

## Anaerobic microbial mobilization and biotransformation of arsenate adsorbed onto activated alumina

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### Abstract

Due to the enactment of a stricter drinking water standard for arsenic in the United States, larger quantities of arsenic will be treated resulting in larger volumes of treatment residuals. The current United States Environmental Protection Agency recommendation is to dispose spent adsorbent residuals from arsenic treatment into non-hazardous municipal solid waste (MSW) landfills. The potential of microorganisms to alter the speciation affecting the mobility of arsenic in the disposal environment is therefore a concern. The purpose of this paper was to evaluate the potential of an anaerobic microbial consortium to biologically mobilize arsenate (As(V)) adsorbed onto activated alumina (AA), a common adsorbent used for treating arsenic in drinking water. Three anaerobic columns (0.271) packed with 100 g dry weight of AA containing 0.657 mg adsorbed As(V) (expressed as arsenic) per gram dry weight were continuously flushed with synthetic landfill leachate for 257 days. The fully biologically active column was inoculated with methanogenic anaerobic sludge (10 g volatile suspended solids l<sup>-1</sup> column) and was operated with a mixture of volatile fatty acids (VFA) in the feed (2.5 g chemical oxygen demand l<sup>-1</sup> feed). At the end of the experiment, 37% of the arsenic was removed from the column, of which 48% was accounted for by arsenical species identified in the column effluent. The most important form of arsenic eluted was arsenite (As(III)), accounting for nearly all of the identified arsenic in periods of high mobilization. Additionally, two methylated metabolites, methylarsonic acid and dimethylarsinic acid were observed. Mobilization of arsenic is attributed to the biological reduction of As(V) to As(III) since literature data indicates that As(III) is more weakly adsorbed to AA compared to As(V). Batch and continuous assays confirmed that VFA, present in landfill leachates, served as an electron donating substrate supporting enhanced rates of As(V) reduction to As(III). Two control columns, lacking inoculum and/or VFA in the feed displayed low mobilization of arsenic compared to the fully biologically active column. Therefore, leachates generated in MSW landfills could potentially result in the biologically catalyzed mobilization of arsenic from As(V)-laden drinking water residuals.

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## 1. Introduction

Due to increasing evidence for cancer risks associated with chronic exposure to low levels of arsenic (NRC, 2001), the United States Environmental Protection Agency (US-EPA) has recently announced that the drinking water standard for arsenic (As) will be reduced from 50 to 10 parts per billion (ppb) (US-EPA 2001). In order to meet the new norm, approximately 4000 water utilities in the US will be required to apply arsenic removal technology. About 90% of the affected utilities are small scale operations serving 10,000 or less consumers. The only economically viable treatment alternative available to small-scale drinking water suppliers will be adsorption of arsenate onto activated alumina or ferrihydrite (amorphous ferric hydroxide) after pre-oxidation to convert any arsenite in groundwater to arsenate (US-EPA, 2001). The adsorbents will eventually become saturated and will need to be discarded or regenerated. For small-scale utilities, the current proposal is to dispose these spent adsorbents into non-hazardous municipal solid waste landfills because they pass the Toxic Characteristic Leaching Procedure (TCLP). The TCLP was used as the sole determinant of the hazard potential of As-laden treatment residuals in the EPA final rule (US-EPA, 2001). The TCLP was originally developed for testing solid wastes with cationic heavy metals. The extraction conditions (0.1 M acetic acid, pH 4.9 for 18 h) were designed to be more severe for these cations than those in the disposal environment. However, arsenate occurs as an oxyanion, which is more readily mobilized as the pH increases (Ghosh et al., 2002). The mobility of arsenic from disposed adsorbents could also potentially increase under reducing conditions, since it is well established that arsenite (As(III)) is less strongly adsorbed by activated alumina compared to arsenate (As(V)) (Lin et al., 2011; Ghosh and Yuan, 1987). The mildly alkaline and anaerobic conditions occurring in a mature landfill (Christensen et al., 2001; Kjeldsen et al., 2002) are therefore potentially more favorable for arsenic mobilization compared to the TCLP (Meng et al., 2001).

Anaerobic microorganisms can potentially play an important role in mobilizing arsenic in spent adsorbents due to their ability to reduce As(V) to As(III). Microorganisms from 16 species of diverse taxonomy have been isolated which are able to utilize As(V) as an electron acceptor for anoxic respiration, yielding energy to support their growth (Oremland and Stolz, 2003). These microorganisms, referred to collectively as dissimilatory arsenate reducing prokaryotes (DARPs), have been isolated from freshwater sediments, estuaries, mine impacted environments, hot springs, soda lakes and gastrointestinal tracts of animals (Oremland and Stolz, 2003). DARPs have been observed in wetland,

lake and pond sediments at approximately  $10^4$  cells  $g^{-1}$  sediment (Harrington et al., 1998; Kuai et al., 2001; Oremland and Stolz, 2003). Additionally, many microorganisms, including bacteria, archaea and fungi, display resistance to arsenate toxicity. A common mechanism of resistance involves reduction of intracellular As(V) to As(III) by arsenate reductases, since As(III) is the substrate of efflux pumps (Oremland and Stolz, 2003; Rosen, 2002).

Based on the considerations mentioned above, the biologically catalyzed reduction of As(V) to As(III) is expected to influence the mobility of arsenic from arsenate-laden drinking water residuals in the disposal environment. In a simple model system, a DARP isolate, *Sulfurospirillum barnesii*, was shown to catalyze the release of soluble As(III) from solid phase As(V) coprecipitated with aluminum hydroxide (Zobrist et al., 2000). The conditions for the microbial reduction of As(V) are favorable in landfills. A diverse population of anaerobic microorganisms, including methanogens, fermentative bacteria, and sulfate- and iron-reducers, is supported in landfill leachates (Christensen et al., 2001). Additionally, the leachates contain organic substrates, such as volatile fatty acids (VFA) (Fischer et al., 1997) that can serve as electron equivalents to sustain the reductive biotransformation of As(V).

The objective of this study was to evaluate the role of anaerobic microbial activity on the long term mobilization of arsenic in drinking water residuals disposed into landfills. As(V) was adsorbed onto activated alumina (AA) at a concentration representative of that from spent adsorbents from drinking water utilities. The arsenate-laden activated alumina (As(V)-AA) was placed into laboratory-scale columns that were fed continuously with defined leachates composed of major inorganic (ammonia and bicarbonate) and organic (VFA) ingredients in landfill leachates, and inoculated with a stable anaerobic consortium. The mobilization of arsenic from the As(V)-AA was measured by monitoring soluble arsenic compounds in the effluent of the column compared to control columns lacking VFA and inoculum.

