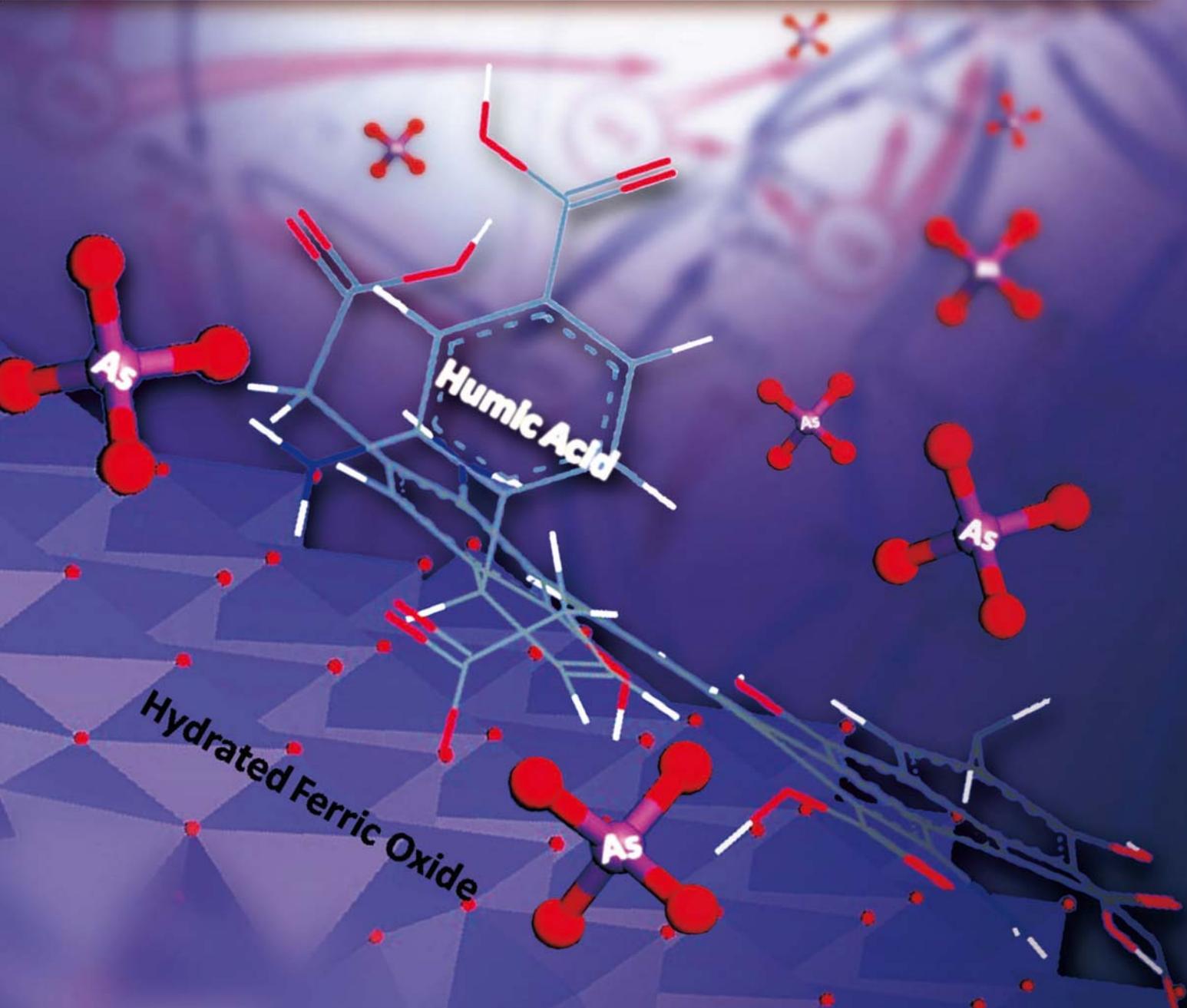


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Arsenic removal from groundwater by acclimated sludge under autohydrogenotrophic conditions

