Published in final edited form as:

Biotechnol Bioeng. 2010 December 1; 107(5): 786–794. doi:10.1002/bit.22883.

The Role of Denitrification on Arsenite Oxidation and Arsenic Mobility in an Anoxic Sediment Column Model with Activated Alumina

Wenjie Sun[*], **Reyes Sierra-Alvarez**, and **Jim A. Field**

Department of Chemical and Environmental Engineering, University of Arizona

Abstract

Arsenite (As(III)) is the predominant arsenic (As) species in reducing environments. As(III) is less strongly adsorbed than As(V) at circumneutral pH conditions by common non-iron metal oxides in sediments such as those of aluminum. Therefore, oxidation of As(III) to As(V) could contribute to an improved immobilization of As and thus help mitigate As contamination in groundwater. Microbial oxidation of As(III) is known to readily under aerobic conditions, however, the dissolved oxygen (O_2) concentration in groundwater may be limited due to the poor solubility of O_2 and its high chemical reactivity with reduced compounds. Nitrate (NO_3^-), can be considered as an alternative electron acceptor, which can support oxidation of As(III) to As(V) by denitrifying bacteria. In this study, two up-flow sediment columns packed with activated alumina (AA) were utilized to demonstrate the role of denitrification on the oxidation of As(III) to As(V) and its contribution to improved As adsorption onto AA. One column was supplied with NO_3^- (C1) and its performance was compared with a control column lacking NO_3^- (C2). During most of the operation when the pH was in the circumneutral range (d 50–250), the release of arsenic was greater from C2 compared to C1. The effluent As concentrations started increasing on d 60 and d 100 in C2 and C1, respectively. Complete breakthrough started on d 200 in C2; whereas in C1, complete breakthrough was never achieved. The effluent and solid phase As speciation was dominated by As(V) in C1, indicating the occurrence of As(III) oxidation due to NO_3^-; whereas in C2, only As(III) was dominant. This study illustrates a bioremediation or natural attenuation process based on anoxic microbial NO_3^--dependent oxidation of As(III) to more readily adsorbed As(V) as a means to enhance the immobilization of As on alumina oxide particles in subsurface environments.

Keywords

Arsenite; Oxidation; Denitrification; Activated Alumina; Adsorption; Attenuation

Introduction

Arsenic (As) is generally found as a contaminant in soil, sediments and water systems (Smedley and Kinniburgh 2002). The occurrence of elevated As in groundwater or surface

[*]Department of Chemical and Environmental Engineering, University of Arizona, P.O. Box, 210011, Tucson, Arizona, USA, Tel. 1-520-626-6781, Fax. 1-520-621-6048, alexsun@email.arizona.edu.

water has been attributed to natural biogeochemical reactions such as weathering and dissolution of As-containing minerals, which promote its release into groundwater (Smedley and Kinniburgh 2002). In natural environments, Arsenic generally occurs as either arsenate (As(V)) encountered under oxidizing conditions, or arsenite (As(III)) in reduced anaerobic environments. At circumneutral pH values, As(V) occurs as deprotonated oxyanions of arsenic acid ($H_2AsO_4^-$ and $HAsO_4^{2-}$); As(III) exists in the non-ionic form (H_3AsO_3) (Cullen and Reimer 1989). Of the two commonly occurring species, As(III) is generally considered more mobile and toxic (Oremland and Stolz 2003; Sierra-Alvarez et al. 2004; Smedley and Kinniburgh 2002).

The main mechanism of immobilizing As is its sorption from the aqueous phase onto the solid phase (Dixit and Hering 2003). Clay minerals are common constituents of the subsurface environment, which act as adsorbents. As(III) and As(V) are adsorbed on the surface of various clay minerals, which largely consists of alternating layers of silica oxide and Al oxide (Lin and Puls 2000; Wang and Mulligan 2006). It is well established that As(III) is less strongly bound compared to As(V) onto clay and minerals with alumina oxides and low content of iron (Goldberg 2002; Lin and Wu 2001; Manning and Goldberg 1997). For example, aolinite and montmorillonite exhibited higher affinities for As(V) than As(III) (Frost and Griffin 1977). Activated alumina (AA) has a 2-fold higher affinity for As(V) than As(III) at pH 7 (Ghosh and Yuan 1987). Therefore, oxidation of As(III) to As(V) could contribute to improved As sorption onto clay mineral and other alumina (hydr)oxides, thereby decreasing As contamination in groundwater.

Some studies have demonstrated an improved removal of As in AA packed columns when a chemical pre-oxidation of As(III) was utilized (Bissen and Frimmel 2003). The microbial oxidation of As(III) under aerobic conditions is well known and has been reviewed in several references (Inskeep et al. 2007; Oremland and Stolz 2003). However, the dissolved oxygen (O_2) concentration in groundwater of anaerobic subsurface zones may be limited due to the poor solubility of O_2 and its high chemical reactivity with reduced compounds. Recently evidence is growing that microbial oxidation of As(III) can also occur under anoxic conditions in the presence of nitrate (NO_3^-) (Oremland et al. 2002; Rhine et al. 2006; Sun et al. 2008) or selenate (Fisher and Hollibaugh 2008). In a previous study, the anoxic oxidation of arsenite (As(III)) linked to chemolithotrophic denitrification was shown to be stable and efficient over prolonged periods of operation ranging up to 3 years in up-flow continuous bioreactors (Sun et al. 2010).

The objective of this study was to evaluate the potential of NO_3^- as a possible oxidant to promote the oxidation of As(III) by denitrifying bacteria, and subsequent effect on As immobility in anaerobic environments. The evaluation was performed using two up-flow sediment columns packed with AA and fed with As(III) either in the presence or absence of NO_3^- The columns were used to simulate anaerobic sediments containing mostly non-iron, alumina oxides.

Materials and methods

Microorganisms

A chemolithotrophic As(III)-oxidizing denitrifying granular biofilm (CAODGB) was used to inoculate the two columns packed with AA. The inoculum was obtained from a 2-l bench-scale upward flow anaerobic sludge bed As(III)-oxidizing denitrifying bioreactor after 466 days of operation (Sun et al. 2010). The bioreactor was fed with As(III) (3.5 mM) as electron donor, NO_3^- (6.5 mM) as electron acceptor, and $NaHCO_3$ (8.0 mM) as major carbon source with a hydraulic retention time (HRT) of 1.1 d. The total suspended solids (TSS) and volatile suspended solids (VSS) content of the sludge was 6.08±0.18% and 5.76±0.23%, respectively, on a wet weight basis. The CAODGB was washed and sieved before use to remove fines.

