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Microbial transformation of arsenic species in municipal landfill leachate

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ABSTRACT

The microbial transformation of arsenic species in municipal landfill leachate (MLL) was investigated with the objective to highlight arsenic transformation in the landfill system. Across the 43 day incubation in MLL, more than 90% arsenate (iAs^V) was found to reduce to arsenite (iAs^{III}) within 20 days, while iAs^{III} was comparably stable although a fraction of iAs^{III} was temporarily oxidated to iAs^V in the first 3 days. Transformation of monomethylarsonic acid (MMA^V) to dimethylarsinic acid (DMA^V) in MLL was slow with only 5% MMA^V methylated to DMA^V after 43 days incubation. A portion of DMA^V and MMA^V in MLL was demonstrated to transform into thiol-organoarsenic and monomethylarsonous acid (MMA^{III}), which were identified to include dimethyldithioarsinic acid ($DMDTA^V$), dimethylmonothioarsinic acid ($DMMTA^V$) and monomethyldithioarsonic acid ($MMDTA^V$) by HPLC–ICPMS and LC–ESI-MS/MS. The microbial formation of $DMDTA^V$, $DMMTA^V$ and $MMDTA^V$ is postulated to relate to hydrogen sulfide generated by bacteria in MLL. Differences in arsenic transformation in sterilised and non-sterilised MLLs demonstrate bacteria play a crucial role in arsenic transformation in the landfill body. This study reveals the complexity of arsenic speciation and highlights the potential risk of forming highly toxic thiol-organoarsenic and MMA^{III} in the landfill environment.

1. Introduction

Speciation of arsenic governs its toxicity and mobility in the environment. Inorganic species (arsenate (iAs^V) and arsenite (iAs^{III})) are generally more toxic than their organic arsenic counterparts, such as monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V). For instance, iAs^{III} is lethal to rats at a LD_{50} dose of 1.5 mg kg^{-1} and is more toxic than iAs^V, MMA^V and DMA^V, while arseno sugars are non-toxic [1]. Thiol-organoarsenic is perceived as an extremely toxic arsenic species with dimethyldithioarsinic acid ($DMDTA^V$) and dimethylmonothioarsinic acid ($DMMTA^V$) being substantially more toxic than iAs^{III} to human cells [2–4].

Arsenic can undergo a variety of microbial transformation reactions, including oxidation, reduction and methylation by naturally occurring and diverse bacteria species in the environment [5–7]. For instance, bacterium *Bacillus arsenoxydans* was observed to microbially oxidise iAs^{III} to iAs^V, while bacterium *Pseudomonas fluorescens* microbially reduces iAs^V to iAs^{III} [6]. The reduction of iAs^V to iAs^{III} and further methylation generally increase its mobility and consequently its availability within the environment [8–10]. Challenger postulated iAs^V biomethylation involved a repeated sequence of pentavalent arsenic reduction to trivalent arsenic, followed by oxidative methylation to methyl arsenic species [11,12].

Recently, thiol-organoarsenic species, including $DMDTA^V$ and $DMMTA^V$, have been reported in a wide range of systems, such as in marine algae [13], vegetation [14], mussels [15], animals [2,16–19], and human beings [20,21]. Ochi et al. [4] and Naranmandura et al. [22] suggested that biologically generated sulfide (HS^-), glutathione (GSH), or sulfide coordinated with a monothiol (protein–S–SH) or dithiol group (protein–S–S–S–protein) may be involved in biological arsenic transformation.

Many arsenic containing wastes, such as chromated copper arsenate (CCA) treated wood, are disposed in municipal landfills [23]. Leachates from these landfills generally contain arsenic and other heavy metals and pose a contamination threat to ground and surface water. Various arsenic species, including iAs^{III}, iAs^V, MMA^V, DMA^V, AsB and TMAO, as well as volatile $(CH_3)_3As$ and $(CH_3)_2AsH$, have been observed in landfill leachate and gases [10,24,25]. In