Siqing Xia*, Shuang Shen, Xiaoyin Xu, Jun Liang, Lijie Zhou

State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

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ABSTRACT

Arsenic in the environment is attracting increasing attention due to its chronic health effects. Although arsenite (As(III)) is generally more mobile and more toxic than arsenate (As(V)), reducing As(V) to As(III) may still be a means for decontamination, because As(III) can be removed from solution by precipitation with sulfide or by adsorption or complexation with other metal sulfides. The performance of As(V) bio-reduction under autohydrogenotrophic conditions was investigated with batch experiments. The results showed that As(V) reduction was a biochemical process while both acclimated sludge and hydrogen were essential. Most of the reduced arsenic remained in a soluble form, although 20% was removed with no addition of sulfate, while 82% was removed when sulfate was reduced to sulfide. The results demonstrated that the reduced arsenic was re-sequestered in the precipitates, probably as arsenic sulfides. Kinetic analysis showed that pseudo first-order kinetics described the bio-reduction process better than pseudo second-order. In particular, the influences of pH and temperature on As(V) reduction by acclimated sludge under autohydrogenotrophic conditions and total soluble As removal were examined. The reduction process was highly sensitive to both pH and temperature, with the optimum ranges of pH 6.5–7.0 and 30–40°C respectively. Furthermore, Arrhenius modeling results for the temperature effect indicated that the As(V) reduction trend was systematic. Total soluble As removal was consistent with the trend of As(V) reduction.

Introduction

Arsenic, a known carcinogen in humans, is often found in contaminated groundwater as a result of weathering of rocks, industrial waste discharges, agricultural use of arsenical herbicides and pesticides, etc. (Bhumbla and Keefer, 1994). Exposure to arsenic-contaminated drinking water has caused serious health problems in various countries, including Japan (ATSDR, 2002), Bangladesh (Wasserman et al., 2004), Shanxi Province, China (Wang et al., 2007) and Bengal (Guha et al., 2004). Because of the health effects of arsenic, most countries in the world have adopted the guideline of 10 µg/L for public water systems (WHO, 2001).

Arsenic is a metalloid (or inorganic semi-conductor) that can occur with oxidation states of -3, 0, +3, and +5. The As(V) and As(III) forms are the most common in natural waters (Cullen and Reimer, 1989; Jekel, 1994). The ratio of the concentrations of As(V) and As(III) is controlled primarily by redox potential and pH (Tallman and Shaikh, 1980; Vance and Jacobs, 1995). At pH values of most natural waters and under reducing conditions, As(III) is present as non-ionic arsenious acid (H₃AsO₃). On the other hand, As(V) dominates under oxidizing conditions and is mainly present in the oxyanionic forms (H₂AsO₄⁻, HAsO₄²⁻) (Cullen and Reimer, 1989b; Smedley et al., 1996; Yan et al., 2000).

Physical and chemical methods are the main treatment processes for arsenic-contaminated water, including anion exchange (Korngold et al., 2001; Vagliasindi and Benjamin, 1998), reverse osmosis (US EPA, 1999), chemical

* Corresponding author. E-mail: siqingxia@gmail.com

precipitation (Borho and Wilderer, 1996; Wickramasinghe, 2004; Tipping, 1981; Meng et al., 2002) and adsorption (Soner et al., 2002; Guha and Chaudhuri, 1990). In addition to physical and chemical methods, the activated sludge process and use of bacteria algal symbionts were two typical biosorption methods for arsenic removal (Goldstone et al., 1990; Jahan et al., 2006). In terms of microbial processes, Luo et al. (2008) reported one of sulfate-reducing bacteria that can reduce sulfate to sulfide as well as As(V) to As(III), so that arsenic can be effectively removed as As-sulfide insoluble precipitate. Lim et al. (2008) studied the iron-reducing bacterium *Shewanella* sp., which can reduce As(V) to As(III) with an organic electron donor. The ability of sulfate-reducing bacteria to reduce arsenic as well as other metals and metalloids has been well documented (Jong and Parry, 2003; Simonton et al., 2000).

