exhausted to atmosphere through the solenoid valve which reset the liquid level, and the timer was activated to allow gas to escape and to allow liquid to reach a steady level. The timer was set manually at 3 seconds. At the completion of this time, the solenoid valve switched to its original position. During the vent cycle, the three way solenoid valve isolated the reactor from the meter. Gas production rate was estimated by dividing the volume of gas required to cause one click by the time interval between two clicks. Calibration was checked before and after each experimental run. All the tubes used in the experiment were Master flex tygon tubes (Cole Parmer).

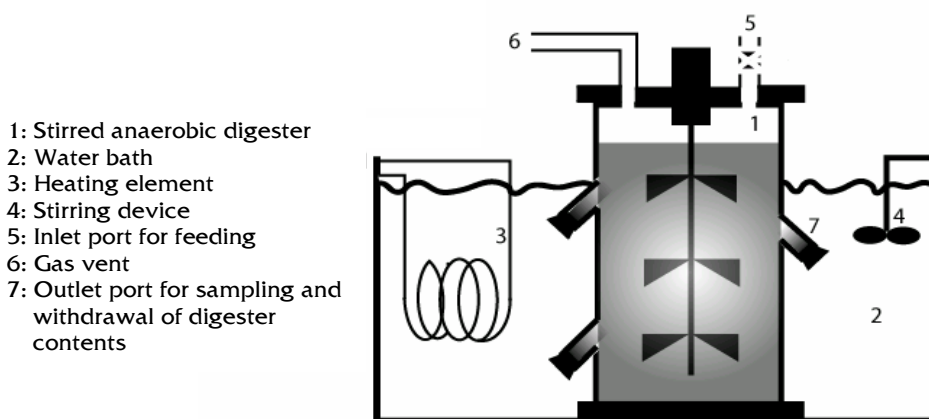


Figure 1. Schematic diagram of the apparatus for volatilization of arsenic

## 2.6. Arsenic analysis

Arsenic was analysed using ICP at Marine and Fresh Water Research Laboratory, Murdoch University. Soluble arsenic was analysed after filtering (Whatman No. 41) samples from the digester. Total arsenic in the digestate was analysed as follows: 0.5ml of concentrated  $\text{HNO}_3$  was added to 10ml of sample and digested in a fume hood at  $100^\circ\text{C}$  for 2 hours. If any solid remained, more  $\text{HNO}_3$  was added and digested again. The tubes were then cooled and 1.25ml of 50%  $\text{HCl}$  added and digested again for about 15 minutes at  $100^\circ\text{C}$ . Sample tubes were cooled and volume made to 10ml using distilled water and kept overnight in the fridge, centrifuged and the upper layer was analysed for arsenic.

## 2.7. Operation of the digester

Anaerobically digested sewage sludge was added into the digester, continuously stirred and fed once daily with the feed solution. The digester was started up with 3.5L of biosolids in the reactor, addition of molasses feed and samples were taken out of the digester at regular intervals. Samples were collected from the reactor using syringe and the activity of the culture was monitored by measuring total gas production and pH. When the reactor was stabilised as indicated by a constant gas production rate and pH, arsenic in the form of arsenite was spiked into the reactor at pre-determined intervals. The digester was operated for about three and half months. Total and soluble arsenic were analysed as described above.

## 2.8. Analysis of gaseous arsenic

The gas from the plexiglass digester was bubbled through 40%  $\text{HNO}_3$  in a Dressel bottle (Figure 2). The resulting  $\text{HNO}_3$  solution was analysed for arsenic as described above.