## 2. Material and methods

### 2.1. Microorganisms

Anaerobic methanogenic granular sludge was obtained from industrial anaerobic treatment plants treating recycled paper wastewater (Industriewater, Eerbeek, The Netherlands) and alcohol distillery wastewater (Nedalco, Bergem op Zoom, The Netherlands). Both sources of sludge were washed and sieved to remove fine particles before use in the tests. The content of volatile suspended solids (VSS) in the Eerbeek and

Nedalco sludge was 12.9% and 10.0%, respectively. The anaerobic sludges were stored under nitrogen gas at 4 °C.

## 2.2. Activated alumina

AA (regenerable AA-400G, Alkan Chemicals, Cleveland, Ohio) was loaded to equilibrium with a final aqueous arsenate concentration of approximately  $14 \mu\text{g l}^{-1}$  at pH 7.0. The activated alumina was added to an aqueous solution (pH 7.0) containing  $131.4 \text{ mg of arsenic l}^{-1}$  (as  $\text{Na}_2\text{HAsO}_4 \cdot 7 \text{ H}_2\text{O}$ ),  $5.85 \text{ g NaCl l}^{-1}$  (equivalent to 0.1 M NaCl), 200 g dry weight AA and shaken at 150 rpm under a  $\text{N}_2$  atmosphere for 48 h. The loading conditions were determined from adsorption isotherms of As(V) onto activated alumina (Ghosh et al., 2002). The adsorbed concentration of arsenate was 0.657 mg As(V) per gram dry weight of activated alumina.

## 2.3. Flow-through columns

Arsenic mobilization under transport conditions was investigated in three columns (each 270 ml) (Fig. 1) continuously fed with synthetic landfill leachate. The columns were placed in a climate controlled room at  $30 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ . All the reactors were packed with 100 g dry weight of AA containing 0.657 mg adsorbed arsenate (expressed as arsenic) per gram dry weight. Two of the columns, R1 and R2 were inoculated with  $10 \text{ g VSS l}^{-1}$  of anaerobic sludge. A third column, R3, was not inoculated and served as a control. A synthetic wastewater formulated to simulate landfill leachates was used to feed column R1 and consisted of ( $\text{mg l}^{-1}$ ):  $\text{NH}_4\text{Cl}$  (668);  $\text{NaHCO}_3$  (2000);  $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$  (10);  $\text{KH}_2\text{PO}_4$  (37);  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (10);  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (68); yeast extract (20) and 1 ml/l of a trace element solution containing (in mg/l):  $\text{FeCl}_3 \cdot 4 \text{ H}_2\text{O}$ , 2000;  $\text{CoCl}_2 \cdot 6 \text{ H}_2\text{O}$ , 2000;  $\text{MnCl}_2 \cdot 4 \text{ H}_2\text{O}$ , 50;  $\text{AlCl}_3 \cdot 6 \text{ H}_2\text{O}$ , 90;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 30;  $\text{ZnCl}_2$ , 50;  $\text{H}_3\text{BO}_3$ , 50;  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4 \text{ H}_2\text{O}$ , 90;  $\text{Na}_2\text{SeO}_3 \cdot 5 \text{ H}_2\text{O}$ , 100;  $\text{NiCl}_2 \cdot 6 \text{ H}_2\text{O}$ , 50; EDTA, 1000; HCl 36% (1 ml). The R1 feed also contained a mixture of VFA ( $2.5 \text{ g COD l}^{-1}$ ) as an electron donating substrate and carbon source consisting of (in  $\text{mg l}^{-1}$ ): acetate (576); propionate (193), butyrate (423); valerate (163), and caproate (233). The pH of the influent was adjusted to 8.0 with NaOH or HCl, as required. The feed for R2 and R3 was prepared similarly to that of R1, except that VFA mixture and yeast extract were omitted. The synthetic leachate was designed using the average composition of major organic and inorganic species found in actual landfill leachate samples (Christensen et al., 2001; Clement and Merlin, 1995; Kjeldsen et al., 2002). The medium was prepared with minimal sulfur and phosphate content to avoid precipitation of arsenite as  $\text{As}_2\text{S}_3$ , and reduce competition of phosphate with

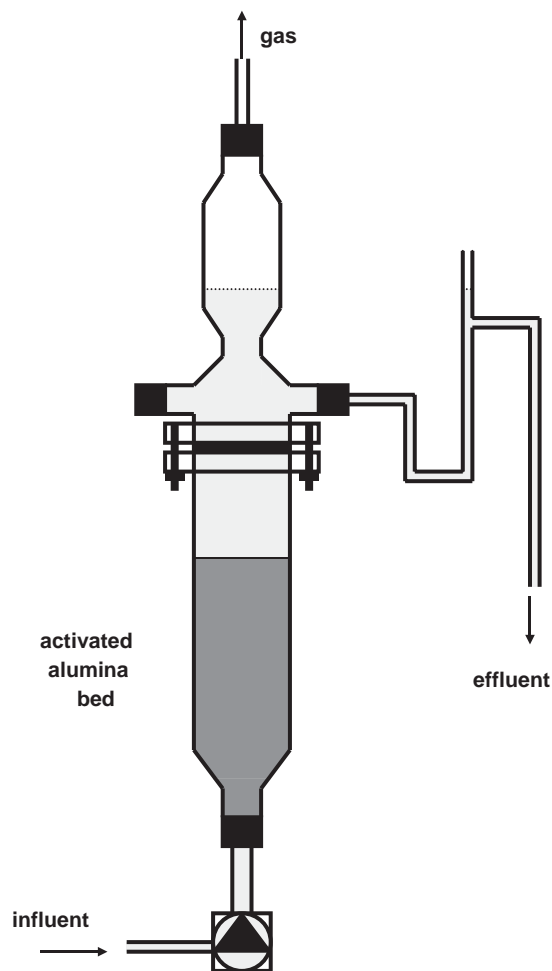


Fig. 1. Anaerobic column used in the continuous experiments.

arsenate for adsorption sites on the AA, respectively. During the last period of the experiment, the influent was supplemented with the humic model compound anthraquinone-2,6-disulfonate (AQDS) at concentrations of  $50 \mu\text{M}$  (day 163–200) or  $500 \mu\text{M}$  (day 201–253). AQDS was previously shown to stimulate the biological reduction of aqueous phase As(V) (Field et al., 2004). Starting on day 241, the phosphate concentration in the influent was increased to  $327 \text{ mg PO}_4^{3-} \text{ l}^{-1}$  to evaluate competition of phosphate with adsorbed As(V).