Basal medium

The standard basal medium at the beginning of the column operation was prepared using ultra pure water (Milli-Q system; Millipore) and contained the following compounds (mg l^{-1}): NH_4HCO_3 (3.16); $NaHCO_3$ (672); $CaCl_2$ (10), $MgSO_4•7H_2O$ (10); K_2HPO_4 (300); $KH_2PO_4•2H_2O$ (800); and 0.02 ml l^{-1} of a trace element solution containing (in mg l^{-1}): $FeC1_3•4H_20$ (2,000); $CoCl_2•6\ H_20$ (2,000); $MnCl_2•4H_20$ (500); $AlCl_3•6\ H_20$ (90); $CuCl_2·2H_20$ (30); $ZnCl_2$ (50); H_3BO_3 (50); $(NH_4)_6Mo_7O_{24}•\ H_2O$ (50); $Na_2SeO_3•5H_2O$ (100); $NiCl_2•6H_20$ (50); EDTA (1,000); resazurin (200); HCl 36% (1 ml). The pH of the basal medium was adjusted to 7.2 using concentrated NaOH or HCl, as needed. On the day 25, the concentration (mg l^{-1}) of bicarbonate and phosphate buffer was lowered to 100 ($NaHCO_3$), 204 ($KH_2PO_4•2H_2O$) and 204 (K_2HPO_4), respectively.

Batch assays to determine the terminal product of denitrification

Microbial reduction of NO_3^- coupled to As(III) oxidation was assessed in shaken batch bioassays inoculated with 1.5 g VSS l^{-1} CAODGB. Serum flasks (160 ml) were supplied with 120 ml of a basal mineral medium (pH 7.0–7.2) with $NaHCO_3$ added as 2150 mg l^{-1}, and 0.2 ml l^{-1} of a trace element solution as described before. The medium was supplemented with As(III) as electron donor (3.5 mM) and NO_3^- as the electron acceptor (2.5 mM). Details of the bioassays are in the Supplemental Information.

Attenuation of As(III) under chemolithotrophic denitrifying conditions in columns packed with AA

Anoxic As(III) oxidation and immobilization of As(V) formed under denitrifying conditions was investigated in two glass columns (each 420 ml) continuously fed with the synthetic basal medium. The columns were placed in a climate controlled room at 30±2°C and covered with aluminum foil to avoid growth of phototrophic microorganisms. Each reactor was packed with 350 grams dry weight of AA (regenerable AA-400G, Alkan Chemicals, Cleveland, Ohio) and inoculated with 2.94 g VSS l^{-1} CAODGB. The full-treatment column (C1) was fully biologically active as it was inoculated with the CAODGB and fed with basal medium, As(III) (6.7 uM) and NO_3^- (2.5 mM). The control column (C2) was inoculated and fed As(III) in the same fashion as C1, but lacked NO_3^-. Both reactors were supplied with

bicarbonate (100 mg l^{-1}) as the major carbon source, except that 18.8 mg l^{-1} acetate was added to support the microbial consumption of any possible traces of dissolved oxygen that could have potentially entered from the medium and caused unwanted aerobic oxidation of As(III). The influent of both reactors was maintained at all times under a N_2 atmosphere supplied via a gas bag (SKC-West Inc, Fullerton, CA) to minimize any exposure to O_2.

The columns were operated with an empty bed hydraulic retention time (HRT) averaging 24 h (from day 0–30), and 12 h for the remainder of the experiment. Fresh liquid samples were collected periodically from the influent and effluent lines and prepared immediately for analysis to minimize possible changes in As speciation upon exposure to the atmosphere. The pH value was determined immediately after sampling. Samples for analysis of As speciation, total arsenic, NO_3^- and NO_2^- were centrifuged (10,000 rpm, 10 min) or membrane filtered (0.45 μm) prior to dilution.

Arsenic extraction

The total As content and As speciation in solid matrices (*i.e.,* activated alumina, biomass) were measured following extraction of the samples with 25 ml of NaOH (2.0 M) in anaerobic tubes (30.0 ml) under N_2 gas immersed in a shaking water bath at a temperature of 90±2°C for 12 h. After the extraction, the liquid samples were immediately adjusted to pH lower than 1.0 with 2.5 M HNO_3 to avoid oxidation at alkaline conditions when the samples first became exposed to air. Subsequently, the samples were adjusted to a final pH of 6.0–6.5 with 2.0 M NaOH and membrane filtered (0.45 μm) and stored in polypropylene vials at −20°C. A simple assay was set up to confirm the preservation of As speciation during the alkaline extraction and sample preservation processes. The results shown (Table 1) demonstrated that the extraction process on solid-phase AA did not change the As speciation.

Analytical methods

As(III) and As(V) species were analyzed by high performance liquid chromatography–inductively coupled plasma–mass spectroscopy (HPLC-ICP-MS). NO_3^-, NO_2^- and As(V) were analyzed by suppressed conductivity ion chromatography (IC) using a Dionex 500 system. N_2 and N_2O were analyzed for the batch assays using a Hewlett Packard 5890 Series II gas chromatograph (GC) fitted with a Carboxen™ 1010 Plot column (30 m × 0.32 mm) and a thermal conductivity detector (TCD). The acetate concentration in liquid samples was determined by gas chromatography (GC) using an HP5290 Series II system equipped with a flame ionization detector (FID) and a Nukol fused silica capillary column. Details of the HPLC-ICP-MS, IC, GC-TCD and GC-FID methods are provided in the supplementary information.

Other analytical determinations (e.g., pH, TSS, VSS, etc.) were conducted according to Standard Methods (APHA 1999).

Results

Terminal products of autotrophic denitrification linked to As(III) oxidation to As(V)

A batch experiment was set up with the CAODGB used to inoculate the columns in order to confirm that N_2 gas was the end product of NO_3^- reduction linked to As(III) oxidation in this microbial consortium. The results shown in Figure 1A demonstrate the time course of As(III) removal and As(V) formation. In all treatments, the formation of As(V) corresponded to an almost stoichiometric elimination of As(III). The results indicated that the enriched microbial consortium could readily oxidize all As(III) to As(V) within 50 days in the presence of NO_3^- as electron acceptor.. By the end of the experiment, 97.79±3.19% of the initial As(III) in the treatments was oxidized to As(V). The possible products from NO_3^- reduction, including NO_2^-, NO, N_2O, N_2 and NH_4^+, were also monitored to determine the fate of NO_3^- in the anoxic oxidation of As(III). Accumulations of NO_2^-, NO, N_2O or NH_4^+ were not detected in any of the treatments. Figure 1B demonstrates that N_2 was the only end product formed from NO_3^- reduction linked to the oxidation of As(III) to As(V). In the treatment, the formation of N_2-N corresponded to 109.9±3.8% of the measured net removal of NO_3^--N, also confirming that N_2 was the only product from NO_3^- due to As(III). The molar ratio of As: NO_3^- involved in the reaction was calculated from the As(V) formed (ΔAs(V)) to the NO_3^- (ΔNO_3^-) consumption corrected for NO_3^- removed in the endogenous control and N_2-N (ΔN_2-N) formation (corrected for the endogenous N_2 formation). The calculated ratios of ΔAs(V) to ΔNO_3^- and to ΔN_2-N were 2.59±0.14 and 2.35±0.05 respectively, which are very close to the theoretical stoichiometric ratio of 2.5 (eq. 1) for As(III) oxidation linked to complete denitrification. These results confirm that the column inoculum was fully capable of complete denitrification to the benign N_2 end product when utilizing As(III) as the electron donor.

