Behaviour of arsenic species in batch activated sludge process: biotransformation and removal

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Abstract The behaviour of As(III), As(V), MMA^v and DMA^v in batch activated sludge process were investigated. Experiments were carried out by using aerobic and anoxic reactors with an initial As concentration of 100 μg l^{-1}. Under aerobic condition, As(III) was oxidized to As(V) within 9 hours, some part of MMA^v was methylated to DMA^v and some other part was demethylated to As(III), which in turn was immediately oxidized to As(V). Under anoxic condition, As(V) was reduced to As(III) within the same time-course. No significant transformation occurred during experiments conducted with DMA^v. It was found that all reactions were biologically mediated. The overall As removal was low ($<20\%$) during the experiments. Although a relationship seems to exist between the sludge concentration and As removal, it is concluded, under the conditions of our study, that the activated sludge process cannot remove arsenicals efficiently. However, it can control their transformations well. Thus, if associated with an appropriate technology, the activated sludge can be used for As pre-oxidation to treat As contaminated wastewaters. Finally, care must be taken on possible presence of MMA^v in the influent of any wastewater treatment plant as it can be easily oxidized by the activated sludge.

Keywords Activated sludge process; biological control; inorganic; organic arsenic compounds

Introduction

Heavy metals are well known for their toxicity to living organisms. Among them, arsenic (As) is one of the most toxic. It can exist in various species on which its toxicity depends. Inorganic arsenic such as arsenite [As(III)] and arsenate [As(V)] are much more toxic than organic ones such as monomethylarsonic acid [MMA^v] and dimethylarsinic acid [DMA^v], and arsenobetaine and arsenocholine are considered to be non-toxic. Arsenite and arsenate are the major arsenic species found in aqueous environments while minor amounts of MMA^v, MMA^{III}, DMA^v and DMA^{III} can also be present (Le, 2002).

The chemistry of As depends on pH and redox potential. At neutral pH, As(III) as H$_3$AsO$_3$ is usually uncharged, As(V) as H$_2$AsO$_4^-$ or HAsO$_4^{2-}$ is anionic (Inskeep *et al.*, 2002), and MMA^v and DMA^v are also anionic (Carbonell-Barrachina *et al.*, 2000). Because of the difference in the chemistry among the As species, speciation analysis rather than total concentration analysis is gaining more application.

Arsenicals reach wastewater treatment plants via natural processes or anthropogenic sources. Precipitation/co-precipitation preceded by As oxidation using chemical reagents are commonly used to treat arsenic contaminated wastewaters. These processes, however, are reported to be difficult to manipulate, may be expensive, inefficient to treat low concentrations and can produce secondary hazardous wastes. Innovative technologies such as biological treatment are now being under investigations and they may be used to treat arsenic more frequently in the future (US EPA, 2002).

The activated sludge process is the most widely used biological wastewater treatment process. The use of A/O or A^2/O processes is now expanding as they combine

processes is well reported and their removal mechanisms nearly understood (Cheng *et al.*,
1975; Brown and Lester, 1979; Goldstone *et al.*, 1990a, b; Chipasa, 2003; Karvelas *et al.*,
2003; Wang *et al.*, 2003). It was reported that most metal cations are rapidly adsorbed
onto the sludge surfaces by physico-chemical reactions that do not depend on the activity
of the microorganisms. On the other hand, Goldstone *et al.* (1990c) have investigated the
behaviour of arsenic in full-scale activated sludge process. The overall As removal was
about 35% when settled sewage was used. However, they did not differentiate the As
species present. So far, no literature has reported more details about arsenicals behaviour
in the activated sludge process or its modifications.

Thus, the purpose of this research is to investigate how different arsenic compounds
will behave in the activated sludge process. Such information will be useful in the devel-
opment of biological processes to treat As contaminated wastewaters.

