

The effects of organic materials on microbial mediation of arsenic in contaminated sediment

Jong-Un Lee¹, Sang-Woo Lee², Kyoung-Woong Kim²

¹Chonnam National University, Gwangju, Korea

²Kwangju Institute of Science and Technology, Gwangju, Korea

Abstract: Indigenous bacterial mediation of As in contaminated sediment after biostimulation with a variety of carbon sources was investigated under aerobic and anaerobic conditions. Under the aerobic condition with lactate supply, indigenous bacteria increased the amount of total As extracted from the sediment and most dissolved As existed as As(V). Under the anaerobic, glucose-supplied condition, dissolved As diminished with time likely due to production of As sulfide(s) and subsequent precipitation, which resulted from bacterial reduction of SO_4^{2-} . The results implied that bacterial natural attenuation of As in subsurface has a potential to be practically applied.

1. Introduction

Long-term mining and smelting of As-bearing ores resulted in significant contamination of nearby air, soil, sediment, and aquatic systems with As. Soil and sediment contaminated with As can serve as potential sources of As pollution to distant downward ecosystems through stream and river. The enrichment of As in solid substrates is generally due to accumulation of dissolved As to ferrihydrite and alumina through adsorption and coprecipitation.

In natural settings, As predominantly exists in two oxidation states: As(III) and As(V). Arsenite (As(III)) which occurs as a main species under reducing environments has higher mobility and toxicity than arsenate (As(V)). Some bacteria can alter electrochemical species of As by either detoxification or dissimilation under aerobic and/or anaerobic conditions. Such influence of bacteria on changes in redox state of As plays an important role in the biogeochemical cycling of this toxic element in nature (Oremland and Stolz, 2003).

While many studies on transport and fate of As in oxidizing surface environments were carried out so far, only a few investigations on As behavior in reducing deep subsurface have been reported. In addition, development of bioremediation technology for As-contaminated subsurface has been rarely tried. Biodegradation of toxic organic compounds to harmless ones can be readily attained since the organics act as carbon sources for biomass increase. However, hazardous inorganic contaminants including As are not biodegradable. Also when the As contamination occurs at deep subsurface, employment of conventional physicochemical treatment methods will need a high cost and sometimes access itself to the sites will be impossible.

Microbiological natural attenuation may be considered as one of the best alternative. As described above, some bacteria can decrease solubility and toxicity of As by changing its oxidation state. Arsenic can be sequestered from groundwater and porewater and stabilized onto surfaces of solid matrices such as iron oxides and hydroxides. In this case, the ultimate goal is not removal or reduction of As from subsurface but containment underground and subsequent isolation from ecosystem. If alteration of As species by indigenous bacteria is kinetically slow, biostimulation which involve artificial supply of nutrients can be considered.

The fundamentals of microbiological natural attenuation of U, Cr, and Se are laid on the electrochemical reduction of these redox-sensitive elements since toxicity and mobility of them become lower in the reduced state. In comparison, in the case of As, As(III) shows higher toxicity and mobility, and thus reducing environments can instigate enhanced transport of As to biosphere through groundwater flow. Especially, since deep sediment and aquifer and tailings heaps artificially covered with soil will readily form reducing circumstances in their lower parts, the environmental concern about As behavior becomes significant.

In the present study, speciation and behavior of As in naturally As-contaminated sediment in response to As-bacteria interaction were investigated under aerobic and anaerobic conditions. Indigenous bacteria in the sediment were activated through biostimulation by supply of various carbon sources. Natural sediment systems are subject to supply of a variety of organic materials, and a seasonal variation of temperature can additionally increase in bacterial activity. Through this study, a potential of natural attenuation of As by indigenous bacteria as a novel remediation technology was to be examined.

2. Materials and Methods

Sediment contaminated with 340 mg/kg of As was taken near an abandoned Au mine in Bosung, Chonnam. To examine modes of As occurrence in the sediment, a sequential extraction technique was applied.

For experiments under an aerobic condition, 3% (w/v) of the sediment samples were added to sterile deionized water (300 mL) and supplied with 3% (w/v) of acetate, lactate or glucose separately. The slurry was incubated at 30 °C on a 150 rpm shaker up to 22 days. To distinguish the effects of indigenous microorganisms, autoclaved, sterile control samples under same conditions were included in the experimental set. During the incubation, aliquots of the solutions were taken at set time intervals and pH was measured. After completion of incubation, total dissolved As, As(III), Fe and some heavy metals (Pb, Zn, Cu, and Cr) were determined and the sediment samples were removed and the sequential extraction technique previously described was applied. The experimental procedure included 2-3 replicates.