The bio-reduction of As(V) can occur with a number of different electron donors. Many researchers have used organic electron donors, such as acetate, lactate, pyruvate, butyrate, and ethanol (Luo et al., 2008; Lim et al., 2008; Jong and Parry, 2003; Oremland et al., 1994; Dowdle et al., 1996; Macy et al., 1996; Newman et al., 1997a, 1997b; Blum et al., 1998; Harrington et al., 1998; Stolz and Oremland, 1999). Nevertheless, using an organic electron donor not only significantly increases excess sludge production, but also involves handling of chemicals that are expensive (Xia et al., 2009). Compared with organic electron donors, H₂ shows advantages as an ideal electron donor, including nontoxicity and lower production of excess biomass (Rittmann et al., 2004). Many oxidized contaminants can be reduced to less toxic or less mobile species by acclimated microorganisms under autohydrogenotrophic conditions (Nerenberg, 2005). The classical example is nitrate, which can be reduced to nitrite and nitrogen gas stepwise (Nerenberg and Rittman, 2004). Recently, more and more oxidized contaminants were shown to be reduced. For instance, bromate (BrO₃⁻) can be reduced to Br⁻ ion (Downing and Nerenberg, 2007); selenate (SeO₄²⁻) can be reduced to less mobile elemental selenium (Se⁰) (Chung et al., 2006a); and chromium can be reduced from hexavalent chromate (CrO₄²⁻) to less toxic Cr³⁺, which precipitates as Cr(OH)₃ and is removed from solution (Chunget al., 2006b).

In this article, we mainly investigated the feasibility of arsenate bio-reduction by acclimated sludge under autohydrogenotrophic conditions, as well as its kinetics. The effects of some key factors like pH and temperature on the bio-reduction process were explored. The precipitate was analyzed to determine whether the reduced arsenate was precipitated.

1 Materials and methods

1.1 Experimental setup

Plastic physiologic saline bottles with the effective volume of 250 mL were used as the reactors. The cap was butyl synthetic rubber, with air tightness, and the solution volume was 200 mL, including feed medium and inoculation sludge. Stock solution was added into the reactor with a sterile syringe. Residual air in the reactor was expelled through a syringe needle, and hydrogen was injected through another needle linked to a hydrogen tank. The reactor was covered with aluminum foil and incubated on a shaking table at 160 r/min. Each time after sampling, any residual air in the bottles was expelled, and new hydrogen gas was injected to ensure adequate supplies of hydrogen through the reaction process.

1.2 Feed medium and stock solution

The composition of the feed medium was as follows: (in g/L) NaNO₃ 0.060, NaHCO₃ 0.016, Na₂HPO₄·12H₂O 4.441, KH₂PO₄ 1.035, FeSO₄·7H₂O 0.001, CaCl₂·2H₂O 0.001; and (in mg/L) ZnSO₄·7H₂O 0.013, H₃BO₃ 0.038, CuCl₂·2H₂O 0.001, Na₂MoO₄·2H₂O 0.004, MnCl₂·4H₂O 0.004, CoCl₂·6H₂O 0.025, NiCl₂·6H₂O 0.001. Nitrate, as the most common pollutant in groundwater, was added to cultivate the microorganisms. NaHCO₃ was added as an inorganic carbon source for the microorganisms and phosphate buffer (KH₂PO₄ + Na₂HPO₄) was used to prevent any sharp pH rise, since denitrification and the reduction of sulfate as well as arsenate may cause a significant rise of pH value. All feed media were purged with N₂ gas to eliminate dissolved O₂. The pentavalent arsenic stock solution contained deionized water with 1000 mg/L of As(V) (from Na₃AsO₄·12H₂O).

1.3 Inoculum, starting up, and feasibility research

Aerobic sludge, as inoculation sludge, was obtained from the anoxic pond of Quyang Sewage Treatment Plant (Shanghai, China). The sludge concentration was about 3000 mg/L and the ratio of volatile suspended solids (VSS) to suspended solids (SS) was 65%–70%. Inoculation sludge (20 mL) was first rinsed three times with a sodium phosphate buffer (KH₂PO₄ + Na₂HPO₄ + KCl + NaCl), then added into the reactor, which contained the feed media and 10 mg N/L nitrate to accumulate microorganisms. Once nitrate and nitrite were completely removed, the denitrification process, which was maintained for 24 hr, was finished, and during the whole acclimation process the pH did not change sharply due to the presence of phosphate buffer (< 0.1 pH unit); then pentavalent arsenic stock solution was added into the reactors. To investigate the potential for bio-reducing As(V), a test group and four control groups were examined. The test group was investigated with three replications, with starting As(V) concentrations 2 mg/L and sulfate 30 mg/L, to ensure the reliability of results. The four control groups were investigated without

inoculums, sulfate, arsenate and hydrogen (using nitrogen instead), respectively, while other conditions were equal. All groups were examined at pH 7.0 and 35°C.