Methods

Experimental procedures

Stock solutions containing arsenic compounds of $1000 \, mg \, l^{-1}$ as As were prepared
with deionised water and were stored without acidification at $4 \, °C$. $NaAsO_2$ [As(III)] and
$Na_2HAsO_4 \cdot 7H_2O$[As(V)] were from Kanto Chemicals (Tokyo, Japan). $Na_2CH_3AsO_3 \cdot 6H_2$-
O [MMA[v]] and $Na(CH_3)_2AsO_2$ [DMA[v]] were from Trichemical Limited (Japan).

The activated sludge used in the experiments was collected from the aeration tank in
the Tonan Wastewater Treatment Plant in Morioka, Japan.

Upon arrival to the laboratory, a desired volume of activated sludge was washed three
times with deionised water to remove soluble components. After settling, the liquid phase
was removed while the solid phase was kept for the experiments.

Solutions containing each As species of $100 \, \mu g \, l^{-1}$ were prepared by diluting the
stock solutions with deionised water. The pH of the solutions was adjusted to 7 with
$0.1 \, M$ NaOH and then samples were collected to determine the initial concentrations.
Different amounts of the washed sludge were added to the solutions in 2-L shaking flasks.
The mixtures were thoroughly agitated on a shaker-table for 12 hours at 120 rpm. The
experiments were carried out under both aerobic and anoxic conditions. Under aerobic
condition, the DO concentration was maintained higher than $2 \, mg \, l^{-1}$. Anoxic condition
was obtained by purging the mixtures with N_2 gas until DO concentration dropped to
around $0 \, mg \, l^{-1}$. In this condition, the flasks were covered with a plastic rubber to avoid
oxygen infiltration.

After the start of the experiments, samples were harvested after 10 minutes, 1, 3, 6, 9
and 12 hours. The samples were centrifuged for 10 minutes at 3500 rpm, filtrated using a
membrane filter with a pore size of $0.45 \, \mu m$ (Millipore) and stored at $4 \, °C$ prior to anal-
ysis for metal and DOC concentration. Routine parameters (pH, DO and ORP) were
checked at each sampling time and readjusted if necessary.

Two blank experiments were conducted to determine the nature of possible reactions
among arsenicals, water and sludge. One was carried out with a solution containing all
As species with no sludge addition, and the other with addition of autoclaved sludge.

Analytical methods

Analyses were done within 24 hours after samples collection. DOC concentrations were
determined by a TOC Analyzer (TOC-5000A, Shimadzu, Japan). As(III), As(V), MMA[v],
DMA[v] were determined by an HPLC-ICP-MS system (Shimadzu, Japan – HP 4500,
Yokogawa Analytical Systems, Tokyo, Japan) based on the method developed by
Martinez-Bravo *et al.* (2002). This method was initially developed for the simultaneous

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determination of As, Se and Cr species in surface waters. In our study, analyzing time was set to 20 min and injected volume to 500 µl. The linearity of working curves was always checked for all As species tested. Correlations coefficients obtained after linear regression were higher than 0.99. Arsenic in the filtrate (sum of all arsenic species present) was directly determined by ICP-MS to confirm the total concentration.

Recovery tests for each As species spiked at different concentrations in sludge liquid phase matrices by the HPLC-ICP-MS system are shown in Table 1. When no species was added to the filtrate, no As species was detected by the system. Table 2 shows the recovery of mixtures of As species in sludge liquid phase matrices by an ICP-MS. The results obtained from the recovery tests show that the methods are suitable for the analysis of As species in the filtrate of activated sludge.

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Results and discussion

DOC concentrations

Under all experimental conditions, the DOC concentrations were relatively low and varied from $3–5\,\text{mg}\,\text{l}^{-1}$ at the initial phase to $16–20\,\text{mg}\,\text{l}^{-1}$ at the end of the experiments.

As species behaviour in autoclaved sludge and solution without sludge

Figure 1 shows the behaviour of As species under aerobic and anoxic conditions when autoclaved sludge was added to the solutions containing the four As species simultaneously.

