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Arsenic Methylation by Microorganisms in Sludge Tank of Arsenic Removal

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Abstract

An arsenic removal unit was constructed by the Miyazaki University research group in Bangladesh. The sludge drained from the arsenic removal unit was directed to a sludge tank and allowed to settle down. Then the supernatant in the tank was released to an artificial pond. Volatile organic methylated arsenic converted by biomethylation is examined as natural attenuation method. Microorganisms in the sludge tank were evaluated growth characteristics and arsenic methylation. As a result, dimethylarsinic acid was detected in the broth, and the amount of total arsenic compounds in the broth decreased. It is thought that this loss was contributed by biogenic activity induced by inorganic arsenic biomethylation to gasified volatile organic species, such as monomethylarsine and dimethylarsine. These results suggested that microorganisms in the sludge tank may be utilized for natural attenuation of arsenic sludge from the arsenic removal unit.

Keywords: Arsenic methylation, Organic methylated arsenic, Biologic gasification,

1. INTRODUCTION

Jessore district is one of the worst arsenic affected regions in Bangladesh. Miyazaki University research group had been constructed arsenic removal unit in some areas as an alternative safe water source for the arsenic affected people (M. M. Hussainuzzaman, et al., 2006). The arsenic removal unit was used a coagulation-sedimentation process, which arsenic is adsorbed on the iron oxide particulates' surface, to remove arsenic from groundwater. Therefore, prolonged accumulation of iron sludge containing very high concentrations of arsenic has been occurred by the long-term operation of the unit. In order to eliminate the clogging problem for the sludge accumulation, the valve drainage in the gravel chambers has been performed regularly (H. Yokota, et al., 2001).

A sludge disposal methodology becomes an important problem currently. In order to solve this problem, a sludge tank has been constructed for settling the sludge after being drained out of the gravel chambers. The drained water containing sludge is directed to the tank where they get time to settle down and then the supernatant water has been released to the artificial pond. Even though the arsenic concentration of the supernatant water flowed to the artificial pond was low, the arsenic concentration in the pond would be increased over time (M. Miyatake, et al., 2009).

Biomethylation of arsenic is usually considered as detoxification of arsenic because toxicity of most organic methylated arsenic is much less than that inorganic arsenic. Volatile organic methylated arsenic converted by biomethylation is examined as natural attenuation method (S.M. Islam, et al., 2005).

For the purpose of natural attenuation and detoxification of arsenic by biomethylation in the sludge tank, we have estimated arsenic methylation by microorganisms in the sludge tank.

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2. MATERIAL AND METHODS

2.1 Sample collection and number of living cells

Sludge was collected from the sludge tank on the arsenic removal unit constructed at Marua village in Bagladesh. The sludge was suspended with sterilized water, and the supernatant was measured on arsenic and iron concentration, and number of living cells. The number of living cells was estimated by plate culture method using standard agar medium.

2.2 Experiments during bacterial culture

Medium was used Nutrient broth (Nissui Pharmaceutical Co., Ltd., Japan) added 0.1% (w/v) glucose, 0.05 (w/v) NH_4NO_3, 0.005% (w/v) L-methionine, 0.25% (w/v) sodium lactate and 0.0005% (w/v) vitamin B_{12}. Sodium arsenite (Wako Pure Chemical Industries, Ltd., Japan) was used as an arsenic As(III) source, and disodium arsenate (Wako Pure Chemical Industries, Ltd., Japan) was used as an arsenic As(V) source. Culture was prepared by 100 ml of culture broth inoculating from the supernatant at a proportion of 2.0% (v/v) into nutrient broth containing As(III) or As(V) at concentrations of 0.100 mg As/l, and incubated aerobically with agitation at 100 strokes/min or anaerobically (static culture) at 30°C for 4 days. Bacterial cells were separated from the culture broth by centrifugation (10,000 × g for 15 min) at fixed intervals, and the respective concentrations of arsenic species in the broth supernatant were measured. Harvested cells were washed twice with distilled water and then lyophilized, after which the weight was measured. In order to determine the arsenic content of cells, lyophilized bacterial cells obtained from media were subjected to alkaline degradation in a 2.0 mol/l aqueous solution of NaOH at 100°C for 3 hours, after which the arsenic concentrations were measured.

2.3 Analysis

Quantitative analysis of arsenic was carried out using an atomic absorption spectrophotometer AA6650 with an arsenic speciation pretreatment system ASA-2sp (Shimadzu Co., Japan) in order to determine the respective concentrations of arsenic species. Iron concentration was determined by phenanthroline absorption spectrophotometry (Shimadzu UV-1700). All experiments were carried out three times, and measurements were performed twice. All of the obtained values were in the range of ±3% from average values. All results are given as average values.are not acceptable. Photographs are also to be treated as figures and numbered.