1.4 Influencing factors

pH and temperature were considered to be two important factors during the process. Previous results showed that the optimum pH for autotrophic reduction of various oxide contaminants was in the range of 7.0–8.2 (Lee and Rittmann, 2003; Chung et al., 2006b). Furthermore, over the past two decades, evidence has been mounting that points to a missing sink for As(III) in various aquatic and sedimentary environments (Aggett and O'Brein, 1985; Moore et al., 1988; Kuhn and Sigg, 1993; Laverman et al., 1995; Newman et al., 1997a) and demonstrated that the stability of As_2S_3 was highly sensitive to small changes in pH. In view of these findings, the initial pH was set to 6.0, 6.5, 7.0, 7.5, and 8.0, with starting As(V) concentration and temperature at 2 mg/L and 35°C respectively. The temperature can influence biochemical processes by enhancing microbial activity. Taking into account the temperature of groundwater, which is around 20°C, the experimental temperatures were set to 10, 20, 25, 30 and 35 and 40°C with As(V) initial concentration 2 mg/L and pH 7.0.

1.5 Sampling and analysis

All the fluid samples were filtered with a 0.45 μm polyether sulfone filter membrane (Anpel Company, Shanghai, Chi-

na) and kept in the refrigerator at 4°C. The samples were divided into two portions. The pH of one portion (for As analysis) was adjusted to below 2 by the addition of pure sulfuric acid (to prevent oxidation of trivalent arsenic to pentavalent arsenic) (Sato et al., 2002). The other portion without treatment was analyzed to determine NO_3^- -N, NO_2^- -N, and SO_4^{2-} , which was carried out by ion chromatography (ICS-1000, Dionex, USA) using an AS-20 column, an AG-20 precolumn, and a 150-mg/L injection loop (Xia et al., 2011). Total soluble As was measured by ICP-MS (Agilent Technologies 7700 Series, Japan) and the different valence states of As were determined by an LC-ICP-MS equipped with a CRC8 reversed-phase column (Agilent, 3 μm diam. particles, 3 mm i.d. \times 150 mm length) (Wolf et al., 2011). The pH was measured with a pHs-29A meter (HACH, USA). SEM-EDS analyses were performed by using an analytical scanning electron microscope equipped with an energy dispersive spectrometer (EDS) (XL30, Philips, Netherlands) according to the manufacturer's instructions.

2 Results and discussion

2.1 Feasibility of As(V) bio-reduction

Figure 1 summarizes the experimental results for the test group and control groups. In the test group, hydrogen and