The SS concentration was $700\,\text{mg}\,\text{l}^{-1}$ under both aerobic and anoxic conditions.

As shown, a small decrease in all species concentration was noticed immediately after the addition of autoclaved sludge. Then, no change in the form and the concentration was observed until the end of the experiments under both aerobic and anoxic conditions.

The behaviour of As during experiments with no sludge addition (data not shown) was exactly similar to that shown in Figure 1 with the exception that the immediate decrease at the beginning of the experiments was not observed.

The slight decrease in the concentrations might be due to the adsorption of As species onto sludge surfaces by specific anionic adsorption, while the stability of the form of As

Table 1 Recovery tests for As species by the HPLC-ICP-MS system

Conc. added (μg l^{-1}/species)	Recovery (%)			
	As(III)	As(V)	MMA	DMA
10	107.2	101.0	92.0	98.6
25	112.3	101.5	95.5	104.4
50	109.2	105.0	94.5	102.2
75	116.4	104.9	98.0	104.6
100	118.8	107.7	102.3	108.6

Table 2 Recovery tests for Total As concentrations by ICP-MS

Total soluble As added (μg l^{-1})	Conc. calculated (μg l^{-1})	Recovery (%)
0	N.D.	–
40 (10 each species)	35.7	89.1
100 (25 each species)	103.6	103.6
200 (50 each species)	204.5	102.2
300 (75 each species)	294.9	98.3
400 (100 each species)	394.8	98.7

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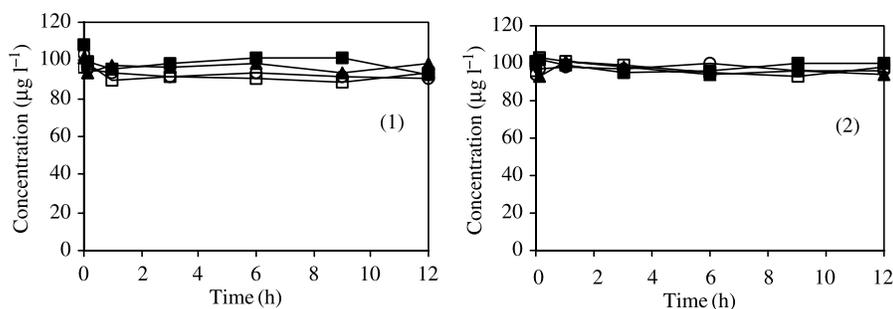
Figure 1 Behaviour of As species in autoclaved sludge under: (1) aerobic and (2) anoxic conditions (■: As(III); ▲: As(V); ○: MMA^v; □: DMA^v)

species indicated that no chemical reactions occurred between As species, water and the sludge under both aerobic and anoxic conditions.

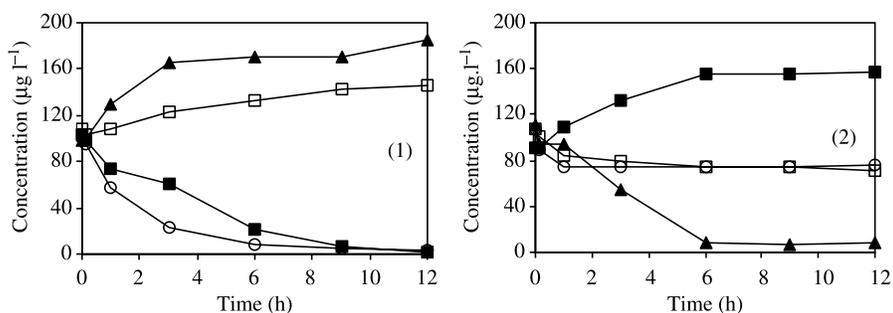### As species behaviour in activated sludge

Figure 2 shows the behaviour of As species under aerobic and anoxic conditions when activated sludge was added to the solutions containing the four As species. The SS concentration was $1100 \, mg \, l^{-1}$ under both aerobic and anoxic conditions.