$$5H_3AsO_3 + 2NO_3^- \rightarrow 8H^+ + 5HAsO_4^{2-} + N_2 + H_2O \quad \text{(eq. 1)}$$

Attenuation of As(III) in AA packed columns

A simple experimental model system was utilized in which AA was placed into an anaerobic up-flow packed bed columns and exposed to continuous feeding of As(III) with basal medium in the presence or absence of NO_3^-. During the first 50 d period, the pH of the effluent was high, in the range of 9.5–10.0 as shown in Figure 2, due to the release of the alkalinity from the AA. After lowering the concentration of bicarbonate and phosphate, as well as continued washing of the AA, the pH stabilized around 7 after d 50. The cumulative release of total As from the two AA packed bed columns fed with As(III) with NO_3^- (C1) and without NO_3^- (C2) is illustrated in Figure 3. Within this initial period, the total As removal efficiencies for C1 and C2 of 65 to 86%, respectively; were not optimal. The removal efficiencies improved dramatically when the pH decreased to 7.2.

From d 50 onwards, circumneutral pH conditions prevailed. In the initial period of the circumnuetral pH phase (d 52–100), the newly established As removal efficiencies had increased to 98 and 89% for C1 and C2, respectively (Table 2 and Figure 3). The total As in the effluent of C2 started to increase on d 60, and continued to increase until almost all of

the As in the influent broke through on d 200. The release of As was noticeably more delayed in C1. Increases in the effluent total As concentration only started after d 100 and even at the end of the experiment on d 250 there was still no complete break through of As from C1 (Figure 3). By the final period of reactor operation (d 202–250), the total As removal efficiencies had decreased to 41 and 5% in C1 and C2, respectively (Table 2).

The mass of As released from C1 and C2 in each 50 d interval of reactor operation is shown in Table 2. The table elucidates a trend in the As mobility difference between C1 and C2. When the AA adsorbent was highly unsaturated during the first 50 d interval of the circumnuetral phase, the mobility difference is very pronounced being 6-fold greater in C2 compared to C1. As the adsorbing sites become progressively more occupied with As, the C2/C1 As release ratio progressively decreases so that by the final interval of 202–250 d, the ratio had declined to 1.7. The cumulative C2/C1 release ratio during the entire circumnuetral phase of the column operation was 2.2. The difference in mobility may be attributed to the impact of NO_3^- on the speciation of As.

The daily concentration of arsenic species (As(III) and As(V)) in the influent and effluent of the two columns is illustrated in Figure 4 for C1 and C2. The results show that influent As(III) was completely removed in C1 from d 50 to 100, after which time, the As started to partially pass through the column with As(V) as the dominant species in the effluent. In C2, the influent As(III) passed through the column without any alteration to the As speciation. The results indicate the removal of As was greater in C1 compared to C2 in accordance with the expectation that anoxic oxidation of As(III) by NO_3^- would lead to As(V) formation, which in turn would be adsorbed more efficiently.

Figure S2 illustrates the average speciation of As in the influent and effluent of column C1 and C2 during the periods from d 50 to 100 and 202–250. The graph demonstrates that the As in the influent and the effluent of C2 was predominately composed of As(III); whereas the effluent of column C1 contained only As(V). There was incomplete recovery of As(V) in the C1 effluent due to its continued adsorption by AA. In contrast, the influent and effluent of column C2 were dominated by As(III). At the end of the experiment, the As(III) concentrations in the effluent of C2 was similar to that of the influent since the As(III) adsorption capacity of the column was most-likely completely exhausted.

The NO_3^- consumption and NO_2^- formation in column C1 is shown in Figure S3 as a function of column operation time. During the operation period of d 50–250, the NO_3^- consumption averaged at 0.48±0.12 mM, which was accounted for the most part by the oxidation of acetate (averaged acetate removal at 0.32±0.01 mM) and to a lesser extent by the low concentration of As(III). Negligible accumulation of nitrite was observed during the whole period. The batch experiment (Figure S4) also demonstrated that the oxidation of As(III) was attributed to denitrification in the presence and absence of acetate. Likewise, the existence of As(III) did not interfere the oxidation of acetate coupled to denitrification.

Residual arsenic in AA and sludge

At the end of the column experiment, the AA was extracted to determine the As adsorbed on the column packing. The extracted As from the column was compared with the quantity of

As retained in the column as estimated from the differences between the influent and effluent As (some of As species and total As) to make the As mass balances. The recovery of As calculated as the ratio of As extracted to As retained for both columns is shown on Table 3. There are three interesting results indicated from Table 3. Firstly, there was more adsorption of As in C1 compared to C2. Secondly, the recovery of adsorbed As species was approximately equal to the cumulative removal of As fed to the columns. Thirdly, the As species extracted from the solid phase was in the form of As(V) in C1 fed with NO_3^-; and in the form of As(III) in C2 lacking NO_3^-. The results are also consistent with a much greater adsorption capacity of AA for As(V) than As(III) as was expected from the isotherms results of earlier studies as well as the results from this study using the same batch of AA used in the columns (Table S1 and Figure S1). The dominance of As(V) in both the liquid and solid phase of C1 confirmed the occurrence of microbial As(III) oxidation by chemolithoautotrophic denitrifiers. Whereas, the effluent and solid phases of C2 contained only As(III).

The adsorbed As concentration profiles of C1 and C2 over the height of the reactors are illustrated in Figure 5. There was clearly a greater level of adsorption at the base of C1 compared to the top of the column. In C2, the As sorption was lower than C1 and there was no obvious profile. The profiles also show that the speciation of As was consistent over the column height, being predominately As(V) in C1 and As(III) in C2.

Discussion

Natural attenuation and bioremediation of arsenic in the presence of nitrate

As(III) is more common in reducing environments, due to microbial catalyzed reduction of As(V) to As(III). The formation of As(III) enhances the release and mobilization of As adsorbed on alumina (hydr)oxides into the aqueous phase. Zobrist et al. (Zobrist et al. 2000) demonstrated that As(V) reduction to As(III) by strain *Sulforospirillum barnesii* can lead to As mobilization from As(V) co-precipitated with aluminum hydroxide. A previous study illustrated that leachates generated from landfills result in the biologically catalyzed mobilization of As from As(V)-laden drinking water residuals on AA due to As(V) reduction (Sierra-Alvarez et al. 2005).