## 3. RESULTS AND DISCUSSION

To understand the effect of arsenic on microorganisms in sewage sludge, increasing amounts of arsenic were spiked into the bioreactor at concentrations of 2 to 2.5ppm at regular intervals for a period of two months. The effect of spiked arsenic concentration on gas production from the mixed culture is presented in

Figure 3. The results clearly show that gas production was not affected up to approximately 34ppm arsenic, out of which 23ppm was in the solid phase and 11ppm in the liquid phase. This suggests that the microorganisms in the sewage sludge were resistant to this high dose of arsenic solution. However, the gas production dropped rapidly at concentrations higher than 34ppm. At 40ppm arsenic, the gas production was almost zero. Thus at this concentration of arsenic the microorganisms were totally inactive. The arsenic concentration in the solid and liquid phases was monitored after each spiking of arsenic in the bioreactor. The distribution of arsenic in solid and liquid phases are as presented in Table 1.

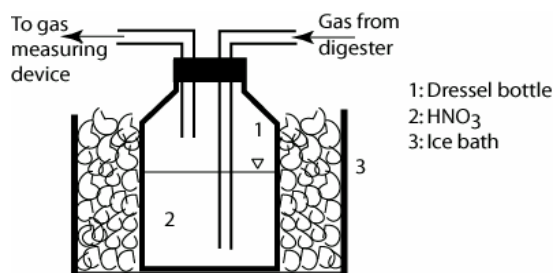


Figure 2. Apparatus for dissolution of volatile arsenic species

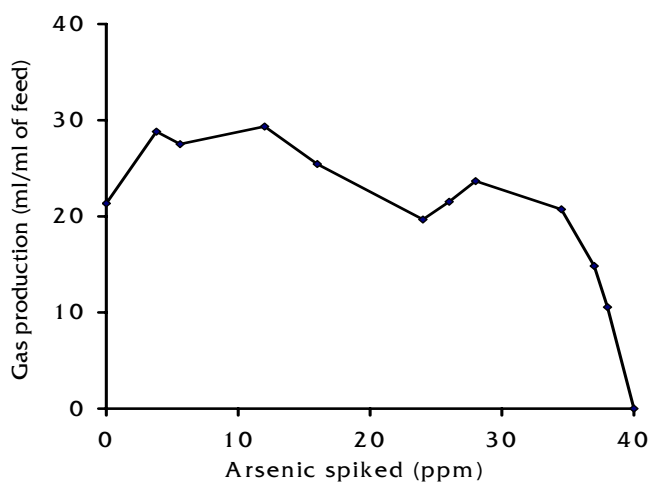


Figure 3. Arsenic spiking and gas production

Table 1  
 Distribution of arsenic in the reactor as a function of time

Time (days)	Total arsenic added (ppm)	Total arsenic in solid + liquid phase (ppm)	Arsenic in solid phase (ppm)	Arsenic in liquid phase (ppm)
1	1.98	1.90	0.52	1.38
8	3.84	3.60	1.30	2.30
15	6.09	5.60	2.30	3.30
17	8.37	8.00	4.00	4.00
21	10.66	10.00	5.20	4.80
24	15.26	13.00	6.60	6.40
27	17.68	12.00	8.20	3.80
28	17.41	17.00	9.30	7.70
35	19.03	18.00	11.00	7.00
36	21.10	18.00	11.00	7.00
38	23.29	21.00	12.00	9.00
41	25.47	24.00	14.00	10.00
43	27.44	24.00	16.00	8.00
45	29.57	26.00	17.00	9.00
48	31.65	28.00	19.00	9.00
50	33.50	28.00	19.00	9.00
52	35.58	34.00	23.00	11.00
55	36.40	35.00	24.00	11.00
57	37.05	35.00	24.00	11.00

The data clearly shows that as the spiking concentration of arsenic is increased, increasing amounts of arsenic end up into the solid phase. The results presented in Table 1 indicate that when a total of 37ppm arsenic were spiked, only 35ppm were present in the combined solid and liquid phase in the bioreactor. The remaining 2ppm is assumed to be the volatalized form of arsenic. The amount of arsenic in the solid phase and liquid phase was determined as 24ppm and 11ppm respectively. With increasing time of exposure in the digester the fraction of total arsenic that was present in the solid phase increased from 28% on day 1 to 68% at day 57. Exposure to anaerobic (or reduced) environment caused soluble arsenic to be present in an insoluble form.

