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Effects of Mn(II) and Fe(II) on microbial removal of arsenic (III)

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Abstract
Goal, scope, and background Arsenic contamination in groundwater creates severe health problems in the world. There are many physiochemical and biological methods available for remediation of arsenic from groundwater. Among them, microbial remediation could be taken as one of the least expensive methods, though it takes longer treatment time. The main objective of this research was to study the improvement on remediation by addition of some essential ion salts such as Mn and Fe.
Materials and methods Staphylococcus aureus, *Bacillus subtilis*, *Klebsiella oxytoca*, and *Escherichia coli* were taken as model microbes from Dhulikhel, 30 km east from Kathmandu, Nepal.
Results and discussion Microbes used in this study showed different abilities in their removal of As(III) with and without addition of Mn and Fe salts. The trend of remediation increased with time. *S. aureus* was found to be the best among the microbes used. It showed almost 100% removal after 48-h culture, both with and without Fe and Mn salts. Rate of removal of As increased with addition of Fe and Mn for all microbes. Removal efficiency was found to increase by about 32% on average after addition of salts in 24-h cultures of *S. aureus*.

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1 Introduction

Arsenic is a nonessential and toxic metalloid. It occurs naturally in organic and inorganic forms. It is also released by human activity through farming, mining, industrial activity, and burning fossil fuels to the environment. As contamination in groundwater used for drinking purpose is a serious threat and almost billion people are affected (Spallholz et al. 2004; Mondal et al. 2006). The predominant form of inorganic arsenic in aqueous oxic environment is arsenate and in anoxic environment are arsenite and sulfide. In organic form, the most common are methyl and dimethyl-arsenate. From an aerobic reduction, there is formation of trimethylarsin from dimethylarsonic acid, while dimethylarsin from methylarsonic acid is formed in an anaerobic condition. The percentage abundance of As (III) is only 40% compared to that of As(V), but As(III) is ten times more toxic than As(V). (Oremland and Stolz 2003). Arsenates enter the cell via the uptake system for phosphate and block the oxidative phosphorylation because they cannot form stable high-energy compounds. Arsenate inhibits adenosine triphosphate synthesis by uncoupling oxidative phosphorylation and replacing the stable phosphoryl group. Arsenite reacts with free thiol groups in proteins and acts as a potent inhibitor of many important metabolic reactions, e.g., the production of coenzyme A and the citric acid cycle. The uptake of arsenite is thought to occur via transporters of glycerol.

There are several techniques to remove As from contaminated water such as coagulation–coagulation, precipitation,

lime softening, adsorption, ion exchange, and electrodialysis (Mondal et al. 2006). The microbial remediation of arsenic might receive increased attention as it requires no chemicals and the microbes are native to the contaminated water. The percentage oxidation has already been reported as 80% at very low pH, i.e., 1.5–3. This should be increased to 6–8 to satisfy drinking water standards. It was reported that microbes, e.g., *Acidithiobacillus caldus*, *Escherichia coli*, *Klebsiella oxytoca, Staphylococcus aureus, Bacillus subtilis*, have resistance to arsenic and are nonpathogenic in normal conditions (Shivaji et al. 2005; Leblanc et al. 1996; Clausen 2000; Groot et al. 2003). Kostal et al. 2004 has shown, by genetic modification of *E. coli*, that the As(V) and As(III) removal efficiency can be improved by 60 and fivefold, respectively. Previous studies show that microbial oxidation could be used to reduce the toxicity of arsenic; microbes have a high tolerance to arsenic and have the capacity to remove the arsenic if they are genetically modified. The remediation rate might be low when microbes are used alone. There is no study undergoing with nonmodified microbes in the presence of Fe(II) and Mn(II) salts.

There are high concentrations of Fe and Mn in groundwater of Nepal, India, Bangladesh, and other arsenic-affected countries. Fe and Mn are released into groundwater with arsenic (Spallholz et al. 2004; Rahman et al. 2005). These salts might help microbes to remove arsenic from the drinking water. The problem of groundwater contamination with arsenic is a topic of extensive discussion and study nowadays. Investigations on the influence of different salts on microbial arsenic removal are very important for remediation. Therefore, the aim of this research was to study the effect of Mn(II) and Fe(II) salts on microbial removal of arsenic from groundwater.