The columns were operated at a temperature of  $30 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  with empty bed hydraulic retention times averaging 12.2 h (from day 0–28), 26.2 h (from days 29–57), and ranging from 32 to 35 h for the remainder of the experiment. The methane production was measured by liquid displacement using inverted 1-l glass serum flasks filled with a 3% (w/v) NaOH solution to scrub out the carbon dioxide from the biogas. Fresh liquid samples

were collected periodically from the effluent line and prepared immediately for analysis to minimize possible changes in arsenic speciation upon exposure to the atmosphere. Filtered samples (0.20  $\mu\text{m}$  membrane filter) were analyzed for arsenic speciation, dissolved organic carbon (DOC), chemical oxygen demand (COD) and pH. The total concentration of arsenic was also determined in selected samples.

#### 2.4. Batch bioassay

Anaerobic reduction of soluble As(V) was assayed in shaken batch bioassays at 30 °C. Serum flasks (135 ml) were supplied with 50 ml of a basal mineral medium (pH 7.2) containing (in mg l<sup>-1</sup>): NH<sub>4</sub>Cl (280); NaHCO<sub>3</sub> (3000); MgCl<sub>2</sub> (78), CaCl<sub>2</sub> (10), MgSO<sub>4</sub>·7 H<sub>2</sub>O (10); K<sub>2</sub>HPO<sub>4</sub> (250); CaCl<sub>2</sub> (10); and 1 ml l<sup>-1</sup> of the trace element solution described above. The bioassays were inoculated with 2.5 g VSS l<sup>-1</sup> of Nedalco granular sludge. As(V) was supplied to the assay medium at 500  $\mu\text{M}$ . In the treatment receiving an exogenous electron donor, a VFA mixture providing a final concentration of 450 mg l<sup>-1</sup> each of acetate, propionate and butyrate (equivalent to 2 g COD l<sup>-1</sup>) was added.

#### 2.5. Analytical methods

Inorganic and organic arsenic species (As(III), As(V), methylarsonic acid (MMA(V)), dimethylarsinic acid (DMA(V)), methylarsonous acid (MMA(III)) and dimethylarsinous acid (DMA(III)) in liquid samples were analyzed by ion chromatography/inductively coupled plasma/mass spectrometry (IC/ICP/MS) using a method adapted from Gong et al. (2001). The HPLC system consisted of an Agilent 1100 HPLC (Agilent Technologies, Inc., Palo Alto, CA) with a reverse-phase C18 column (Prodigy 3u ODS(3), 150 × 4.60 mm, Phenomenex, Torrance, CA). The mobile phase (pH 5.85) contained 4.7 mM tetrabutylammonium hydroxide, 2 mM malonic acid and 4% (v/v) methanol at a flow rate of 1.2 ml min<sup>-1</sup>. The column temperature was maintained at 50 °C. An Agilent 7500a ICP-MS with a Babington nebulizer was used as the detector. The operating parameters were as follows: Rf power 1500 W, plasma gas flow 15 l min<sup>-1</sup>, carrier flow 1.2 l min<sup>-1</sup>, and arsenic was measured at 75 m/z. The injection volume was 10  $\mu\text{l}$ . The detection limit for the various arsenic species was 0.1  $\mu\text{g l}^{-1}$ . All liquid samples were membrane filtered (0.20  $\mu\text{m}$ ) immediately after sampling to minimize exposure to the atmosphere, and stored in polypropylene vials (2 ml) to reduce adsorption of arsenic species to the vial. Filtered samples were then stored at -20 °C till analysis was performed in order to reduce changes in arsenic speciation.

The total concentration of arsenic in liquid samples was determined using an ASX500 autosampler (CETAC

Technologies, Omaha, NE) and an Agilent 7500a ICP-MS. The analytical system was operated at a Rf power of 1500 W, a plasma gas flow of 15 l min<sup>-1</sup> and a carrier gas flow of 1.2 l min<sup>-1</sup>. The acquisition parameters used were: arsenic measured at m/z 75; terbium (IS) measured at m/z 159; 3 points per peak; 1.5 s dwell time for As, 1.5 s dwell time for Tb; number of repetitions = 7.

The total arsenic content in solid matrices (i.e., activated alumina, biomass in the columns) was measured following extraction of the samples with 10 ml of HCl (6.75 N) in a microwave digestion system (MDS2100, CEM Corporation, Matthews, NC) for 35 min.

Conversion of substrates was monitored by measuring the DOC content in influent and effluent samples. DOC measurements were performed using a Total Organic Carbon Analyzer (TOC-500, Shimadzu, Columbia, MD). Other parameters (e.g., pH, volatile suspended solids, COD) were determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998).

### 3. Results

#### 3.1. Batch assay: volatile fatty acids as electron donor for soluble arsenate reduction

A batch experiment was conducted to determine if VFA, representative of the organic substrates present in landfill leachate, would serve as an electron donating substrate supporting the anaerobic respiration of soluble As(V) supplied at 500  $\mu\text{M}$ . Fig. 2 shows that a stable anaerobic microbial consortium could readily reduce As(V) to As(III) in presence and absence of added VFA. The results demonstrate that endogenous substrates present in the added microbial consortium were sufficient to support As(V) respiration. Only one electron milliequivalent would be required to reduce 500  $\mu\text{M}$  of As(V), equivalent to 8 mg of COD l<sup>-1</sup>. The microbial inoculum was supplied at 2.5 g volatile suspended solids (VSS) l<sup>-1</sup>, equivalent to 3500 mg COD l<sup>-1</sup>, thus only 0.22% of the biomass in the inoculum would need to be degradable to support the observed endogenous respiration. Nonetheless, the rate of As(V) reduction was significantly increased in the treatment with VFA added as an exogenous electron donor. Fig. 2 also clearly demonstrates that the observed conversion of As(V) to As(III) was biologically catalyzed since the heat killed inoculum was inactive.

#### 3.2. Set-up of continuous column experiment

Continuous laboratory columns packed with As(V)-AA were extracted continuously with defined leachate to determine the mobilization of arsenic from the columns.