Figure 4 shows the distribution of arsenic in solid, liquid and gaseous forms during the digestion process after one week and 4 weeks of spiking arsenic. As can be seen from the figure that after one week when 4.27mg arsenic had been spiked, 3.02mg arsenic was found in insoluble form, 1.23mg in soluble form and thus by difference 0.02mg of arsenic was assumed to be in gaseous form at the one week interval of the experiment. After four weeks when 17.07mg arsenic had been spiked the distribution was 6.91mg arsenic in insoluble form, 8.4mg in soluble and 1.76mg in gaseous form. These results confirm that the arsenic is volatalized and the extent of volatalization depends on the time of exposure of arsenic to the microorganisms.

In order to reconfirm the arsenic volatalization further, the gas, which had vented from the digester was analysed for its arsenic content. For this purpose the gas was bubbled through dilute HNO<sub>3</sub> solution, which trapped any volatalized arsenic species. Arsenic was indeed detected in the HNO<sub>3</sub> solution at ppm levels (0.5ppm) confirming that arsenic was volatalized by the microorganisms in the sewage sludge.

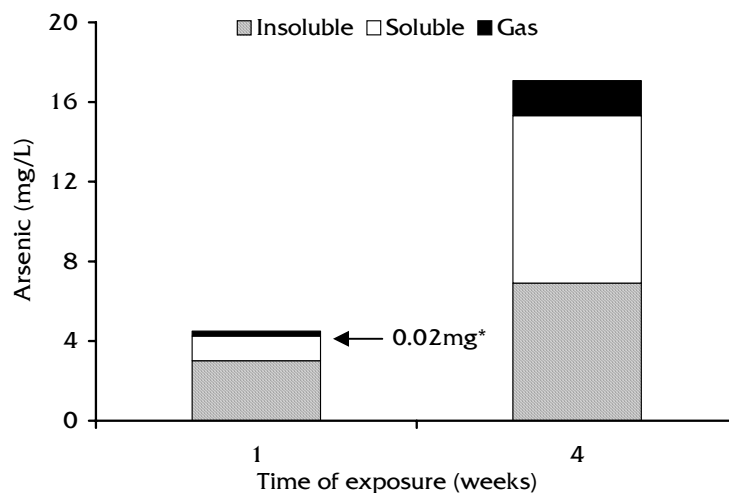


Figure 4. Distribution of insoluble, soluble and gaseous forms of arsenic as a function of time (\* not to scale)

Bachofen *et al.* (1995) also reported that arsine with small amounts of dimethylarsine was detected in the HNO<sub>3</sub> solution while using pure cultures for the volatalization of arsenic and continuous cultures fed with arsenate reduced arsenic mainly to arsine and small amounts of dimethyl arsine using *Methanobacterium thermoautotrophicum*. Pure cultures of organisms which are representatives of anaerobic sewage sludge bacteria, methanogenic *Archaea* and sulphate reducing bacteria produced arsine when culture was spiked with arsenic compounds and most of the reports so far used batch cultures, incubated in dark at 37°C for one week prior to the analysis of volatilised arsenic (Michalke *et al.*, 2000). Our studies show that mixed culture sewage sludge bacteria are tolerant to arsenic at concentration of 34ppm and volatalizes inorganic arsenic into gaseous forms. Identification of the volatalized products will be reported in a future publication.

#### 4. CONCLUSIONS

Microorganisms from an anaerobic sewage sludge digester can volatalize inorganic arsenic. These organisms can tolerate high concentration (34ppm) of arsenic. Arsenic spiked into the anaerobic digester distributes itself into solid, liquid and gaseous forms. The relative distribution of arsenic in the three phases depends on the amount of the spiked arsenic and duration of exposure. As the duration of exposure increases arsenic found in the solid and gaseous phases increases. Arsenic volatilised can be trapped in cold concentrated nitric acid, providing a means for estimating extent of arsenic volatilization.

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