2 Materials and methods

2.1 Isolation of microorganisms from soil samples

Homogeneous suspension of a 0.1-g soil sample collected from Dhulikhel, 30 km east from Kathmandu, Nepal, was prepared by adding 10 mL of double-distilled water. The suspension was centrifuged at 1,000 rpm for 10 min to separate all suspended particles. Clarified samples were serially diluted, spread on nutrient agar plates, and incubated at 298 K for 48 h. Colonies were subcultured continuously until pure cultures were obtained. Several morphological and biochemical studies were performed (Aneja 2004). Pure culture was inoculated in brain hearth infusion broth for 18 h and biochemical tests performed using biochemical test kit from Himedia. Four bacteria *S. aureus*, *E. coli*, *B. subtilis*, and *K. oxytoca* were isolated for the analysis (Wang 2004).

2.2 Preparation of stock culture media and chemical solutions

The stock culture media were prepared by suspending 7.0 g of the nutrient agar (Himedia) in 250-mL double-distilled water and boiling to dissolve the medium completely. It was sterilized by autoclaving at 103-kPa pressure (394 K) for 15 min. One loop full of bacteria was inoculated in 20-mL cold media and incubated at 310 K for 48 h. Four same sets were taken for each analysis to check reproducibility.

For the arsenic stock solution, 13.2 mg of As_2O_3 (M. W.197.84, Himedia, A.R. grade) was dissolved in double-distilled water to prepare 100-mg L^{-1} AS(III) solution. To prepare 100-mg L^{-1} Fe(II) solutions, 49.75 mg of $FeSO_4.7H_2O$ (M.W 277.85, Qualigens, A.R. grade) was dissolved in 100-mL solution. To prepare 100-mg L^{-1} Mn (II) solutions, 36.6 mg of $MnCl_2.4H_2O$ (M.W.197.90, Qualigens, A. R. grade) was dissolved in 100-mL solution.

2.3 Inoculums media preparation (Himedia)

Inoculums media were prepared by suspending 6.5 g of nutrient broth (Himedia) in 500-mL double-distilled water and heated to dissolve medium completely. It was sterilized by autoclaving at 103-kPa pressure (394 K) for 15 min. A loop full of bacteria was inoculated from the stock culture in 100-mL cold media solution. The media was kept in the incubator at 310 K for 48 h.

2.4 Preparation of growth media (Himedia)

The growth media was prepared by suspending 13 g of nutrient broth (Himedia) in 1,000-mL distilled water and heating to dissolve the medium completely. It was sterilized by autoclaving at 103-kPa pressure (394 K) for 15 min.

2.5 Treatment of microorganisms with arsenic and its analysis

Eight different experiments with each microorganism species were carried out with and without iron and manganese solution in 0, 2, 4, and 6-mg L^{-1} concentrations of arsenic after 0, 6, 18, and 48 h. In each case, 0.5 mL of inoculum media solution was mixed with different concentrations of arsenic solution and 1 mL of each Fe and Mn solutions by maintaining 5 mg L^{-1}. Then, growth media solution was added to make up the volume of 20.5 mL. Each experiment was carried out in an incubator at 310 K. pH and Eh were measured after this in all cases. Samples were taken from the incubator and centrifuged at 1,500 rpm for 15 min. The suspended particles were removed and conc. HNO_3 was added to the solution to prevent further microbial consumption of metal. Arsenic(III) present in these acid-treated

solutions was determined by AAS hydride analysis (Thermo Electron Corporation, 949940021001, Solar MAA system).

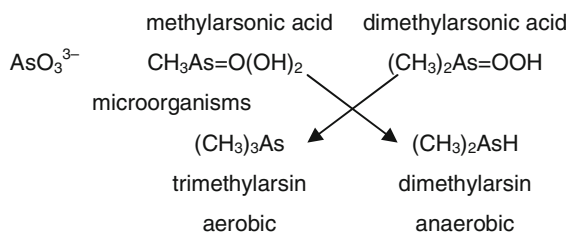
3 Results

Four model microbes showed an effect on the removal of As(III) in 48-h-long experiments with and without Mn and Fe salts (Fig. 1). The average pH and Eh were observed as 6.9 and −0.2 V, respectively. The trend of remediation increased with respect to time. There were considerable variations among these microbes to remove the arsenic. *S. aureus* was the best among them. It showed 100% removal of As at the end of the experiment, both with and without Mn and Fe salts. Rate of removal of As increased with addition of Mn and Fe salts in the system. The addition of salts showed remarkable effects on *B. subtilis.* At the end of the experiment, nearly 100% removal was observed.