Under aerobic condition, the concentrations of As(III) and MMA^v were gradually decreased and they dropped to less than $10 \, \mu g \, l^{-1}$ after 9 hours. On the other hand, the concentrations of As(V) and DMA^v were gradually increased respectively to $185 \, \mu g \, l^{-1}$ and $146 \, \mu g \, l^{-1}$ until the end of the experiments. The sums of the four As species at the initial phase and at the final phase were respectively $408 \, \mu g \, l^{-1}$ and $337 \, \mu g \, l^{-1}$.

Under anoxic condition, it was observed that As(V) was gradually decreased until 6 hours from the initial phase and reached to less than $10 \, \mu g \, l^{-1}$ until 12 hours. MMA^v and DMA^v were slowly decreased until 3 hours and were stabilized to around $75 \, \mu g \, l^{-1}$ until 12 hours. As(III) was gradually increased to around $155 \, \mu g \, l^{-1}$ after 6 hours. The sums of the four As species at the initial phase and at the final phase were respectively $417 \, \mu g \, l^{-1}$ and $312 \, \mu g \, l^{-1}$.

From these results, it was found that all changes in the concentrations began immediately after the sludge addition to the metal solutions.

Previous data obtained in autoclaved sludge have shown that few chemical reactions occurred between the sludge and the As species. Therefore, it is shown that the changes in the concentrations here will be biologically mediated. However, the total As removal under both conditions did not exceed 25%.

Figure 2 Behaviour of As species in activated sludge: (1) aerobic and (2) anoxic conditions (■: As(III); ▲: As(V); ○: MMA^v; □: DMA^v)

To clarify the behaviour of As species and the biological reactions that occurred in the activated sludge, experiments with solutions containing only one As species were carried out.

Effect of aerobic condition on As(III) behaviour

Figure 3 shows the behaviour of As(III) in aerobic activated sludge with various sludge concentrations. Immediately after adding the sludge to the solution containing only As(III), a gradual decrease in As(III) concentration was observed. As As(III) was decreased, As(V) was increased until a complete removal of As(III). Higher concentration of sludge decreased As(III) faster than lower concentration. A complete removal of As(III), associated with its transformation to As(V), was achieved within 9 hours. Since the decrease in As(III) concentration and the increase in As(V) under the aerobic condition were not observed in the autoclaved sludge as shown in Figure 1, it could be concluded that As(III) was oxidized to As(V) biologically. Several heterotrophs and autotrophs isolated from various contaminated environments, including sewage, were already found to oxidize As(III) (Ehrlich, 2002).

Once As(III) was transformed to As(V), a small decrease in As(V) was observed. This decrease was more pronounced with higher sludge concentration. The removal efficiency of total As obtained by direct determination with ICP-MS (data not shown) was increased with the increase in the sludge concentration. The maximum efficiency was 20%.

As(V) has similar structure to phosphate and is anionic. Therefore, it can accumulate to cells via the phosphate transport chain (Rosen, 2002) or bind to metal oxides (US EPA, 2002). The exact relationship between As removal efficiency and SS concentration was not yet established and is under more investigations.