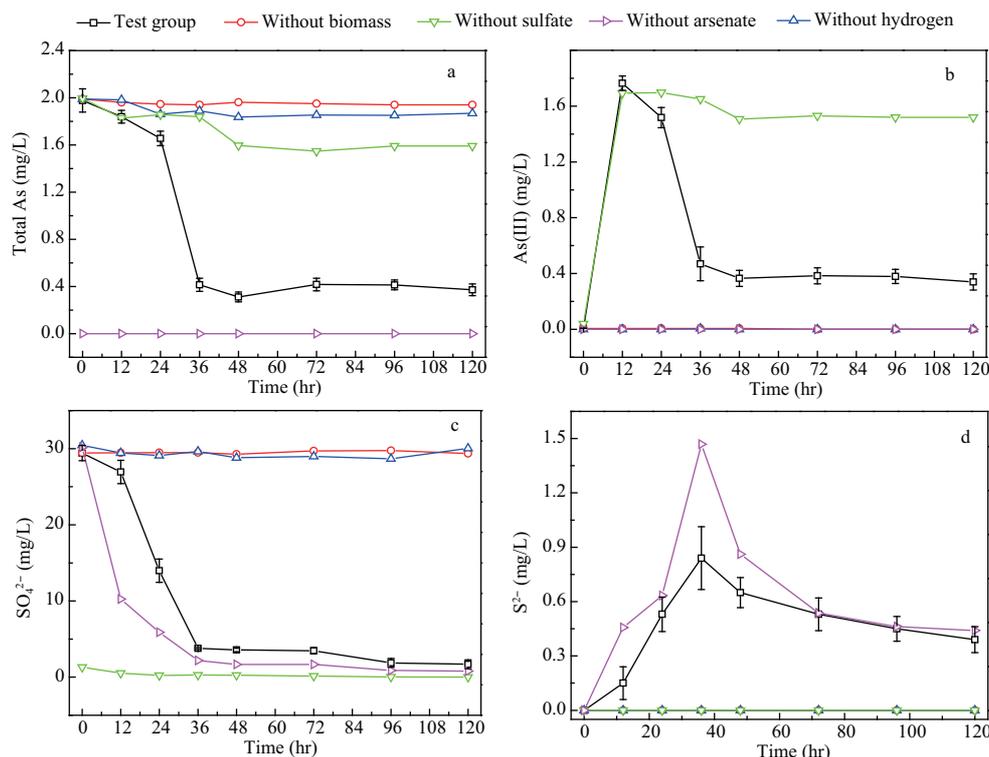


Fig. 1 Dynamic change of total soluble As (a), As(III) (b), sulfate (c) and sulfide (d) in feasibility experiments.

acclimated sludge were both provided, with As(V) 2 mg/L and sulfate initial concentration 30 mg/L. As shown in **Fig. 1a**, the total soluble As concentration dropped from 2 mg/L to 0.37 ± 0.05 mg/L within 120 hr, while the removal rate was $81.2\% \pm 2\%$. According to the results from LC-ICP-MS, the residual arsenic was composed of 0.02 mg/L As(V) and 0.35 mg/L As(III), meaning that the initial As(V) was reduced to As(III). Furthermore, as shown in **Fig. 1b**, the As(III) concentration of the test group samples increased stepwise, following a sharp decrease to a steady-state by 48 hr, while the average reduction rate of total soluble As reached as high as 0.80 ± 0.01 mg/(L·day). The results also confirmed that As(V) was converted to As(III) in the reaction process. As(V) was depleted in the end, so the accumulation of As(III) did not limit the reduction rate of As(V). The stoichiometry of As(V) reduction to form As(III) with hydrogen as the electron donor is shown as Eq. (1):



However, the As(III) detected in the test group was much lower than the amount of reduced As(V), which resulted in a larger net As removal (82%) in the presence of sulfate reduction (**Fig. 1c**). The sulfide concentration increased initially due to microbial reduction of sulfate, but decreased later on. In the control group with no addition of sulfate, **Fig. 1a** indicates a small total As removal (20%), and once steady-state was reached, the total soluble As concentration was 1.6 mg/L, much more than when sulfate reduction occurred.

EDS analysis in the region $V_a = 18.0$ kV was employed to determine the elemental compositions of the precipitates after incubation. Precipitates were collected on day 10 when the batch experiments finished. The insoluble samples were rinsed three times with a sodium phosphate buffer and air-dried (Bankar et al., 2009). The EDS spectrum in **Fig. 2** shows that the carbon, oxygen, arsenic and elemental sulfur peaks were pronounced, with weight percentages of 58.53%, 39.58%, 1.12% and 0.77%, respectively. The results indicated that reduced arsenic was probably precipitated with sulfide or by adsorption or complexation with other metal-sulfides, which is con-

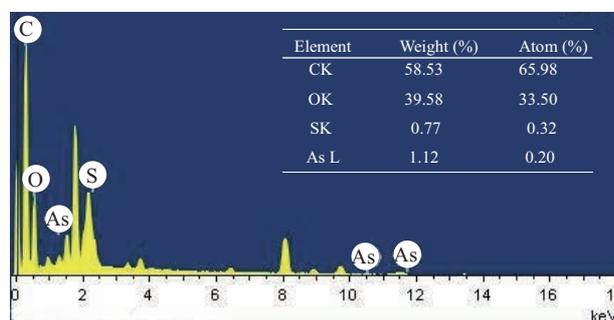


Fig. 2 EDS analysis of the sediment from test group of feasibility experiments.