The removal percentage of arsenic by *S. aureus* in 24 and 48 h, with and without Mn and Fe salts, gave a good picture of the effect of addition of salts. There was only an average of 62% removal of As(III) without salts in 24 h. On an average, there was a 32% increment removal after addition of salts. It seems that there was no effect by addition of salts after 48 h because this microbe had 100% removal capacity even without salts.

4 Discussion

Four microbes used in this work had the capacity to remove As(III) from contaminated water, although the differences between the effects of various microorganism were not significant. *S. aureus* was best among them. Increase in the removal of As(III) shows that microbes can survive in this environment. It was also reported that many microorganisms, including bacteria, archaea, and fungi, show a resistance to arsenic toxicity (Bröer et al. 1993). A common mechanism of resistance involves the reduction of intracellular As(V) to As(III) by As(V) reductases because As(III) is the substrate of efflux pumps (Oremland and Stolz 2003; http://www.boil.lu.se). In some cases, the following mechanism might follow:



The above mechanism shows only the conversion of inorganic As(III) to less toxic organic AS(III) species. But removal of As(III) showed that there was an adsorption of conversion of As(III) to As(V) by microbes. Therefore, it might show the ability of the microbes to oxidation of As (III) to less toxic As(V) by stepwise methylation conversion (Hall 1997; Thomas et al. 2004):

$As(III)O_3^{3-}+CH_3^+$	$\rightarrow CH_3^+As(V)O_3^{2-}+2e$
$CH_3^+As(III)O_2^{2-}+CH_3^+$	$\rightarrow (CH_3)_2As(V)O^{2-}+2e$
$(CH_3)_2As(III)\ O^-+CH_3^+$	$\rightarrow (CH_3)_3As(V)O^-$

This less toxic As(V) might accumulate on them as it is known that As(III) is toxic not only to human beings but also to microbes at an elevated level. Preetha and Viruthagiri (2007) explained the accumulation of chromium in microbes in the study. Maybe in the same way, these microbes could accumulate in cells by way of different metabolisms. They might take As(III) in dissolved form and convert it into less toxic forms (the mechanism is still unknown).

It is clearly seen that the removal of As(III) increased with an increase in time. There was a removal of 62% by microbes alone for 24 h and nearly 100% for 48 h for *S. aureus*. But, by the addition of Mn and Fe salts, nearly 100% removal was observed in 24 h. From this, it can be concluded that 24 h is an optimum time for this process. In this reaction period, all microbes increased the removal of As(III) by salts. This might be due to oxidation of As(III) by using available oxygen in water or Fe(III) (that is obtained from Fe(II)) as an electron acceptor and CO_2 or HCO_3^- as a carbon source. The As(III) oxidation also provides energy for the microbial growth (Santini et al. 2000). It was reported that the microbes' population increases through the addition of nutrients like Mn and Fe. It is also clear that Fe- and Mn-oxyhydrate (formed from the added Mn(II) and Fe(II) salts) are very good adsorbents for arsenite. The variable oxidation states of the Fe and Mn help to change the oxidation state of the arsenic. Because Fe and Mn are nutrients to the microbes and can change its oxidation state easily, this salt may help the microbes to reduce the toxicity of arsenic towards it by biophysical processes. The current study could not explain the exact mechanism by which As(III) removal took place. The removal, however, might be a biophysiochemical remediation process.