3. RESULTS AND DISCUSSION

3.1 Arsenic and iron concentration, and number of living cells in sludge

As a result of investigating the arsenic and iron concentration which have been solubilized in sludge tank, arsenic concentration was 0.286 mg/l and iron concentration was less than 0.1 mg/l. Moreover, a small amount of organic methylated arsenic was able to be detected. The number of living cells in sludge was 1.2×10^7 CFU/ml.

3.2 Effects of arsenic on bacterial growth and the behavior of arsenic during culture

Changes over time in the cell amount, when culture were carried out in the presence of 0.100 mg As/l of either As(III) or As(V), are shown in Fig. 1.When it cultivated aerobically, the cell amount on culture added As(V) had less than that for As(III) by the 3rd day of culture. This result indicates that As(V) inhibits the bacterial growth more than As(III). On the other hand, the difference of the cell amount on culture added As(III) and As(V) was not seen in anaerobic culture. The cell amount on aerobic culture had less than that on anaerobic culture by the 4th day of culture. On both of aerobic and anaerobic culture added As(V), the cell amount increased from the 3rd day to the 4th day of culture

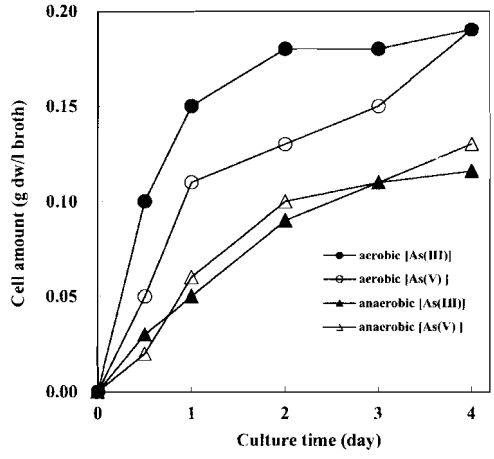

Fig. 1 Effect of arsenic on cell growth. Culture were carried out in the presence of 0.100 mg As/l of either As(III) or As(V) for 4 day at 30 °C.

The species of arsenic remaining in the culture medium over time at initial concentrations of 0.100 mg As/l were examined in detail (Fig. 2). The concentration of inorganic arsenic decreased as culture progressed also on each culture condition, and especially in aerobic culture, reduction was rapidly seen from the 1st day to the 2nd day of culture. The concentrations of inorganic arsenic in aerobic culture at both As(III) and As(V) were less than the concentration of dimethylarsinic acid (DMAA) from the 3rd of culture, and

less than 0.009 mg As/l on the 4th day of culture. DMAA was detected from the 2nd day of culture on each culture condition, and was almost constant from the 2nd day to the 4th day of culture. Monomethylarsonic acid (MMAA) was detected slightly on each culture condition except aerobic culture added As(V). Trimethylarsine oxide (TMAO) was not detected on each culture condition. These results suggested that the bacteria responsible for methylation of arsenic existed in sludge. The concentration of total arsenic compounds in the culture medium decreased as culture progressed also on each culture condition, and it decreased to 25% on the 4th day of aerobic culture added As(V). The reduction rate of total arsenic compounds concentration showed the same tendency as change of the cell amount on culture. This was considered that the bacteria which were not resistant to arsenic propagated with the decrease of the arsenic concentration.

3.3 Adsorption of Arsenic by bacteria

The amounts of arsenic contained in the dried cell were measured. As a result, although the arsenic contents in cell increased to the 2nd day of culture, they were almost constant after it at lower than 10% of initial arsenic concentration on each culture condition. The arsenic contents in cell was 0.0089 mg As/l broth on the 4th day of anaerobic culture added As(III).