Abbreviations: iAs^V, arsenate (AsO_4^{3-}); iAs^{III}, arsenite (AsO_2^-); DMA^V, dimethylarsinic acid ($(CH_3)_2As(=O)(OH)$); $DMDTA^V$, dimethyldithioarsinic acid ($(CH_3)_2As(=S)(SH)$); $DMMTA^{III}$, dimethylmonothioarsinous acid ($(CH_3)_2As(SH)$); $DMMTA^V$, dimethylmonothioarsinic acid ($(CH_3)_2As(=S)(OH)$); MMA^{III}, monomethylarsonous acid ($CH_3As(OH)_2$); MMA^V, monomethylarsonic acid ($CH_3As(=O)(OH)_2$); $MMDTA^V$, monomethyldithioarsonic acid ($(CH_3As(=S)(OH)(SH)$); $MMMTA^{III}$, monomethylmonothioarsonous acid ($(CH_3As(SH)(OH)$); $MMMTA^V$, monomethylmonothioarsonic acid ($(CH_3As(=S)(OH)_2$); $MMTTA^V$, monomethyltrithioarsonic acid ($CH_3As(=S)(SH)_2$); TMAO, trimethylarsine oxide ($(CH_3)_3As(=O)$).

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our previous work, we identified two thiol-organoarsenic species, $DMDTA^V$ and $DMMTA^V$, as well as observed the transformation of DMA^V to $DMDTA^V$ and $DMMTA^V$ in a municipal landfill leachate (MLL) [26]. This transformation demonstrated that arsenic species in MLL can differ from those initially present in solid waste disposed in landfill, revealing the need to study the underlying mechanism for arsenic species transformation in a landfill environment. The aims of this work are: (1) to investigate the role bacteria play in transforming arsenic species, including iAs^{III}, iAs^V, MMA^V and DMA^V in MLL; and (2) propose a transformation pathway with particular emphasis on thiol-organoarsenic species formation.

2. Experimental

2.1. Municipal landfill leachate

MLL sampled from a municipal landfill located in the metropolitan region of Sydney, was used in this study. The landfill is lined with natural clay, receives both municipal and non-putrescible solid wastes and has been operating for approximately 20 years. Leachate sample was collected directly from a pipe used to collect leachate across the landfill, with the outlet immediately adjacent to a leachate pond. The sample was stored in a container without headspace at 4 °C and characterised previously (denoted as MLL(A)) [26]. It contained 80 $\mu g\,L^{-1}$ dissolved arsenic, comprising iAs^{III} (3 $\mu g\,L^{-1}$, as As), MMA^V (9 $\mu g\,L^{-1}$), DMA^V (2 $\mu g\,L^{-1}$), $DMDTA^V$ (27 $\mu g\,L^{-1}$) and $DMMTA^V$ (4 $\mu g\,L^{-1}$). The leachate also contained dissolved sulfate (2.1 $mg\,L^{-1}$), sulfide (2.0 $mg\,L^{-1}$), high concentrations of total organic carbon (1600 $mg\,L^{-1}$) and bacterial biomass, dominated by *Bacillus* species. Low redox potential (−310 mV) and the presence of ferrous iron and sulfide indicated the MLL had a strong reducing capacity.

2.2. Reagents

Arsenate ($Na_2HAsO_4{\cdot}7H_2O$) was purchased from Sigma (Germany). Arsenite ($NaAsO_2$) was obtained from Fluka (Switzerland). Monomethylarsonate ($CH_3AsNa_2O_3{\cdot}6H_2O$) and dimethylarsinate ($C_2H_7AsO_2$) were from Supelco (USA). Formic acid (HCOOH) was obtained from Univar (Australia). Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) was obtained from Merck (Germany). Luria-Bertani (LB) medium was prepared by dissolving 10 g tryptone (Sigma, Germany), 5 g yeast extract (Sigma, Germany) and 10 g NaCl (Merck, Germany) in 1 L of deionised water, adjusting the pH to 7.2 with NaOH and sterilising prior to use.

Monomethylarsonous acid (MMA^{III}) and thiol-organoarsenic species ($DMDTA^V$, $DMMTA^V$, monomethyldithioarsonic acid ($MMDTA^V$) and monomethylmonothioarsonic acid ($MMMTA^V$)) were synthesised in-house and structurally characterised prior to use. The preparation and identification of $DMDTA^V$ and $DMMTA^V$ were reported previously [26]. A mixture of MMA^{III}, $MMMTA^V$ and $MMDTA^V$ were synthesised by bubbling H_2S through a standard MMA^V solution (100 $mg\,L^{-1}$) for 4 h and stored in an airtight vial. The proportions of MMA^{III}, $MMMTA^V$ and $MMDTA^V$ in the mixture varied with reaction time and degree of exposure to air during analysis. Details regarding characterisation of the synthesised MMA^{III}, $MMMTA^V$ and $MMDTA^V$ can be found in Supplementary materials.