5 Conclusions

It can be concluded that microbial ability for the removal of As(III) could be increased by addition of Mn and Fe salts and the optimum time for this process was found to be 24 h. This is one of the feasible methods with a low cost for

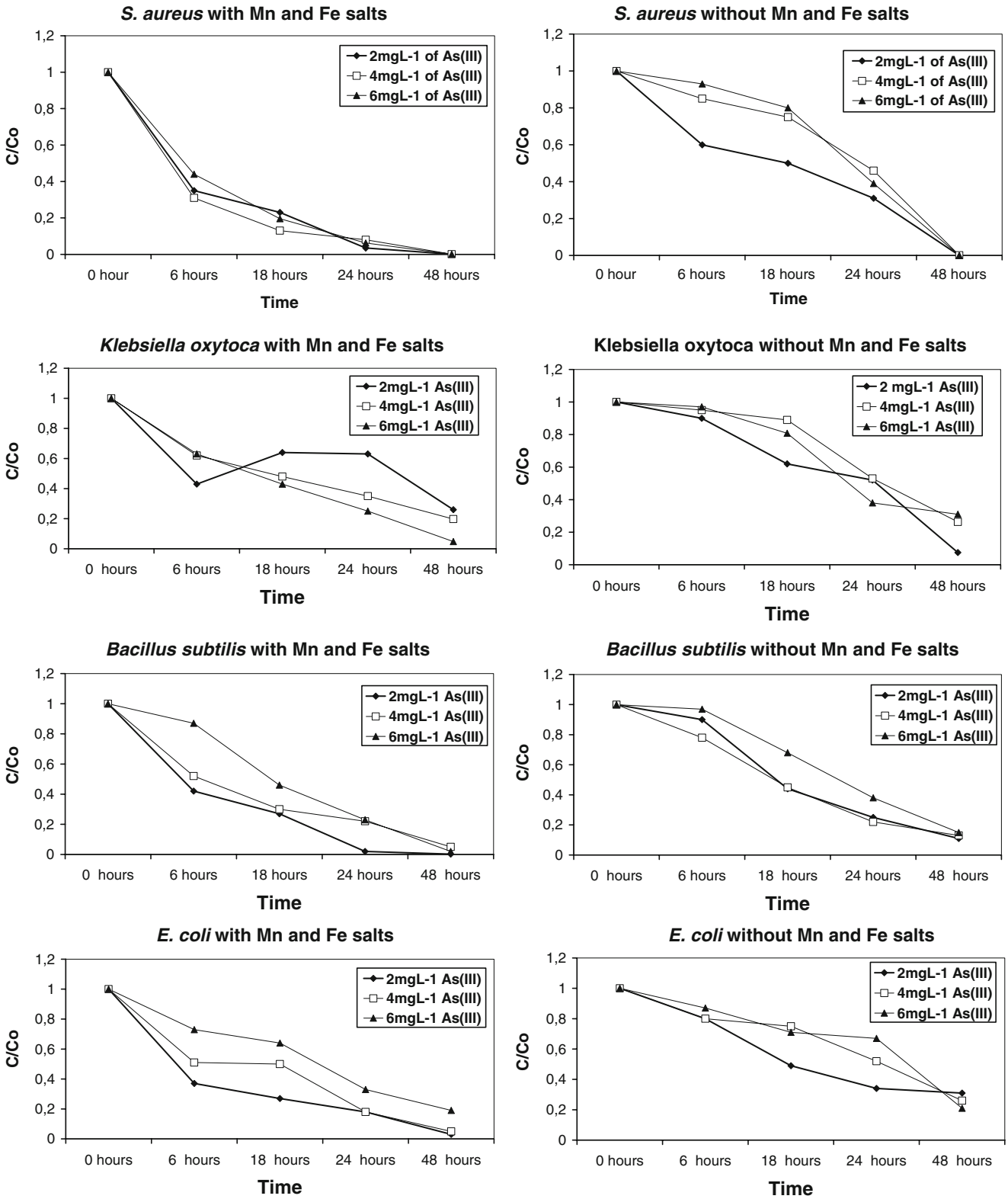


Fig. 1 Comparative removal of arsenic by *S. aureus*, *K. oxytoca*, *B. subtilis*, and *E. coli* with and without Mn and Fe salts in different concentrations of arsenic solution (*C*; [As(III)] at certain time, C_o; original[As(III)])

removal of As(III) from contaminated water containing high contents of Mn and Fe salts.

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