For anaerobic incubation, after the wet sediment and pH 5 buffer (0.1 M $(\text{NH}_4)_2\text{HPO}_4$) were mixed at 1:2 ratio (w/v) and shaken for 30 minutes, 5 mL of supernatant were taken as an inoculum. The sediment was dried and homogenized and 9 g of the sediment was separately put in 300 mL of experimental solutions which contained deionized water or each 5 mM of acetate, lactate, or glucose, pH of which were adjusted at 7.0. The slurry was inoculated with the inoculum described above and shaken at 150 rpm up to 14 days under an anaerobic condition with $\text{CO}_2\text{-H}_2$ atmosphere. Sediment-solution mixtures under the same conditions were autoclaved and used as controls. At set time intervals, pH, total As, As(III), Fe^{2+} and some anions (Cl^- , NO_2^- , NO_3^- , SO_4^{2-}) were determined. To monitor bacterial growth with time, 20 μL of supernatant of the slurry was periodically removed and put in 5 mL of TSB (trypticase soy broth). The TSB was incubated for a day and O.D. (optical density) was measured.

Arsenite was separated from As(V) through a silica-based strong anion cartridge (LC-SAX SPE, Supelco). Hydride generating AAS (HG-AAS, Perkin Elmer 5100) was used for determination of total As and As(III) and As(V) was calculated from the difference between these two. Heavy metals and anions were determined using ICP and IC, respectively. Optical density and Fe^{2+} stained with ferrozine were measured at 600 nm and 562 nm, respectively, using UV-vis spectrometer.

3. Results and Discussion

The results of the sequential extraction technique revealed that As in the studied sediment mainly existed as Fe-associated (40%) and residual (47%) fractions.

After 22-day incubation under the aerobic condition, the remained As concentrations in the sediment showed no significant differences with the sterile controls in the cases of acetate and glucose addition as carbon sources. However, in the case of lactate, non-sterile sediment contained less amount of As (131 mg/kg) than sterile sediment (176 mg/kg), indicating that, whether it was direct or indirect, the effects of indigenous microorganisms fed with lactate facilitated As extraction from the sediment. The result showed that bioleaching of As can be enhanced by supply of carbon source(s) and that such enhancement depends on the specific type of carbon source. The result also implied that practical application of conventional biostimulation techniques to remove toxic organic compounds for the sites which are concomitantly contaminated with organics and As can adversely cause an enhancement of As extraction from solid substrates.

In the case of glucose addition, some slimy materials appeared to occur in the solution with time. Such materials were considered as extracellular polymeric substances (EPS) which were produced by bacterial activity. If this were the case, virtually dissolved As into the solution might be considered as undissolved As since the EPS readily adsorbed As extracted from the sediment.

Species of dissolved As showed an apparent difference between the controls and the non-sterile sediments. While As(III) concentrations were almost similar with As(V) in the sterile controls, nearly all dissolved As was predominantly present as As(V) in the non-sterile sediments (Fig. 1). The result indicated that indigenous bacteria increased the total amount of extracted As and subsequently transformed the dissolved As(III) to As(V) in the solution. In a sense of microbiological remediation of As, such enhancement of total As mobility may constraint the practical application of this technique; contradictorily, since As(V) is more immobile and less toxic than As(III), the observed microbial transformation of As(III) to As(V) will positively serve during the remediation procedure. If the extracted As(V) is adsorbed to or coprecipitated with sediment particles, an effective As sequestration can be expected. However, if the extracted As(V) transports to reducing environments in lower subsurface, it will be reduced and mobilized.

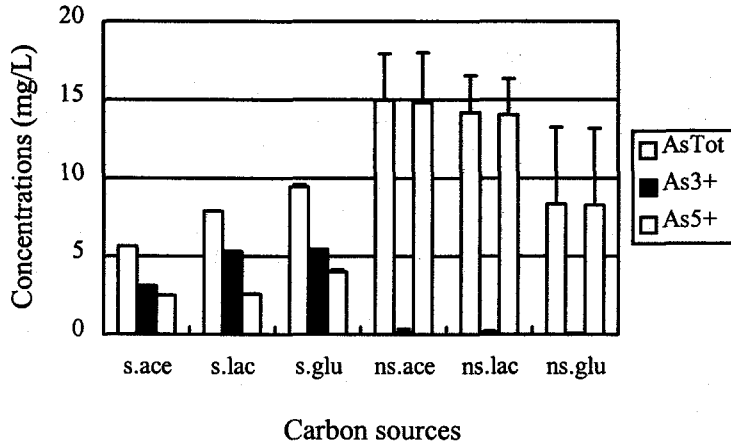


Fig. 1. Speciation of As extracted from the sediment after 22-day incubation with various carbon sources under an aerobic condition. s.: sterile, ns.: non-sterile, ace: acetate, lac: lactate, glu: glucose.

In the case of anaerobic incubation, glucose-added sediment showed a dramatic decrease in total dissolved As after 6 days from the onset of incubation while the other carbon sources showed no significant influence on As mobility (Fig. 2). Since the ratio of As(III)/total As was also high in the non-sterile, glucose solution, the observed decrease in dissolved As was not likely due to oxidation of As(III) to As(V) and subsequent adsorption to solid surfaces. In the same sediment, Fe^{2+} concentration gradually increased after 2 days, likely due to microbial Fe(III) reduction. Ferrous ion can act as a reducing agent of As(V) to As(III). Production of As sulfide(s) (e.g., As_2S_3) from As(III) and S^{2-} and subsequent precipitation was likely responsible for the observed decrease in total dissolved As. The anion determination showed that SO_4^{2-} concentration decreased in the solution with time, indicating bacterial SO_4^{2-} reduction. Dissolved SO_4^{2-} has been reported to be reduced to reactive S^{2-} and to form As trisulfide by bacteria (Newman et al., 1997a; 1997b).