The question arises if the oxidation of As(III) would result in arsenic immobilization in aluminum (hydr)oxides dominated solid phases. In the continuous study presented here, the addition of NO_3^- supported the anoxic oxidation of As(III) to As(V) and its subsequent adsorption on AA. These findings are consistent with the adsorption studies of As in flow through columns of AA comparing As(III) with As(V). In such studies, an earlier break through of As(III) has been documented (Clifford 1990). In addition, the desorption of As(V) from the Al oxides decreased with the increasing aging time (Lin and Puls 2000). In natural soil and sediments, iron (Fe) (hydr)oxides strongly sorb both As(III) and As(V) (Dixit and Hering 2003; Lin and Wu 2001). However, microbial reduction of As(V) and ferric (hydr)oxides can lead to release of Fe and As from iron or non-iron metal oxides (Anawar et al. 2006; Sierra-Alvarez et al. 2005). In previous study, we already validates that microbial nitrate-dependent oxidation of Fe(II) and As(III) enhances the immobilization of

As in the anoxic environments (Sun et al. 2009b). Therefore, the addition of NO_3^- played an important role in improving the immobilization of As.

Microbial NO_3^--dependent oxidation of As(III) to As(V)

Diverse microorganisms, including both heterotrophs and autotrophs, have been reported to have the capacity to oxidize As(III) to As(V) in various environments (Inskeep et al. 2007; Stolz et al. 2006). The heterotrophic As(III) oxidizer most likely provide a detoxification mechanism, since they do not derived cell growth energy from the oxidation of As(III) (Silver and Phung 2005). In comparison, some chemolithotrophic As(III)-oxidizing bacteria can grow using the energy gained from the reaction (Rhine et al. 2007; Santini et al. 2000).

Recently, increasing evidence displayed the occurrence of anaerobic oxidation of As(III) under anoxic conditions with NO_3^- as an efficient alternative electron acceptor. Previous studies have illustrated that As(III) oxidizing denitrifying bacteria are widespread in nature, including As-contaminated lakes (Oremland et al. 2002; Senn and Hemond 2002) and soil (Rhine et al. 2006), as well as sludges and sediments with no known exposure to As (Sun et al. 2008). Denitrifying bacteria from the genus *Azoarcus* and *Diaphorobacter* have been isolated that can link As(III) oxidation to NO_3^- reduction to N_2 (Rhine et al. 2006; Sun et al. 2009a). By comparison of the performances of two columns in this study, microbial anoxic oxidation of As(III) to As(V) by NO_3^- was most likely the main mechanism which enhanced the As immobilization onto the AA.

Adsorption of As(III) and As(V) on AA

Adsorption isotherms of both As(III) and As(V) in this study illustrate that As(V) is more strongly adsorbed by AA compared to As(III) at circumneutral pH conditions (supplemental information), which is consistent with previous findings in literature reports (Table S1).

During the initial period of the column study from d 0–50, the effluent pH was high in the range of 9.5–10.0., The sorption of As on AA in C1 was lower than column C2, even though the As speciation of C1 was dominated by As(V) compared to As(III), which was prevalent in C2. Previous studies have shown that the adsorption of As(III) and As(V) on AA is a highly pH-dependent process (Lin and Wu 2001; Xu et al. 1991). When the effluent pH values were around 9.5–10.0, anionic $HAsO_4^{2-}$ is the main As(V) species. In contrast, As(III) occurs as mixture of the non-ionic H_3AsO_3 and the deprotonated $H_2AsO_3^-$ forms at the same pH range. Since the point of zero charge (pH_{pzc}) for different types of aluminum oxides is around 8.4–9.1 (Lin and Wu 2001), the surface of AA would be negatively charged when the pH is higher than the pH_{pzc}. Therefore the adsorption of the anionic As(V) species would be suppressed due to strong repulsion from the negatively charged surface of AA. In contrast, the less ionic As(III) species would show better adsorption than As(V) because of weaker repulsion at the high pH conditions.

Later, when the pH in the effluent was stabilized and maintained in the circumneutral range, column C1 displayed a much stronger and efficient As removal than column C2. At neutral pH, the surface of AA would be positively charged, which is favorable for the sorption of anions due to charge attraction. The negatively ionic forms of As(V) (as $H_2AsO_4^-$ and

$HAsO_4^{2-}$), would thus have a stronger interaction (specific binding) with the AA surface and have a higher adsorption affinity. In contrast, As(III) would solely be present in its non-ionic form (H_3AsO_3), which would only afford weak van der Waals forces or weak chemisorption between the solute and the alumina surface. This could explain why the As(III) adsorption on AA had broken through much earlier in column C2 than column C1 under circumneutral pH conditions. This also provides a scientific underpinning to why the oxidation of As(III) to As(V) would promote the removal of As from groundwater.

The profile of adsorbed As on AA clearly demonstrated that the adsorption process is kinetically dependent on the As speciation in the columns. The results indicate that As(V) adsorption on AA was kinetically limited in C1, but As(III) adsorption on AA was not kinetically limited in C2. There are two possibilities for the kinetic limitation in C1. Firstly, short circuiting may have occurred causing As(V) to bypass active adsorption sites on the surface of some AA. Secondly, the diffusion of As(V) into microporosity of AA was slower in the upper part of the column due to lower As(V) concentration in the upper reaches of C1.

Implications

Biogeochemical processes affecting the adsorption of As on soil and sediment minerals have been a matter of considerable research interest in order to determine the factors controlling the release of As into groundwater under anoxic environment (Oremland and Stolz 2005). This work used AA as model to determine the impact of microbial conversion of the As speciation on the mobility of As in the presence of non-iron metal (hydr)oxides. Anaerobic environments, are favorable to the microbial reduction of As(V) to As(III) increases the mobility of As in soil and sediments. To counteract the mobilization due to anaerobic conditions, NO_3^- could be utilized as an alternative electron acceptor to O_2 promote the microbial oxidation of As(III) in the saturated subsurface. This study validates a bioremediation or natural attenuation strategy based on anoxic microbial NO_3^--dependent oxidation of As(III) to As(V) which is more readily adsorbed onto aluminum (hydr)oxides in sediments. The net result was a significantly enhanced immobilization of As.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work presented here was funded by a USGS, National Institute for Water Resources 104G grant (2005AZ114G), and by a grant of the NIEHS-supported Superfund Basic Research Program (NIH ES-04940). The use of trade, product, or firm names in this report is for descriptive purposes only and does not constitute endorsement by the U.S. Geological Survey.