Effect of anoxic condition on As(V) behaviour

Figure 4 shows the behaviour of As(V) in anoxic activated sludge with various sludge concentrations. Immediately after adding the sludge to the solution containing only As(V), a gradual decrease in As(V) concentration was observed. As As(V) was decreased, As(III) was increased. Higher concentration of sludge decreased As(V) faster than lower concentration. However, As(V) was not decreased with an SS concentration less than $60 \, \text{mg} \, \text{l}^{-1}$. The phase of As(V) decrease, associated with its transformation to As(III), was achieved within a maximum of 6 hours depending on the sludge concentration. After that, $5-10 \, \mu\text{g} \, \text{l}^{-1}$ of As(V) remained unchanged until the end. The transformation of As(V) to As(III) indicated that As(V) was biologically reduced to As(III) under anoxic condition. The bacterial reduction of arsenate in anoxic environments has been reviewed by Oremland *et al.* (2002).
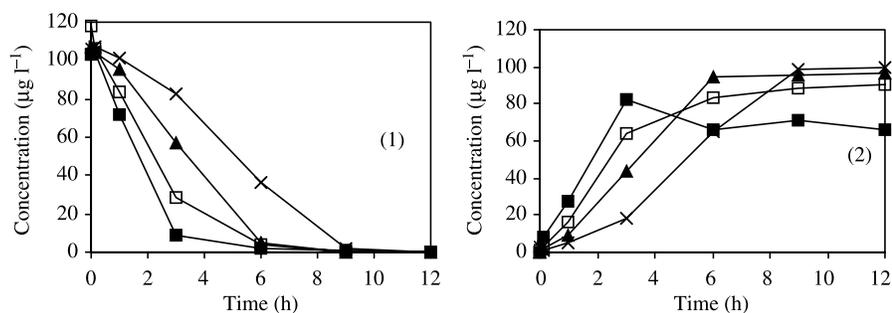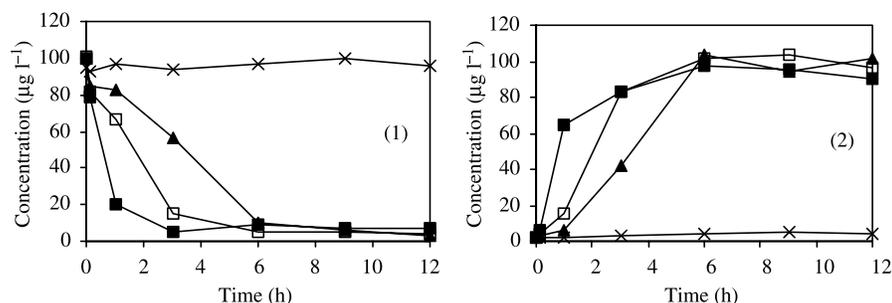

Figure 3 Behaviour of (1) As(III) and (2) As(V) in case of As(III) initially present under aerobic condition. SS $(\text{mg} \, \text{l}^{-1}) = (\times: 100; \blacktriangle: 160; \square: 370; \blacksquare: 720)$

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Figure 4 Behaviour of (1) As(V) and (2) As(III) in case of As(V) initially present under aerobic condition. SS $(mg\,l^{-1})$ = (× : 60; ▲: 190; □: 400; ■: 740)

The removal efficiency of total As obtained by direct determination with ICP-MS (data not shown) was increased with the increase in the sludge concentration. The maximum efficiency was 25%.

In anoxic condition, the removal of arsenic also seems to be related to the SS concentration.

### Effect of aeration conditions on MMA^{v} and DMA^{v} behaviour

Figure 5 shows the behaviour of As species under aerobic and anoxic conditions when MMA^{v} was initially present. Immediately after the sludge addition, the decrease in 20–30% of MMA^{v} occurred under both aerobic and anoxic conditions. This decrease did not correspond to any transformation of MMA^{v} to other species. As expected from the results obtained with the mixtures of As species under aerobic condition, a part of MMA^{v} was biologically methylated to DMA^{v}. However, a part of MMA^{v} was also demethylated to As(III), which in turn was oxidized to As(V). This indicated the simultaneous occurrence of methylation and demethylation of MMA^{v}. Cullen *et al.* (1977) have demonstrated the aerobic methylation of arsenic by isolated micororganisms. To date, no literature has reported the simultaneous occurrence of methylation and demethylation processes.

Under anoxic condition, after the rapid decrease in MMA^{v} concentration from the initial phase, the concentration was stabilized to around $70\,\mu g\,l^{-1}$ until the end and other species were not detected.