sistent with results found by other investigators. They reported that arsenic existed in the precipitate as orpiment (As_2S_3) and realgar (AsS), and extensive X-ray absorption near edge structure and thermodynamic modeling were conducted to confirm the formation of arsenic sulfide (Kocar et al., 2010; Onstott et al., 2011; Xu et al., 2011; Battaglia-Brunet et al., 2012).

In this study, although the exact processes responsible for arsenic removal are not clear, it is evident that when compared to the control group containing no sulfate, sulfate reduction can greatly enhance the total As removal efficiency. Work is continuing to determine what mechanism is in fact responsible for As removal, including secondary ion mass spectrometry or X-ray absorption near edge structure studies.

In the control group with N_2 instead of H_2 , the removal percentage of total soluble As was about 6%, which showed that microbial physical adsorption contributed a small amount to As removal. That the As(III) concentration was nearly zero in this group also illustrated this point, meaning that As(V) could not be reduced by acclimated sludge without hydrogen as electron donor. When there were no microbes present, the As(V) concentration remained unchanged and the As(III) concentration was nearly zero, which demonstrated that As(V) reduction was a biochemical process. Neither As(V) nor As(III) were detected in the control group with no addition of arsenate.

In summary, acclimated sludge and hydrogen were essential. Once these conditions were available, the As(V) reduction was feasible, and it is also recognized that there was high total soluble As removal efficiency with addition of sulfate, because As(III) can be removed from solution by precipitation or complexation with sulfide.

2.2 Reduction kinetics

The kinetics of bio-reduction describes the rate of As(V) reduction. In order to identify the mechanism and the potential rate-controlling steps involved in the process of bio-reduction, the data of As(V) reduction by autohydrogenotrophic organisms was fitted using the Lagergren pseudo first-order and pseudo second-order models (Zubair et al., 2008).

The linearized form of the pseudo first-order equation can be expressed as Eq. (2):

$$\ln\left(\frac{C_t}{C_0}\right) = -k_1 t \quad (2)$$

The pseudo second-order kinetic model can be expressed in linear form as Eq. (3):

$$\frac{1}{C_t} = k_2 t + \frac{1}{C_0} \quad (3)$$

where, C_0 (mg/L) and C_t (mg/L) are the concentrations at the initial condition and at time t (min), respectively, and k_1 (min^{-1}) is the pseudo first-order rate constant, and k_2 ($\text{L}/(\text{mg} \cdot \text{min})$) is the pseudo second-order rate constant.

The coefficient of correlation (R^2) for the pseudo first-order kinetic model ($R^2 = 0.9555$, $k = -0.0407$) is much higher than that for the pseudo second-order model ($R^2 = 0.8086$, $k = 0.0736$). The result implied that the pseudo first-order kinetic model better described the reaction kinetics than the pseudo second-order one. The results fitted with the first-order model are shown in **Fig. 3**. Evidence had been found that for most dissimilatory metal reduction processes, first-order models were good and comparable (Liu et al., 2002).

2.3 Effect of pH

The effect of solution pH on the As removal process was investigated. In this research, at the end of the experiments, the pH did not change sharply due to the presence of phosphate buffer (< 0.2 pH units), so the pH change versus time was ignored.