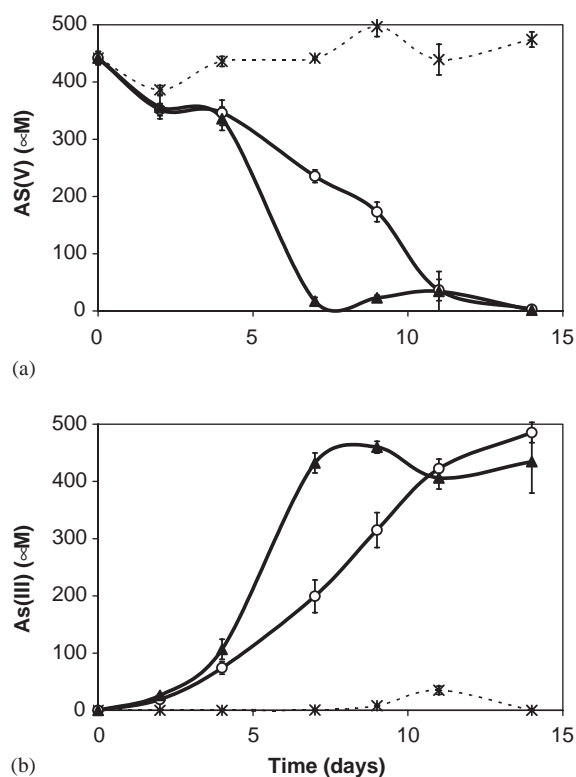


Fig. 2. The reduction of As(V) (500  $\mu\text{M}$ ) catalyzed by a stable mixed anaerobic consortium (2.5 g volatile suspended solids  $\text{l}^{-1}$ ) as inoculum in the absence (—○—) and presence (—▲—) of a VFA mixture (containing 7.5, 6.1 and 5.1 mM of acetate, propionate and butyrate, respectively). A control culture with heat killed inoculum (—\*—) was included for comparison. (A) As(V) concentrations. (B) As(III) concentrations.

The performance of three columns were compared. Each column was operated differently with respect to inoculation and the presence of VFA in the defined leachate. The first column, reactor 1 (R1), was the fully biologically active column inoculated with anaerobic sludge and fed with leachate containing VFA. The second column, reactor 2 (R2), was inoculated but was fed leachate lacking VFA. The third column, reactor 3 (R3) was the abiotic control (not inoculated and lacking VFA). The columns were operated for a period of 257 days and the operation was divided into five periods, which could be distinguished based on the hydraulic retention time and the inclusion of additional amendments as shown in Table 1. R1 operated under fully methanogenic conditions, the average methane production accounted for  $82.5 \pm 12.6\%$  of the influent VFA on a COD basis. The DOC removal averaged  $96.5 \pm 5.6\%$ . The median pH values in the three columns were 8.12, 8.49 and 8.34 for columns R1, R2 and R3, respectively. The range of pH values from the 0.05 to 0.95 percentile

was 7.68–8.62, 7.92–8.77 and 7.78–8.63 for columns R1, R2 and R3, respectively.

### 3.3. Arsenic mobilization

The cumulative release of soluble species of arsenic from the three columns is illustrated in Fig. 3. The figure shows the sum of six species monitored with IC-ICP-MS of which only four were detected as follows: As(V), As(III), methylarsonate (MMA(V)) and dimethylarsinate (DMA(V)). The cumulative release of soluble identified arsenic species was several fold to many fold greater in R2 and R1, respectively, compared to R3, indicating that inoculation of the columns with anaerobic sludge clearly enhanced mobilization. Moreover, the greater release of arsenic from R1 compared to R2 indicates that the mobilization was further enhanced by inclusion of exogenous electron donating substrate (VFA) in the leachate. When the experiment was terminated after 257 days of operation, 17.4%, 9.2% and 3.4% of the initial As(V)-AA added to the columns (As(V)-AA<sub>0</sub>) was recovered as identified soluble arsenic species in the effluents of columns R1, R2 and R3, respectively.

During the first 7 days, a labile fraction of arsenic, accounting for about 1.3% of As(V)-AA<sub>0</sub>, was readily released from the columns as evidenced from the pattern in R3. Thereafter, the impact of VFA in the defined leachate was most evident during the following 14 days of operation (period I). During this period the rate of arsenic release was approximately 8-fold greater in R1 (0.427% As(V)-AA<sub>0</sub> day<sup>-1</sup>) versus R2 (0.053% As(V)-AA<sub>0</sub> day<sup>-1</sup>). An enhanced rate of mobilization was still sustained in period II, when the rate of arsenic release was approximately 2.4-fold greater in R1 (0.101% As(V)-AA<sub>0</sub>/day) versus R2 (0.043% As(V)-AA<sub>0</sub> day<sup>-1</sup>). In the remaining operation periods, the rate of arsenic release became similar in R1 and R2 (0.025 and 0.031% As(V)-AA<sub>0</sub> day<sup>-1</sup>, respectively). The rate of release in the two inoculated columns exceeded the rate sustained in R3 of 0.0087% As(V)-AA<sub>0</sub> day<sup>-1</sup> from day 7 onwards.

### 3.4. Arsenic effluent concentration and speciation

The daily concentration of soluble arsenic species in R1 is shown in Fig. 4 for periods I and II. The graph illustrates that the peak concentration of arsenic reached  $812 \mu\text{g l}^{-1}$  on day 10. The value gradually declined to  $223 \mu\text{g l}^{-1}$  by day 25 and increased again after day 28 to  $393 \mu\text{g l}^{-1}$  due to an increase in the hydraulic retention time at the start of period II. Towards the end of period II, the soluble arsenic levels declined to approximately  $100 \mu\text{g l}^{-1}$ . Fig. 4 also illustrates that the dominant arsenic species was As(III), indicating the occurrence of microbial As(V) reduction.

Table 1  
Operational periods for the continuous columns

Period	Days	HRT <sup>a</sup>		OLR <sup>b</sup>		AQDS <sup>c</sup>	Phosphate
		(h) Avg <sup>d</sup>	Std <sup>d</sup>	(g COD/l/d) Avg	Std		
I	0–28	12.2	1.9	5.05	0.28	0	0.270
II	29–57	26.2	7.7	2.65	0.45	0	0.270
III	72–161	35.5	7.8	1.87	0.41	0	0.270
IV	162–240	32.2	5.5	1.98	0.34	50–500 <sup>e</sup>	0.270
V	241–257	35.0	5.7	1.83	0.31	500	3.44

<sup>a</sup>Hydraulic retention time.

<sup>b</sup>Organic loading rate (for R1) expressed in grams chemical oxygen demand (COD) per liter of reactor per day.

<sup>c</sup>Anthraquinone disulfonate.

<sup>d</sup>Avg = average, Std = standard deviation.

<sup>e</sup>50  $\mu\text{M}$  (day 162–200), 500  $\mu\text{M}$  (day 201–257).