4. CONCLUSION

The concentrations of total arsenic compounds and inorganic arsenic decreased as culture progressed also on each culture condition. On the 4th day of aerobic culture added 0.100 mg As/l of As(V), the concentrations of total arsenic compounds and inorganic arsenic decreased to 0.025 mg As/l (25%) and 0.009 mg As/l (9%). At the time, the arsenic content in cell was 0.0056 mg As/l broth. These

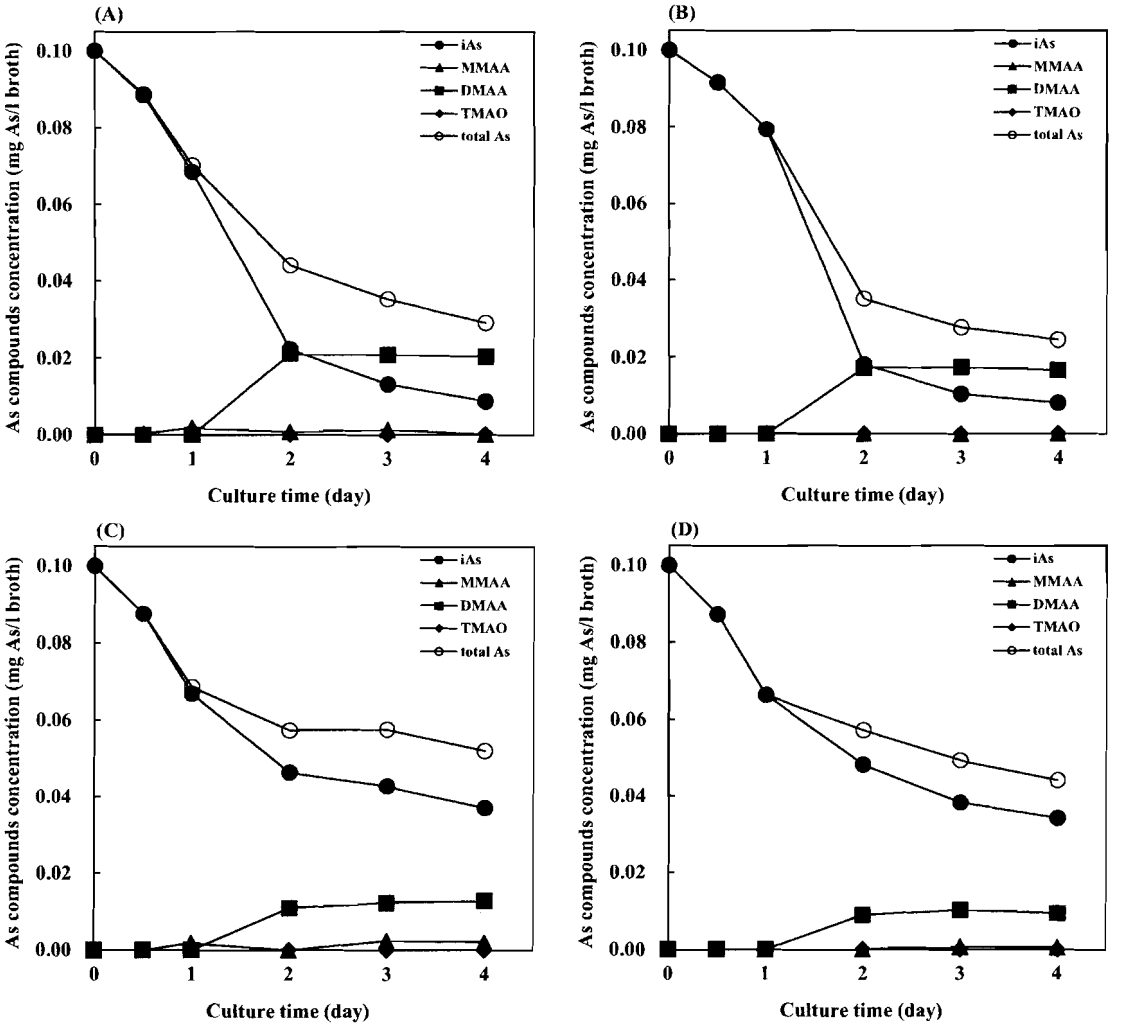

Fig. 2 Time courses of arsenic methylation. Culture were carried out in the presence of 0.100 mg As/l of either As(III) or As(V) for 4 day at 30 °C. (A) Aerobic culture [As(III)]; (B) aerobic culture [As(V)]; (C) anaerobic culture [As(III)]; (D) anaerobic culture [As(V)]. iAs, inorganic arsenic

results showed existences of microorganisms converting to MMAA or DMAA from inorganic arsenic, and to volatile organic methylated arsenic like monomethylarsine or dimethylarsine from MMAA or DMAA in culture broth. Since the volatile organic methylated arsenic made by these microorganisms evaporated from culture broth, it is thought that the concentration of the arsenic in a culture broth decreased. Existence of these microorganisms shows the possibility of natural attenuation and detoxification of arsenic by biomethylation in the sludge tank.

5. ACKNOWLEDGMENT

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