References

Anawar HM, Akai J, Yoshioka T, Konohira E, Lee JY, Fukuhara H, Alam MTK, Garcia-Sanchez A. Mobilization of arsenic in groundwater of Bangladesh: evidence from an incubation study. Environ Geochem Health. 2006; 28(6):553–565. [PubMed: 17120102]

APHA. Standard methods for the examination of water and wastewater. Washington D. C: American Public Health Association; 1999.

Bissen M, Frimmel FH. Arsenic - a review. Part II: Oxidation of arsenic and its removal in water treatment. Acta Hydroch Hydrob. 2003; 31(2):97–107.

Clifford, DA. Ion exchange and inorganic adsorption. In: Pontius, FW., editor. In Water Quality and Treatment. 4. American Water Works Association, McGraw- Hill, Inc; NY, USA: 1990.

Cullen WR, Reimer KJ. Arsenic speciation in the environment. Chem Rev. 1989; 89(4):713–764.

Dixit S, Hering JG. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: Implications for arsenic mobility. Environ Sci Technol. 2003; 37(18):4182–4189. [PubMed: 14524451]

Fisher JC, Hollibaugh JT. Selenate-dependent anaerobic arsenite oxidation by a bacterium from Mono Lake, California. Appl Environ Microbiol. 2008; 74(9):2588–2594. [PubMed: 18326681]

Frost RR, Griffin RA. Effect of Ph on Adsorption of Arsenic and Selenium from Landfill Leachate by Clay-Minerals. Soil Sci Soc Am J. 1977; 41(1):53–57.

Ghosh MM, Yuan JR. Adsorption of inorganic arsenic and organoarsenicals on hydrous oxides. Environ Prog. 1987; 6(3):150–157.

Goldberg S. Competitive adsorption of arsenate and arsenite on oxides and clay minerals. Soil Sci Soc Am J. 2002; 66(2):413–421.

Inskeep WP, Macur RE, Hamamura N, Warelow TP, Ward SA, Santini JM. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. Environ Microbiol. 2007; 9(4):934–943. [PubMed: 17359265]

Lin TF, Wu JK. Adsorption of arsenite and arsenate within activated alumina grains: Equilibrium and kinetics. Water Res. 2001; 35(8):2049–2057. [PubMed: 11337853]

Lin Z, Puls RW. Adsorption, desorption and oxidation of arsenic affected by clay minerals and aging process. Environ Geol. 2000; 39(7):753–759.

Manning BA, Goldberg S. Adsorption and stability of arsenic(III) at the clay mineral-water interface. Abstracts of Papers of the American Chemical Society. 1997; 213:221-GEOC.

Oremland RS, Hoeft SE, Santini JA, Bano N, Hollibaugh RA, Hollibaugh JT. Anaerobic oxidation of arsenite in Mono Lake water and by facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl Environ Microbiol. 2002; 68(10):4795–4802. [PubMed: 12324322]

Oremland RS, Stolz JF. The ecology of arsenic. Science. 2003; 300(5621):939–944. [PubMed: 12738852]

Oremland RS, Stolz JF. Arsenic, microbes and contaminated aquifers. Trends Microbiol. 2005; 13(2): 45–49. [PubMed: 15680760]

Raven KP, Jain A, Loeppert RH. Arsenite and arsenate adsorption on ferrihydrite: Kinetics, equilibrium, and adsorption envelopes. Environ Sci Technol. 1998; 32(3):344–349.

Rhine ED, Ni Chadhain SM, Zylstra GJ, Young LY. The arsenite oxidase genes (aroAB) in novel chemoautotrophic arsenite oxidizers. Biochem Biophys Res Commun. 2007; 354(3):662–667. [PubMed: 17257587]

Rhine ED, Phelps CD, Young LY. Anaerobic arsenite oxidation by novel denitrifying isolates. Environ Microbiol. 2006; 8(5):899–908. [PubMed: 16623746]

Santini JM, Sly LI, Schnagl RD, Macy JM. A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. Appl Environ Microbiol. 2000; 66(1):92–97. [PubMed: 10618208]

Senn DB, Hemond HF. Nitrate controls on iron and arsenic in an urban lake. Science. 2002; 296(5577):2373–2376. [PubMed: 12089437]

Sierra-Alvarez R, Cortinas I, Yenal U, Field JA. Methanogenic inhibition by arsenic compounds. Appl Environ Microbiol. 2004; 70(9):5688–5691. [PubMed: 15345461]

Sierra-Alvarez R, Field JA, Cortinas I, Feijoo G, Moreira MT, Kopplin M, Gandolfi AJ. Anaerobic microbial mobilization and biotransformation of arsenate adsorbed onto activated alumina. Water Res. 2005; 39(1):199–209. [PubMed: 15607178]

Silver S, Phung LT. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol. 2005; 71(2):599–608. [PubMed: 15691908]

Smedley PL, Kinniburgh DG. A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem. 2002; 17(5):517–568.

Stolz JE, Basu P, Santini JM, Oremland RS. Arsenic and selenium in microbial metabolism. Annu Rev of Microbiol. 2006; 60:107–130. [PubMed: 16704340]

Sun WJ, Sierra R, Field JA. Anoxic oxidation of arsenite linked to denitrification in sludges and sediments. Water Res. 2008; 42(17):4569–4577. [PubMed: 18762312]

Sun WJ, Sierra-Alvarez R, Fernandez N, Sanz JL, Amils R, Legatzki A, Maier RM, Field JA. Molecular characterization and in situ quantification of anoxic arsenite-oxidizing denitrifying enrichment cultures. FEMS Microbiol Ecol. 2009a; 68(1):72–85. [PubMed: 19187211]

Sun WJ, Sierra-Alvarez R, Hsu I, Rowlette P, Field JA. Anoxic oxidation of arsenite linked to chemolithotrophic denitrification in continuous bioreactors. Biotechnol Bioeng. 2010; 105(5):909–917. [PubMed: 19953675]

Sun WJ, Sierra-Alvarez R, Milner L, Oremland R, Field JA. Arsenite and ferrous iron oxidation linked to chemolithotrophic denitrification for the immobilization of arsenic in anoxic environments. Environ Sci Technol. 2009b; 43(17):6585–6591. [PubMed: 19764221]

Wang SL, Mulligan CN. Natural attenuation processes for remediation of arsenic contaminated soils and groundwater. J Hazard Mater. 2006; 138(3):459–470. [PubMed: 17049728]

Xu H, Allard B, Grimvall A. Effects of acidification and natural organic materials on the mobility of arsenic in the environment. Water Air Soil Pollut. 1991; 57–8:269–278.

Zobrist J, Dowdle PR, Davis JA, Oremland RS. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. Environ Sci Technol. 2000; 34(22):4747–4753.