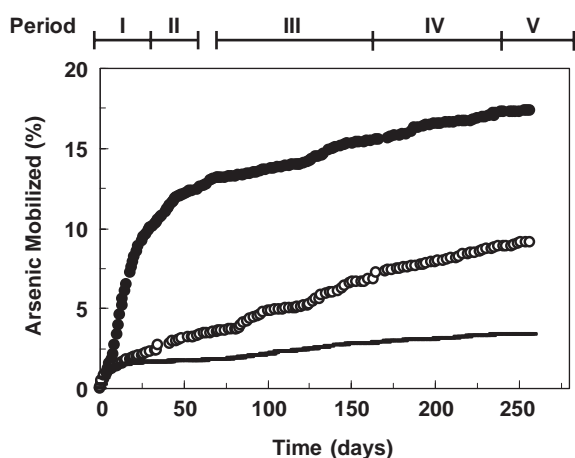


Fig. 3. The cumulative mobilization of arsenic (sum of all identified species) with the effluent of columns R1 (●), R2 (○) and R3 (—) expressed as a percent of the initial sorbed As(V) (% As(V)-AA<sub>0</sub>). The periods are indicated with roman numerals.

Table 2 summarizes the speciation of the identified soluble arsenic compounds in the three columns for each period of operation. Initially the most important species in the three columns was As(III), accounting for approximately 80% or more of the released identified species in periods I and II. In the case of R1 and R2, the occurrence of As(III) can be rationalized by biological reduction supported with VFA and endogenous substrates in the inoculum biomass as electron donors, respectively. In the period from day 23 to 43, As(V) was essentially non-detectable and As(III) accounted for nearly 100% of the species in both columns.

In period III, the relative proportion of As(III) decreased while that of As(V) increased in all the columns. In column R1, As(III) continued to account for an important fraction, 26–43% of the observed arsenic species, in periods III, IV and V. However in R2

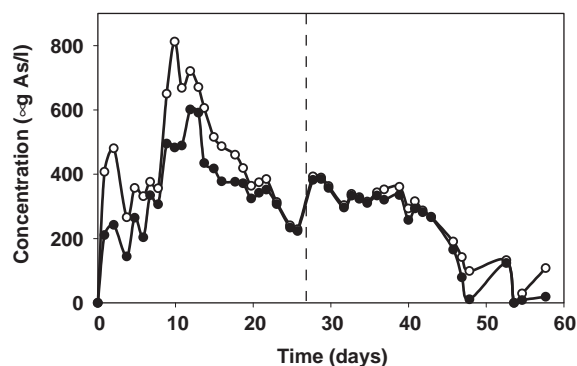


Fig. 4. Daily concentrations and speciation of arsenic identified in the effluent of column R1 during periods I and II. Legend: ○, sum of all species identified; ●, As(III). The vertical dashed line indicates the switch from period I to II when the hydraulic retention time was changed from 12.1 to 26.2 h.

and R3, As(III) became less important and the main species observed was As(V) during the final periods of column operation.

Column R1 was also characterized by the production of methylated species, MMA(V) and DMA(V) (Table 2). Initially in period I, low concentrations of MMA(V) were observed. The highest MMA(V) concentrations were observed in period II, reaching 21  $\mu\text{g As l}^{-1}$  on day 40. Thereafter in periods III, IV and V, MMA(V) fluctuated for the most part between 1 and 5  $\mu\text{g As l}^{-1}$ . The systematic production of DMA(V) began towards the end of period I on day 23; thereafter, the values generally fluctuated between 2 and 25  $\mu\text{g As l}^{-1}$  with an average concentration of 8.5  $\mu\text{g As l}^{-1}$ . The peak concentration of DMA(V) of 33  $\mu\text{g As l}^{-1}$  was observed on day 144. The proportion of the methylated species generally increased during R1 operation as shown in Table 2, accounting for 43.1% of the observed arsenic species in period V. However, this was due

Table 2

Period average values of total identified arsenic concentration in the column effluents and the period average distribution of identified species (% of total identified arsenic concentration)

Period	As-total <sup>a</sup>		As(III)		MMA(V)		DMA(V)		As(V)	
	$\mu\text{g l}^{-1}$ Avg <sup>b</sup>	Std <sup>b</sup>	% Avg	Std	% Avg	Std	% Avg	Std	% Avg	Std
<i>Column R1</i>										
I	453.7	158.4	79.6	14.9	0.5	0.5	0.3	0.5	19.6	15.1
II	269.8	104.7	84.8	25.7	3.5	4.3	2.8	6.9	8.9	19.2
III	92.5	66.0	31.8	25.7	2.2	1.4	17.4	13.0	48.5	27.4
IV	56.6	26.2	26.0	20.1	4.4	5.0	15.3	9.3	54.2	17.1
V	19.3	15.9	43.2	32.2	4.3	3.7	38.8	27.8	13.8	12.1
<i>Column R2</i>										
I	112.0	82.5	91.2	8.0	0.0	0.1	0.0	0.0	8.7	8.0
II	61.0	26.9	86.8	17.9	0.0	0.0	0.0	0.0	13.2	17.9
III	118.8	83.0	33.5	26.9	0.8	1.2	1.6	4.2	64.1	26.6
IV	79.2	39.7	7.5	7.6	0.3	0.5	0.3	0.9	91.9	7.6
V	61.2	31.6	4.1	6.1	0.0	0.0	0.4	0.5	95.5	6.1
<i>Column R3</i>										
I	73.6	112.0	85.7	19.5	0.1	0.5	0.1	0.5	14.1	19.6
II	10.5	4.2	80.5	18.8	0.0	0.0	0.0	0.0	19.5	18.8
III	30.4	19.5	53.0	30.9	0.6	1.5	0.5	1.9	45.9	30.4
IV	21.3	9.6	12.9	7.4	0.6	1.9	0.5	1.3	86.1	7.6
V	12.6	6.6	25.1	18.4	0.0	0.0	0.4	1.1	74.5	18.6

<sup>a</sup>Sum of soluble species identified with IC-ICP-MS in column effluents.

<sup>b</sup>Avg = average, Std = standard deviation.

mostly to a decrease in concentration of total identified arsenic species rather than an increase in the concentration methylated species.

During the operation of column R1 and R2, unidentified arsenic containing peaks were observed occasionally in the chromatograms of the IC-ICP-MS. A minor peak and major peak were observed at 4.3 and 7.5 min retention time, respectively. Selected samples collected between day 71 and 90 from columns R1 and R2 were analyzed by both IC-ICP-MS and direct ICP-MS. A ratio was established between the sum of identified species from IC-ICP-MS and the total arsenic from ICP-MS. This ratio averaged  $0.72 \pm 0.12$  and  $0.67 \pm 0.14$  mg As-identified  $\text{mg}^{-1}$  As total for columns R1 and R2, respectively; indicating that during this period, about a third of the arsenic was not identified.