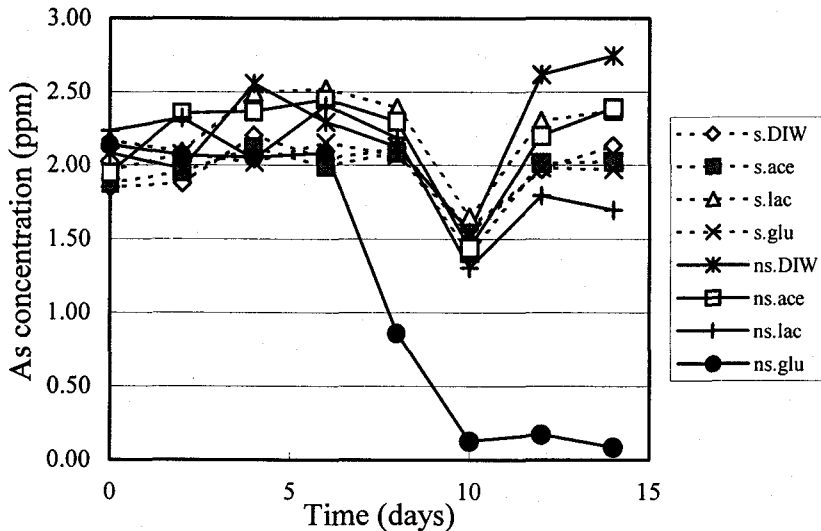


Fig. 2. The variation in total arsenic concentrations versus time during anaerobic incubation. s.: sterile, ns.: non-sterile, DIW: deionized water, ace: acetate, lac: lactate, glu: glucose.

4. Geological and Environmental Implications

According to combined results of both aerobic and anaerobic incubation of the contaminated sediment after supply of a variety of carbon sources, As stabilization in the subsurface was ultimately expected (Fig. 3).

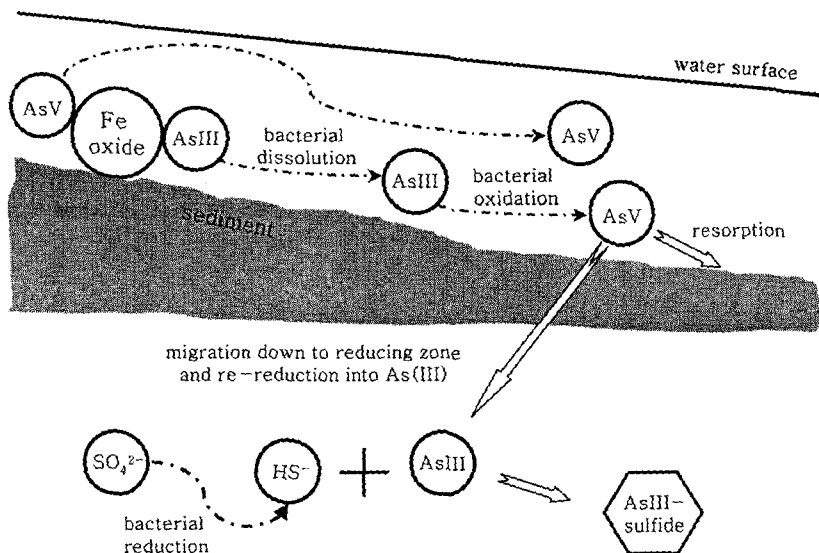


Fig. 3. Schematic diagram of a model representing bacterial effects on As behavior in sediment in a range of depth.

Arsenic moving downward in subsurface will be transformed to As(III) and thus mobility will increase; this can lead to negative effects with a viewpoint of application of natural attenuation. However, based on the results of this study, biotic reduction of the other main compounds such as Fe(III) and SO_4^{2-} could affect As behavior and finally stabilize it. Conclusively, natural attenuation technology appeared to have a potential to be successfully applied in remediation of As which shows an enhanced mobility under deep, reducing environments unlike U, Cr, and Se. In addition, since biostimulation using appropriate carbon source(s) was effective to activate indigenous bacteria, proper carbon source(s) should be determined in prior to practical application.

References

- Newman, D.K., Kennedy, E.K., Coates, J.D., Ahmann, D., Ellis, D.J., Lovley, D.R., and Morel, F.M.M., 1997a, Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov., Arch. Microbiol., Vol. 168, 380-388.
- Newman, D.K., Beveridge, T.J., and Morel, F.M.M., 1997b, Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*, Appl. Environ. Microbiol., Vol. 63, 2022-2028.
- Oremland, R.S. and Stolz, J.F., 2003, The ecology of arsenic, Science, Vol. 300, 939-944.