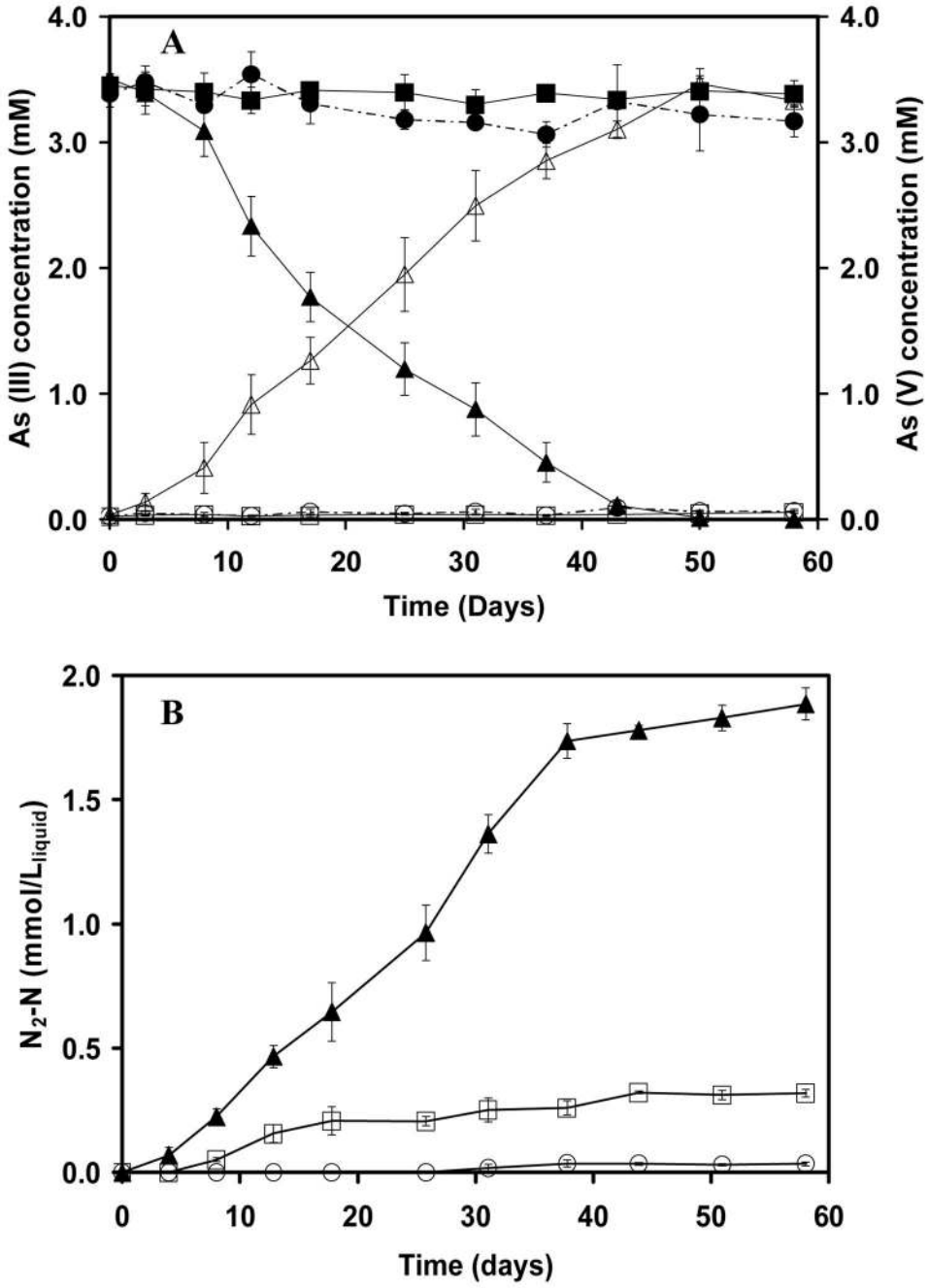

Figure 1.

Batch experiment demonstrating linkage of As(III) oxidation to complete denitrification to N_2 gas by CAODGB. Elimination of As(III) and formation of As(V) (panel A), As(III) (●) and As(V) (○) by the abiotic treatment, As(III) (▲) and As(V) (△) by CAODGB supplied with As(III) and NO_3^-, As(III) (■) and As(V) (□) by CAODGB supplied with As(III), but without NO_3^-; formation of N_2 (panel B), Abiotic control (○); CAODGB supplemented with As(III) and NO_3^- (▲);CAODGB with NO_3^-, but without As(III) (□), in all the treatments under denitrifying conditions.

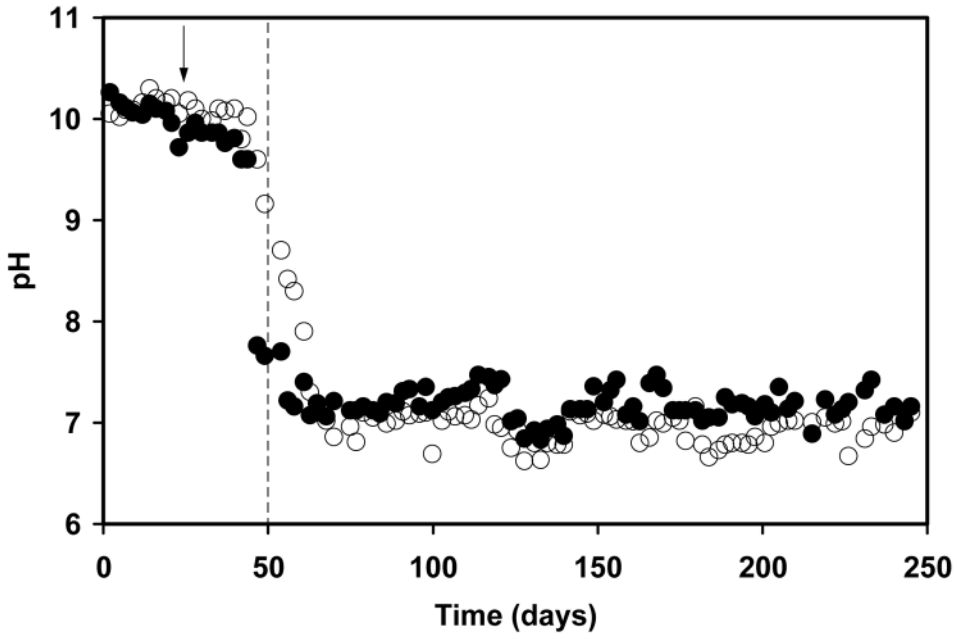

Figure 2.

Effluent pH of two AA packed columns fed with a mineral medium containing 6.67 μM As(III). Column C1 (fed with 2.5 mM NO_3^-), (●); Column C2 (without NO_3^-): (○). The vertical line on day 50 indicates the time point after which the effluent pH stabilized in the circumneutral range, when prior to day 50 the pH ranged from 9.5 to 10. The vertical arrow indicates the time point when HRT decreased to 0.5 d and the concentration of bicarbonate and phosphate buffer were lowered to 1.2 and 2.4 mM, respectively.