### 3.5. Effect of AQDS and phosphate

AQDS, a known electron shuttle (Cervantes et al., 2002) was tested as a feed additive to column R1 in periods IV and V. As can be seen from Fig. 2, the addition of AQDS to the feed at concentrations of 50–500  $\mu\text{M}$ , had no noticeable impact on the release of arsenic from As(V)-AA. In period V, the phosphate

concentration of the defined leachate of all three columns was increased from 0.27 to 3.44 mM. Phosphate is a competitive anion with As(V) for adsorption sites (Darland and Inskeep, 1997). However as can be seen in Fig. 2, there was no noticeable increase in the mobilization of arsenic from any of the columns during period V.

### 3.6. Residual arsenic in activated alumina and sludge

At the end of the column experiment, AA and sludge were recovered and extracted to determine the residual concentration of arsenic remaining in the columns. The recovery of arsenic in the AA from the three columns is compared with reference material. The reference material is As(V)-AA prepared in the same fashion as the As(V)-AA used to pack the columns at the start of the column experiments. The results indicated a progressively decreasing recovery of arsenic in proportion to the identified species of arsenic which were mobilized. Since the extraction efficiency of the reference material was  $75.3 \pm 4.2\%$ . An estimate of the recovery of arsenic in residual AA was made by normalizing the measured recovery values against the reference material as show in the mass balance (Table 3). A similar extraction was

Table 3

Mass balance of arsenic from identified species mobilized in column effluents, residual arsenic in sludge and residual arsenic in activated alumina

Column	Residual arsenic in activated alumina		Residual arsenic in sludge		Identified arsenic species mobilized	Total arsenic recovery
	% reference <sup>a</sup> Avg <sup>c</sup>	Std <sup>c</sup>	% As(V)-AA <sub>t0</sub> <sup>b</sup> Avg	Std	%As(V)-AA <sub>t0</sub>	%As(V)-AA <sub>t0</sub>
R1	62.8	3.6	0.65	0.39	17.4	80.9
R2	70.1	4.3	2.06	0.25	9.2	81.4
R3	92.4	2.8	NA <sup>d</sup>		3.4	95.8

<sup>a</sup>Percent arsenic extracted compared to reference material, freshly prepared As(V)-AA.

<sup>b</sup>Percent arsenic extracted compared to initially added As(V)-AA.

<sup>c</sup>Avg = average, Std = standard deviation.

<sup>d</sup>Not applicable.

performed with residual sludge recovered from columns R1 and R2, resulting in a minor recovery (Table 3). The sum of the recoveries in the AA, sludge and the cumulative identified species mobilized in the column effluents indicates a global recovery of arsenic of about 81% in columns R1 and R2 and 96% in column R3 (Table 3). Based on the recovery of arsenic in the residual AA and sludge, approximately 37%, 28% and 8% of arsenic was mobilized from the R1, R2 and R3 columns, respectively. The cumulative arsenic identified as species in the eluted effluent accounted for 48%, 33% and 45% of the mobilized arsenic in R1, R2 and R3 columns, respectively.

## 4. Discussion

### 4.1. Implications

The results from this study provide compelling evidence that As(V) adsorbed on activated alumina is susceptible to mobilization in anaerobic environments. In this study a simple experimental model system was utilized in which As(V)-laden activated alumina was placed into anaerobic columns and exposed to continuous flushing with defined leachates. After 257 days of operation, 17.4% of the initial As(V) adsorbed was recovered as identified species of arsenic in the column effluent. The residual activated alumina contained 37% less arsenic than unextracted reference material. The biological activity of the columns played an important role in the mobilization process as evidenced by the considerably lower removal of adsorbed As(V) in organic substrate-free or uninoculated control columns.

### 4.2. Mobilization of arsenic

Processes affecting the mobilization of arsenic adsorbed onto soil and sediment minerals have been a

matter of considerable research interest in order to determine factors governing the release of arsenic into groundwater (Smedley and Kinniburgh, 2002). An important trigger of arsenic mobilization is the sudden occurrence of reducing conditions resulting in the dissimilatory reduction of iron minerals and subsequent release of adsorbed arsenic. A recent field study has demonstrated that creating anaerobic conditions in sediments was clearly associated with the mobilization of arsenic (Harvey et al., 2002).

The mobilization of arsenic under reducing conditions can be rationalized by two mechanisms. The first mechanism is due to reductive dissolution of Fe(III) in iron minerals to soluble Fe(II) by dissimilatory iron-reducing bacteria (Lovley, 1997). The microbial mediated dissolution of iron results in the disappearance of the adsorbing matrix, releasing As(V) as has been demonstrated in simple model systems with pure cultures of iron-reducing bacteria (Cummins et al., 1999; Zobrist et al., 2000). Under reducing conditions, the released As(V) will subsequently be rapidly reduced to As(III) by anaerobic consortia in the environment (Dowdle et al., 1996; Field et al., 2004).

The second mechanism of mobilization is due to the direct biological conversion of sorbed As(V) to As(III). An As(V)-respiring bacterium was shown to mobilize As(V) sorbed to aluminum hydroxide precipitates, converting it to aqueous phase As(III) (Zobrist et al., 2000). There is a strong body of evidence suggesting that As(III) is less strongly adsorbed by alumina compared to As(V) (Ghosh and Yuan, 1987; Lin and Wu, 2001). As(V) is adsorbed predominantly by aluminum oxides by strong inner sphere complexes with a bidentate binuclear configuration (Arai et al., 2001). In contrast, As(III) is adsorbed by both inner-sphere and weaker outer-sphere complexes. Thus, the biological reduction of aqueous As(V) in equilibrium with sorbed As(V) would clearly shift the equilibrium more towards the aqueous phase. During the column study presented here,



direct reduction of As(V) to As(III) was clearly the principal mechanism of arsenic mobilization. During the periods of maximum release of soluble arsenic, As(III) was the dominant arsenic species in the aqueous phase.