As shown in **Fig. 4a**, the As(V) reduction rate was relatively higher at pH = 6.5 and 7.0 than at pH = 6.0, 7.5 and 8.0 under the same conditions. Steady-state reduction of As(V) to As(III) was evident by 12 hr and the maximum

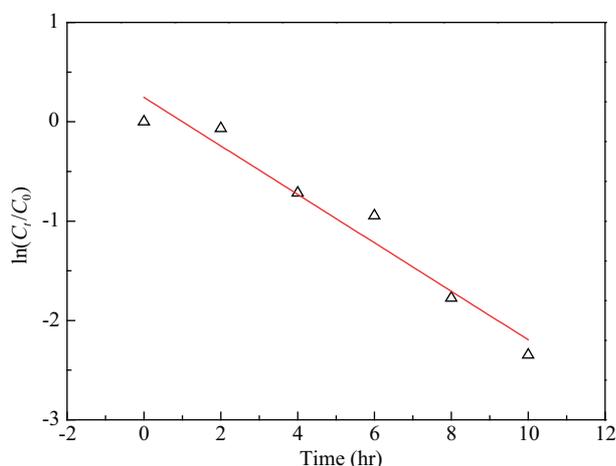


Fig. 3 Pseudo first-order kinetic plots for As(V) reduction.

As(V) reduction rate reached as high as 0.16 mg/(L·hr) at pH 7.0. Finally, As(V) concentrations all declined to nearly zero at the different pH values. The results showed that it was more suitable for As(V) reduction to control the pH between 6.5–7.0. The results in **Fig. 4b** show that the maximum total soluble As removal percentage took place at pH 7.0, with a removal percentage above 85% and concentration 0.29 mg/L. In contrast to As(V) reduction, once total soluble As reached steady-state removal, the total soluble As concentrations at pH = 6.5, and 7.0 (0.34 and 0.29 mg/L, respectively) were less than for pH = 6.0, 7.5 and 8.0 (0.49, 0.41, and 0.47 mg/L, respectively). As a whole, the maximum removal percentage was obtained between pH 6.5–7.0.

2.4 Effect of temperature

The influence of temperature on the biochemical reduction of As(V) is shown in **Fig. 5a**. In summary, the As(V) reduction rate was relatively faster at 30, 35 and 40°C after 36 hr the removal rates tended to balance with the reduction rates of 1.27, 1.29, and 1.31 mg/(L·day) respectively, and As(V) reduction percentages were all above 95%. When the temperature decreased to 20 or 25°C the performance of the microbes was not good, for the reduction rates were lower after 36 hr (0.80 and 1.17 mg/(L·day), respectively). This demonstrated that low temperature could possibly limit microbial metabolism and reduce its activity so as to inhibit the bio-reduction of As(V) to As(III). It is noteworthy that the As(V) concentration was fairly high at the temperature of 10°C with the average reduction rate of 0.18 mg/(L·day). Furthermore, the removal curve of total soluble As in **Fig. 5b** was consistent with the trend of As(V) reduction. Since sulfate reduction was also inhibited by lower temperature, total soluble As concentrations at 10 and 20°C (1.72 and 1.32 mg/L, respectively) were much higher than at 25, 30, 35 and 40°C (0.48, 0.50, 0.39 and 0.39 mg/L, separately) once total soluble As removal achieved steady-state.

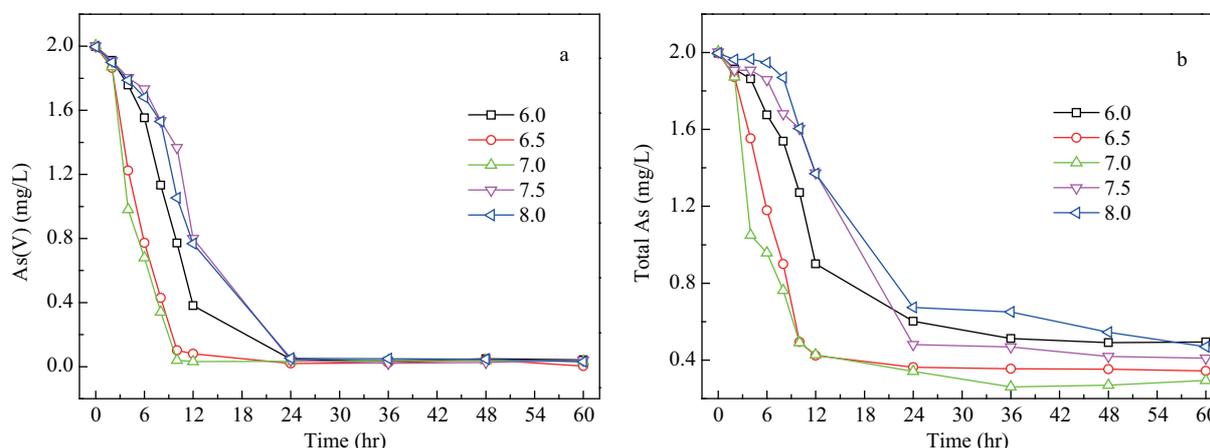


Fig. 4 Effect of solution pH on (a) As(V) reduction and (b) total soluble As removal.