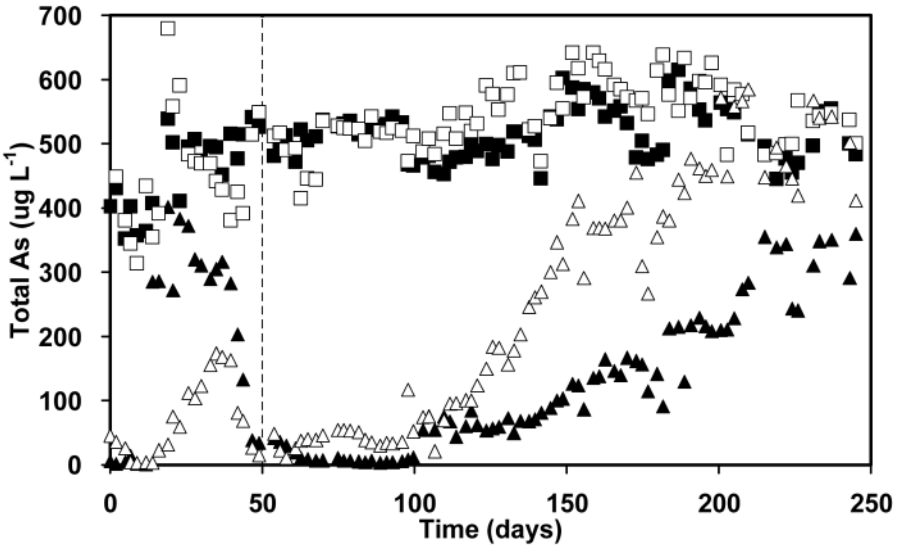

Figure 3.

Removal of total arsenic in two AA packed columns fed with a mineral medium containing 6.67 μM As(III). Column C1 (fed with 2.5 mM NO_3^-): (■) influent, (▲) effluent; Column C2 (without NO_3^-): (□) influent, (△) effluent. The vertical line on day 50 indicates the time point after which the effluent pH fell within the circumneutral pH range.

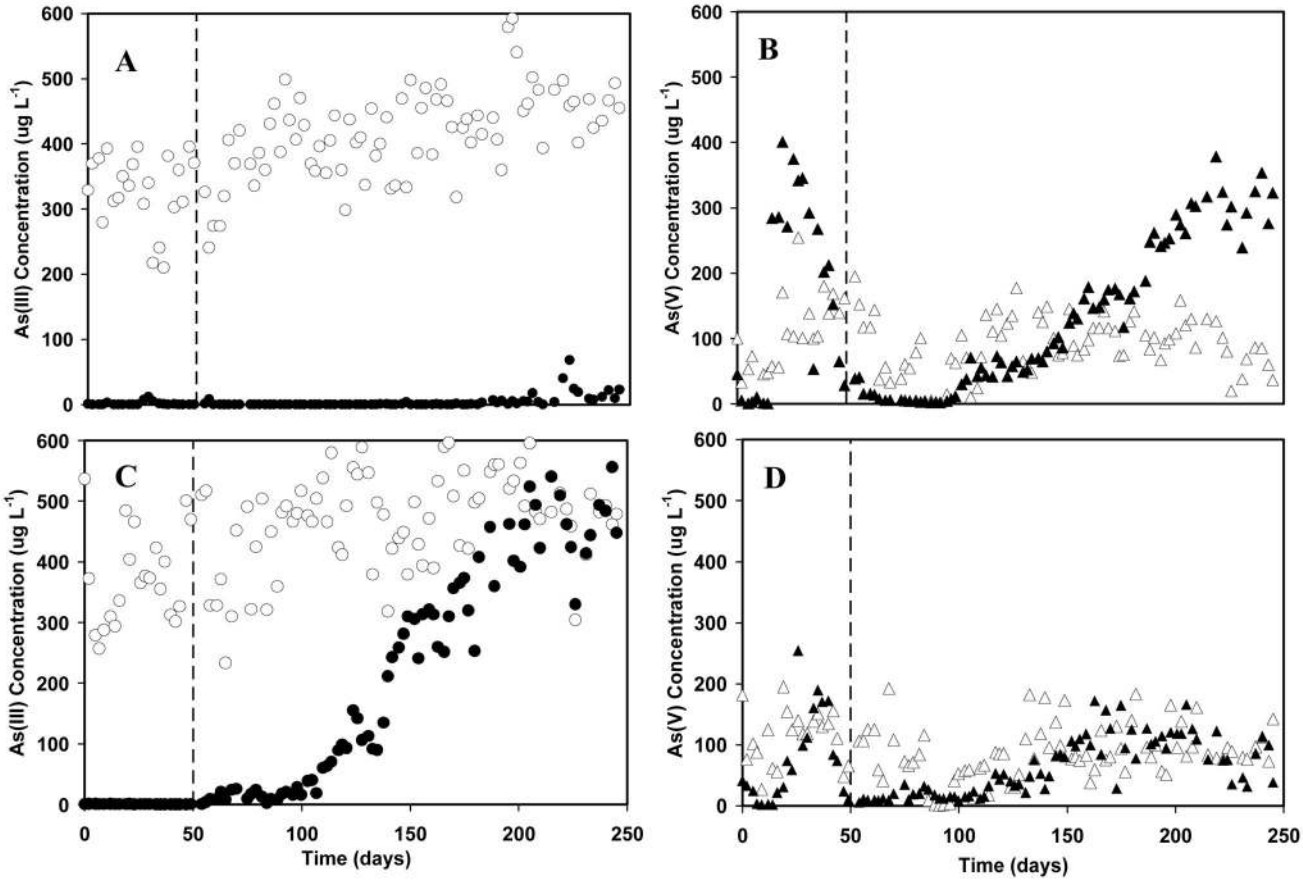

Figure 4.

Daily concentrations of As(III) and As(V) in the influent and effluent of column C1 (Panels A and B, respectively) and column C2 (Panels C and D, respectively) as a function of time: As(III) concentrations: (○) influent; (●) effluent. As(V) concentrations: influent (△); effluent (▲).