#### 4.3. Effect of electron donating substrates

The biological reduction of As(V) to As(III) requires some kind of electron donating substrate. In this study we have observed that the slow hydrolysis of endogenous substrates in sludge biomass, used as an inoculum, was sufficient to support the microbial mediated reduction of As(V). This observation applied to soluble As(V) in batch experiments as well as sorbed As(V) in the column experiment. However, supplementing the microbial medium with simple organic substrates in the form of a mixture of VFA significantly increased the rate of soluble As(V) reduction as well as the rate of its mobilization from activated alumina. VFA are the most important organic substrates present in MSW landfill leachate (Fischer et al., 1997). Therefore, these organic constituents in landfill leachates can be expected to promote the mobilization of arsenic in landfilled drinking water residuals. The use of VFA as an electron donor to support As(V) reduction is consistent with previous observations from isolated cultures of DARPs. Two strains *Sulfurospirillum arsenophilum* strain MIT-13 and *Desulfotomaculum auripigmentum* strain OREX-4 were shown to utilize butyrate as electron donor (Newman et al., 1997; Stolz et al., 1999). Another strain, *Chrysiogenes arsenatis*, could utilize acetate as an electron donor (Macy et al., 1996). Oxidation of [ $^{14}\text{C}$ ]-acetate to  $^{14}\text{CO}_2$  was also shown to be coupled to As(V) reduction in anaerobic sediments (Dowdle et al., 1996).

The presence of VFA in the leachate medium sustained increased rates of As(V) mobilization for approximately 2 months. Thereafter, the rates became comparable in the two inoculated columns, one with and the other without VFA supplementation in the influent medium. This observation can be interpreted as a shift in conditions from an initially electron-donor limited system to a system in which either lacked a nutrient required for As(V) reduction or the bioavailability of sorbed As(V) became limiting.

#### 4.4. Arsenic bioavailability

Not all sorption sites are expected to bind with As(V) and As(III) with equal strength. For example, As(III) is known to be adsorbed by both weak and strong outer- and inner-sphere complexes of aluminum oxides, respectively (Arai et al., 2001). At the end of the experiment, increasing the phosphate concentration in the column feed created a large increase in the ionic strength but this had no effect on arsenic mobilization. The lack of any effect suggests that weak electrostatic

adsorption of As(V) or As(III) susceptible to competition from phosphate were not important at that time. Aging time is also known to affect surface speciation of As(V) on aluminum oxides. Decreasing desorption kinetics has been noted for of aluminum oxides exposed for long periods to As(V) (Arai and Sparks, 2002).

#### 4.5. Methylated arsenicals

In addition to As(III), two methylated metabolites (MMA(V) and DMA(V)) were routinely observed in this study from the bioconversion of sorbed As(V). These metabolites accounted for up to 43% of the total identified arsenical species. Methylation of As(V) by anaerobic microorganisms was recently reviewed (Bentley and Chasteen, 2002). Most of the studies report on the conversion of As(V) to small amounts of volatile methylarsines, which were first described in a pure culture of a methanogen, *Methanobacterium bryantii* (McBride and Wolfe, 1971). Recently, several pure cultures of anaerobes, including a methanogen (*Methanobacterium formicicum*), a fermentative bacterium (*Clostridium collagenovorans*) and sulfate-reducing bacteria (*Desulfovibrio vulgaris* and *D. gigas*), were also implicated in the formation of methylarsines in small yields (Michalke et al., 2000; Wickenheiser et al., 1998). There are few reports indicating the accumulation of MMA(V) and DMA(V) under anaerobic conditions, although such compounds are observed as intermediates in the pathway to methylarsines in methanogens (Michalke et al., 2000; Wickenheiser et al., 1998). Accumulation of MMA(V) and DMA(V) was reported in the pore water of lake sediments of a gold mine impacted lakes (Bright et al., 1996). Anaerobic microcosms established from the sediments of one of the studied lakes were shown to methylate As(V) forming MMA(V) and DMA(V) in yields ranging from 0.75% to 10.5% (Bright et al., 1994). High yields of methylated arsenicals of up to 40% were found by incubating rat cecal content with either As(V) or As(III) (Hall et al., 1997). It has been proposed that MMA(V) formation by methanogens is directly formed from the oxidative methylation of As(III) according to the Challenger mechanism (Bentley and Chasteen, 2002; McBride and Wolfe, 1971). The proposal is consistent with the widely accepted view that As(III) is the preferred substrate of methylation reactions (Bentley and Chasteen, 2002). Likewise the proposal is consistent with the formation of As(III) from As(V) prior to methylation (Hall et al., 1997; Wickenheiser et al., 1998).

## 5. Conclusions

The results of this study indicate that As(V) adsorbed onto AA is susceptible to biologically catalyzed

mobilization in anaerobic environments. The main findings supporting this conclusion are as follows:

- (1) 37% of As(V) sorbed onto AA was removed from AA in 237 days in an anaerobic flow through columns inoculated with anaerobic sludge and flushed with synthetic landfill leachate.
- (2) Sorbed As(V) was subject to two types of biotransformation reactions: reduction to As(III); and methylation to MMA(V) and DMA(V).
- (3) Approximately half of the arsenic removed was recovered as identified arsenical species (As(III), As(V), MMA(V) and DMA(V)) in column effluent samples, of which As(III) was the most important species in periods of high arsenic mobilization rates.
- (4) Mobilization of arsenic is attributed to the biological reduction of As(V) to As(III) since literature data indicates that As(III) is more weakly adsorbed to AA compared to As(V).
- (5) VFA present in landfill leachate serves as a good electron donating substrate supporting enhanced rates of biological As(V) reduction to As(III).
- (6) Control columns lacking inoculum and/or VFA in the feed displayed low mobilization of arsenic compared to the fully biologically active column.