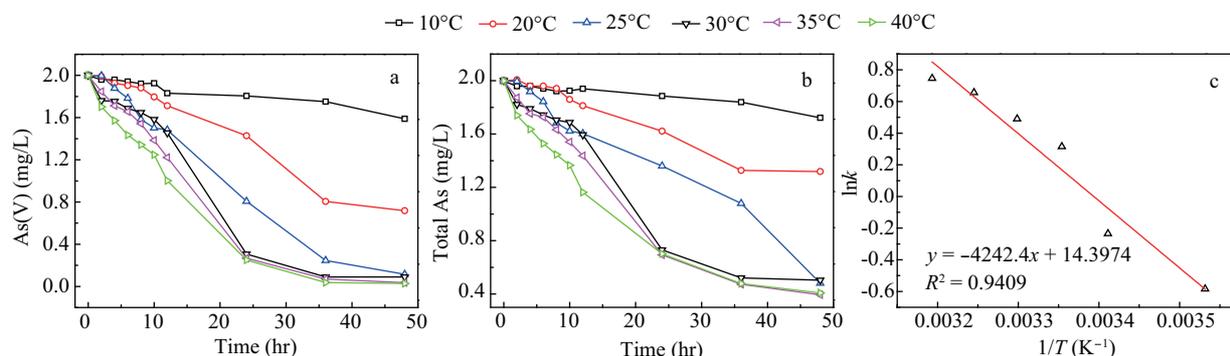


Fig. 5 Effect of temperatures on As (V) reduction (a), total soluble As removal (b) and Arrhenius modeling results for the temperature influence (c).

The results were also fitted with the Arrhenius model as shown in **Fig. 5c**. The linearized form of the Arrhenius equation is given as follows:

$$\ln k = -E_a/RT + \ln A \quad (4)$$

where, k is the rate coefficient; E_a (J/(g·mole)) is the activation energy; and T (K) is the absolute temperature and A is the pre-exponential factor, which is the pre-exponential constant, and it is 1.79×10^6 in this study. Clearly, As(V) reduction was sensitive to temperature and the trend was systematic.

As a whole, a high As(V) reduction rate can be achieved between 30–40°C and the optimum temperature was 40°C in the researched range. Furthermore, the reduction process would be inhibited below 30°C. The results demonstrated that higher temperature, which could maintain higher microbial activity, led to a faster reduction rate. Consequently, in actual operations the temperature range should be kept above 25°C to accelerate the biochemical reduction process.

3 Conclusions

The present article investigated the bio-reduction of As(V) to As(III) by acclimated sludge and total soluble As removal from synthetic groundwater when sulfate reduction occurred. First, feasibility research demonstrated that As(V) reduction was a biochemical process while acclimated sludge and hydrogen were essential. Secondly, As(III) was detected and most of the reduced arsenic remained in a soluble form, although 20% was removed with no addition of sulfate, while 82% was removed when sulfate reduction occurred. The results showed that As(V) was first reduced to As(III) and then precipitated in the reactor, probably as arsenic sulfides, which was also verified by EDS of the sediment. It was observed that there was high total soluble As removal efficiency with addition of sulfate. Kinetic analysis showed that the pseudo first-order kinetic model better described the bio-reduction process

than the pseudo second-order model. Thirdly, research on influencing factors indicated the reduction process was highly sensitive to both pH and temperature, with the optimum ranges of pH 6.5–7.0 and 30–40°C respectively. Furthermore, Arrhenius modeling results for the temperature influence indicated that the As(V) reduction trend was systematic. Total soluble As removal was consistent with the trend of As(V) reduction.

Acknowledgments

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