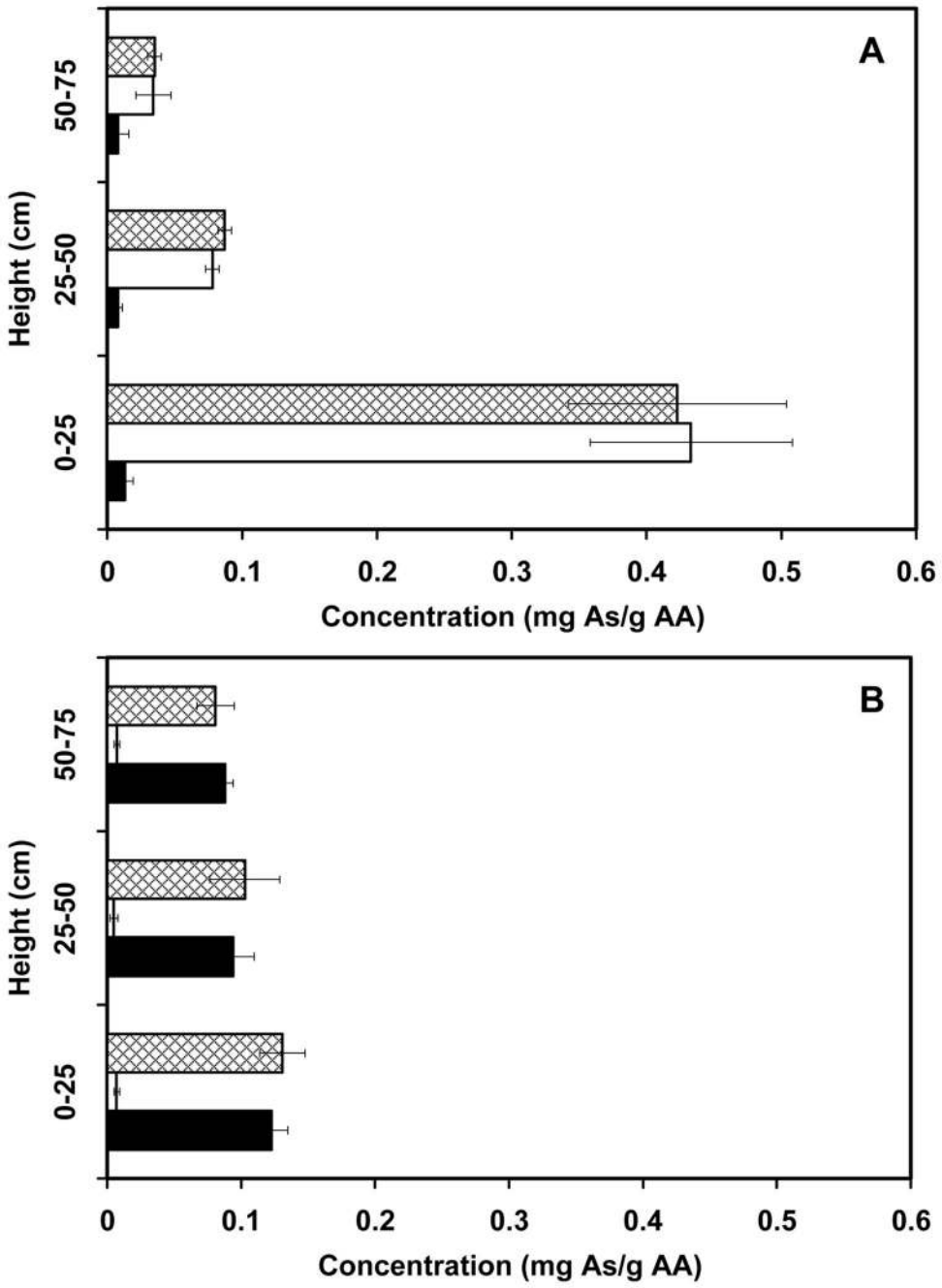

Figure 5.

The profile of sorbed arsenic in two AA packed columns at the end of the continuous experiment. Panel A: Column C1 fed with 6.67 μM As(III) and 2.5 mM. Panel B: Column C2 fed with 6.67 μM As(III) but lacking NO_3^-. Total As: (cross hatched block); As(V): (empty block); As(III): (solid block).

Table 1

Alkaline extraction protocol for extraction of solid-phase As on the AA[¥].

Initial As speciation	Adsorbed As(μg As g^{-1} AA)		Extracted As(μg As g^{-1} AA)		Sum of As recovery[†] Extracted As recovery (%)
	As(III)	As(V)	As(III)	As(V)	
AA-As(III)[*]	98.7±7.5	ND[‡]	96.5±8.2	ND	97.7±1.09
AA-As(V)	ND	109.5±0.6	ND	104.2±6.2	95.2±1.68

[¥]Known amounts of As(III) and As(V) were allowed to become adsorbed on AA to test the efficiency and conservation of speciation during the extraction protocol

[†]The sum of As species recovery was calculated from the extracted sum(As(III)+As(V))/adsorbed sum (As(III) and As(V)) × 100.

[‡]ND means that not detectable.

[*]The samples of AA with adsorbed As was separately prepared with either As(III) or As(V), respectively. The AA-As(III) means that the soluble As(III) was homogenously mixed with AA and the adsorbed As(III) was calculated from the difference of initial and equilibrium concentration of As(III). The adsorbed As(III) was subject to extraction with NaOH (2.0 M) at a temperature of 90±2°C for 12 h. The same procedure was used for adsorbed As(V) on AA as AA-As(V).

Table 2

Mass of the total As fed with influent and released with the effluent in C1 and C2 during specific intervals of the column operation.

Phase	Period (days)	Column C1			Column C2			As release ratio for C2/C1	
		Influent (mg)	Effluent (mg)	Removal (%)	Influent (mg)	Effluent (mg)	Removal (%)	interval‡	cumulative†
alkaline	0–50	11.61	4.03	65.3	12.18	1.73	85.8	0.43	NA*
circumnuetral	52–100	16.65	0.29	98.3	16.27	1.75	89.2	6.03	NA*
	102–150	16.63	2.35	85.9	18.20	6.80	62.6	2.89	3.24
	152–200	21.72	6.06	72.1	23.22	15.06	35.1	2.48	2.71
	202–250	16.70	9.94	40.5	17.29	16.49	4.6	1.66	2.15

‡ratio of mass of As released from C1 compared to C2 during interval

†ratio of cumulative mass of As released from C1 compared to C2 during circumnuetral phase

*NA, not applicable.

Table 3

Mass balance of arsenic at the end of the experiment.

Parameter[¥]	Column[*]	Cumulative As retained in the column[‡] (mg)	As extracted from column packing (mg)	Recovery[†] (%)
Sum of As species	C1	69.22	67.05	96.9
	C2	48.25	40.54	84.0
Total As	C1	65.01	63.65	97.9
	C2	50.37	39.42	78.3

[*] C1: Column fed with NO_3^- (2.5 mM) and As(III) (6.67 μM). C2: Column fed with As(III) only (6.67 μM).

[†] The recovery of arsenic calculated as the ratio of As extracted to As retained

[‡] The cumulative As retained in the column was calculated from the mass difference of As between the influent and effluent.

[¥] The Sum of As species calculated from the sum of As(III) and As(V) concentrations measured on HPLC-ICP-MS for As speciation; the Total As directly measured on ICP-MS.