Figure 6 shows the behaviour of As species under aerobic and anoxic conditions when DMA^{v} was initially present. Differently from the behaviour of other three species, DMA^{v} was not significantly affected by the aeration conditions. A slight decrease in the concentration at the initial phase (20%) was followed by a lag time of about 3 hours. Then,
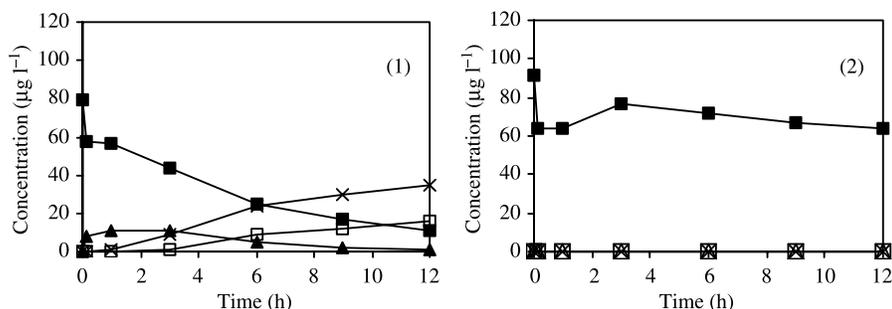
Figure 5 Behaviour of As species in case of MMA^{v} initially present under (1) aerobic and (2) anoxic conditions (▲: As(III); □: As(V); ■: MMA^{v}; × : DMA^{v})
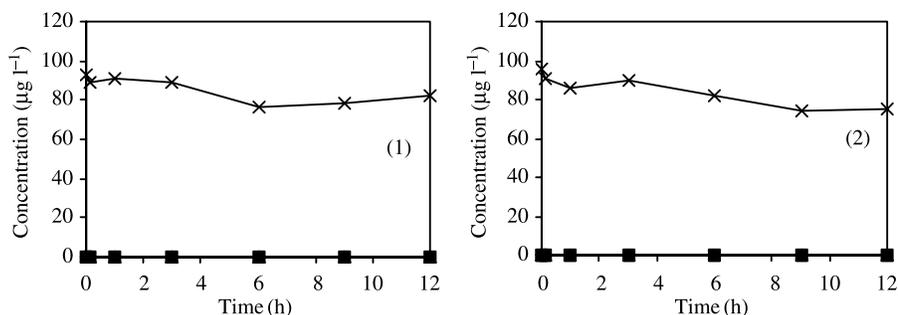
Figure 6 Behaviour of As species in case of DMA^v initially present under (1) aerobic and (2) anoxic conditions (▲: As(III); □: As(V); ■: MMA^v; ×: DMA^v)

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DMA^v gradually decreased until the end. Neither MMA^v nor As(III) or As(V) were produced under both conditions.

Conclusions

Under the conditions of this study, the overall removal of As was low in both autoclaved and non-autoclaved activated sludge during all experiments. On the other hand, the activated sludge exhibited a high ability in controlling the transformations of As species by oxidation-reduction and methylation-demethylation reactions. These transformations were found to be immediate and biologically mediated, and were not inhibited even though one or more species were present. Under aerobic condition, As(III) was oxidized to As(V) and MMA^v was simultaneously methylated to DMA^v and demethylated to As(III), which was in turn oxidized to As(V). Under anoxic condition, As(V) was reduced to As(III). DMA^v was not transformed under both aeration conditions.

Therefore, the activated sludge process is not appropriate for the removal of arsenic by direct adsorption onto sludge flocs. The findings from this study, however, indicate that it is suitable for the pre-oxidation of arsenic species to As(V). Then, As(V) could be removed from the wastewater by co-precipitation with metal coagulants. Such coagulants might be added at the final stage of the aeration tank or to the effluent from the secondary clarifier. During the operations, it is important to keep an aerobic condition in the aeration tank and the secondary clarifier as As(V) can be rapidly reduced to As(III) under anoxic condition.

Finally, future research should be conducted to investigate: (a) the effects of nutrients and organic matter addition on the biotransfomations of arsenic species in the activated sludge process, and (b) the occurrence and the behaviour of arsenic species in full-scale treatment plants receiving wastewater contaminated with arsenic.

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