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### References

- APHA, 1998. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.
- Arai, Y., Sparks, D.L., 2002. Residence time effects on arsenate surface speciation at the aluminum oxide-water interface. *Soil Sci.* 167, 303–314.
- Arai, Y., Elzinga, E.J., Sparks, D.L., 2001. X-ray absorption spectroscopic investigation of arsenite and arsenate adsorption at the aluminum oxide-water interface. *J. Colloid Interf. Sci.* 235, 80–88.
- Bentley, R., Chasteen, T.G., 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol. Mol. Biol. Rev.* 66, 250–271.
- Bright, D.A., Brock, S., Cullen, W.R., Hewitt, G.M., Jafaar, J., Reimer, K.J., 1994. Methylation of arsenic by anaerobic microbial consortia isolated from lake sediment. *Appl. Organomet. Chem.* 8, 415–422.
- Bright, D.A., Dodd, M., Reimer, K.J., 1996. Arsenic in subArctic lakes influenced by gold mine effluent: the occurrence of organoarsenicals and ‘hidden’ arsenic. *Sci. Total Environ.* 180, 165–182.
- Cervantes, F.J., de Bok, F.A.M., Tuan, D.D., Stams, A.J.M., Lettinga, G., Field, J.A., 2002. Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. *Environ. Microbiol.* 4, 51–57.
- Christensen, T.H., Kjeldsen, P., Bjerg, P.L., Jensen, D.L., Christensen, J.B., Baun, A., Albrechtsen, H.J., Heron, C., 2001. Biogeochemistry of landfill leachate plumes. *Appl. Geochem.* 16, 659–718.
- Clement, B., Merlin, G., 1995. The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ.* 170, 71–79.
- Cummings, D.E., Caccavo, F., Fendorf, S., Rosenzweig, R.F., 1999. Arsenic mobilization by the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY. *Environ. Sci. Technol.* 33, 723–729.
- Darland, J.E., Inskeep, W.P., 1997. Effects of pH and phosphate competition on the transport of arsenate. *J. Environ. Qual.* 26, 1133–1139.
- Dowdle, P.R., Laverman, A.M., Oremland, R.S., 1996. Bacterial dissimilatory reduction of arsenic(V) to arsenic(III) in anoxic sediments. *Appl. Environ. Microbiol.* 62, 1664–1669.
- Field, J.A., Sierra-Alvarez, R., Cortinas, I., Feijoo, G., Moreira, M.T., Kopplin, M., Gandolfi, A.J., 2004. Facile reduction of arsenate in methanogenic sludge. *Biodegradation* 15, 185–196.
- Fischer, K., Chodura, A., Kotalik, J., Bieniek, D., Kettrup, A., 1997. Analysis of aliphatic carboxylic acids and amino acids in effluents of landfills, composting plants and fermentation plants by ion-exclusion and ion-exchange chromatography. *J. Chromatogr. A* 770, 229–241.
- Ghosh, A., Mishra, D., Ela, W., 2002. Toxics leaching from residuals of arsenic treatment under landfill conditions, editors. Proceedings of the American Water Works Association, Annual Conference. American Water Works Association, New Orleans, pp. 16–20.
- Ghosh, M.M., Yuan, J.R., 1987. Adsorption of inorganic arsenic and organoarsenicals on hydrous oxides. *Environ. Prog.* 6, 150–157.
- Gong, Z.L., Lu, X.F., Cullen, W.R., Le, X.C., 2001. Unstable trivalent arsenic metabolites, monomethylarsonous acid and dimethylarsinous acid. *J. Anal. At. Spectrom.* 16, 1409–1413.
- Hall, L.L., George, S.E., Kohan, M.J., Styblo, M., Thomas, D.J., 1997. In vitro methylation of inorganic arsenic in mouse intestinal cecum. *Toxicol. Appl. Pharmacol.* 147, 101–109.
- Harrington, J.M., Fendorf, S.E., Rosenzweig, R.F., 1998. Biotic generation of arsenic(III) in metal(loid)-contaminated freshwater lake sediments. *Environ. Sci. Technol.* 32, 2425–2430.
- Harvey, C.F., Swartz, C.H., Badruzzaman, A.B.M., Keon-Blute, N., Yu, W., Ali, M.A., Jay, J., Beckie, R., Niedan, V., Brabander, D., Oates, P.M., Ashfaq, K.N., Islam, S., Hemond, H.F., Ahmed, M.F., 2002. Arsenic mobility and

- groundwater extraction in Bangladesh. *Science* 298, 1602–1606.
- Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., 2002. Present and long-term composition of MSW landfill leachate: a review. *Crit. Rev. Environ. Sci. Technol.* 32, 297–336.
- Kuai, L., Nair, A.A., Polz, M.F., 2001. Rapid and simple method for the most-probable-number estimation of arsenic-reducing bacteria. *Appl. Environ. Microbiol.* 67, 3168–3173.
- Lin, T.F., Wu, J.K., 2001. Adsorption of arsenite and arsenate within activated alumina grains: equilibrium and kinetics. *Water Res.* 35, 2049–2057.
- Lovley, D.R., 1997. Microbial Fe(III) reduction in subsurface environments. *Fems Microbiol. Rev.* 20, 305–313.
- Macy, J.M., Nunan, K., Hagen, K.D., Dixon, D.R., Harbour, P.J., Cahill, M., Sly, L.I., 1996. *Chrysiogenes arsenatis* gen nov, sp nov; a new arsenate-respiring bacterium isolated from gold mine wastewater. *Int. J. Syst. Bacteriol.* 46, 1153–1157.
- McBride, B.C., Wolfe, R.S., 1971. Biosynthesis of dimethylarsine by a methanobacterium. *Biochemistry* 10, 4312–4317.
- Meng, X.G., Korfiatis, G.P., Jing, C.Y., Christodoulatos, C., 2001. Redox transformations of arsenic and iron in water treatment sludge during aging and TCLP extraction, editors. *Env. Sci. Technol.* 35, 3476–3481.
- Michalke, K., Wickenheiser, E.B., Mehring, M., Hirner, A.V., Hensel, R., 2000. Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. *Appl. Environ. Microbiol.* 66, 2791–2796.
- Newman, D.K., Kennedy, E.K., Coates, J.D., Ahmann, D., Ellis, D.J., Lovley, D.R., Morel, F.M.M., 1997. Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. *Arch. Microbiol.* 168, 380–388.
- NRC, 2001. Arsenic in drinking water: 2001 update. National Academy Press, Washington, DC.
- Oremland, R.S., Stolz, J.F., 2003. The ecology of arsenic. *Science* 300, 939–944.
- Rosen, B.P., 2002. Biochemistry of arsenic detoxification. *Febs Lett.* 529, 86–92.
- Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* 17, 517–568.
- Stolz, J.F., Ellis, D.J., Blum, J.S., Ahmann, D., Lovley, D.R., Oremland, R.S., 1999. *Sulfurospirillum barnesii* sp nov and *Sulfurospirillum arsenophilum* sp nov., new members of the *Sulfurospirillum* clade of the epsilon Proteobacteria. *Int. J. Syst. Bacteriol.* 49, 1177–1180.
- US-EPA, 2001. National Primary Drinking Water Regulations: arsenic and clarifications to compliance and new source contaminants monitoring. *Federal Register* 66, 6976–7066.
- Wickenheiser, E.B., Michalke, K., Drescher, C., Hirner, A.V., Hensel, R., 1998. Development and application of liquid and gas-chromatographic speciation techniques with element specific (ICP-MS) detection to the study of anaerobic arsenic metabolism. *Fresenius J. Anal. Chem.* 362, 498–501.
- Zobrist, J., Dowdle, P.R., Davis, J.A., Oremland, R.S., 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environ. Sci. Technol.* 34, 4747–4753.