2.3. Preparation of arsenic-spiked leachate

The leachate was filtered through a sterilised Whatman 541 filter paper (pore size, 20–25 μm) to remove the larger particulate matters and equilibrated to room temperature (22 °C) for 24 h prior to use. Sterilised leachate was prepared by autoclaving the filtered MLL at 121 °C for 15 min.

The transformation of arsenic species in MLL was investigated using MLL individually spiked with iAs^V, iAs^{III}, DMA^V and MMA^V standard solutions. Arsenic spiking was performed in a nitrogen glove box to maintain an anaerobic condition. Spiked samples comprised 75% (v/v) MLL and a single arsenic species (2.0 $mg\,L^{-1}$) with water constituting the remaining solution. The pH of arsenic-spiked leachates was the same as the original MLL. Control samples containing 10 $mg\,L^{-1}$ (as As) of a single arsenic species and 75% (v/v) sterilised MLL were also prepared. MLL and sterilised MLL samples spiked with iAs^V were prepared in duplicate to estimate analytical error. Compared with the spiked arsenic levels (2.0 or 10 $mg\,L^{-1}$), the background concentration of each arsenic species (from the original MLL) was small (<1%) and their contribution to species transformed from the spiked species was negligible.

The role bacteria played in arsenic transformation was also assessed in LB medium inoculated with bacteria from MLL and individually spiked with iAs^V, iAs^{III}, DMA^V and MMA^V. Each spiked sample contained 80% (v/v) LB medium, 10% (v/v) MLL (sterilised MLL for control samples) and a single arsenic species (5.0 $mg\,L^{-1}$, as As) with water constituting the remaining solution. The spiked arsenic concentrations (5.0 $mg\,L^{-1}$ in LB medium and 2.0 $mg\,L^{-1}$ in non-sterilised MLL) were observed to have a negligible toxic impact on the MLL bacteria in this study (data not shown).

2.4. Speciation and identification of arsenic species

Aliquots of arsenic-spiked samples were kept in the dark in airtight tubes without headspace and continuously agitated (250 rpm) at room temperature (22 °C). At selected time intervals, leachate samples were filtered using a 0.45 μm membrane filter and acidified with HCl (0.2%, v/v) prior to speciation.

Arsenic speciation was assessed by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC–ICPMS). Two chromatography methods, a Hamilton PRP-X 100 anion exchange (PSDVB/trimethylammonium) column (250 × 4.6 mm id, 10 μm particle diameter, 100 Å pore size, 30 °C) using a mobile phase of 20 mM $NH_4H_2PO_4$ (pH 5.6, flow rate 1.5 $mL\,min^{-1}$) and a Thermo HyPURITY end-capped C18 column (250 × 4.6 mm id, 5 μm particle diameter, 120 Å pore size, 25 °C) using a mobile phase of 5 mM formic acid (pH 2.9, flow rate 1.3 $mL\,min^{-1}$), were employed for separating arsenic species, hereafter referred to as chromatography methods "PRP-X 100" and "HyPURITY C18". The ICPMS (Elan 6100DRC, Perkin-Elmer SCIEX, Canada), equipped with a dynamic reaction cell (DRC), was used to selectively monitor AsO at *m/z* 91 and SO at *m/z* 48 for estimating the concentration of iAs^{III}, iAs^V, MMA^V, DMA^V and unknown arsenic species, as well as the ratio of sulfur/arsenic (S/As) in unknown arsenic species. Recovery for the HPLC–ICPMS (method PRP-X 100) was found to be between 85% and 115% using a standard solution containing iAs^{III}, iAs^V, MMA^V and DMA^V. A similar recovery level was also found for a wastewater reference material (CWW-TM-C, High-Purity Standards) containing iAs^{III} and iAs^V that had been certified for total arsenic concentration.

The unknown arsenic species were matched to the synthesised arsenic compounds and identified by their retention time (RT), S/As ratio, fragment patterns and molecular ions using HPLC–ICPMS and liquid chromatography–electrospray ionisation tandem mass spectrometry (LC–ESI-MS/MS). The details of HPLC–ICPMS and LC–ESI-MS/MS methods used for speciation and identification have been described previously [26].

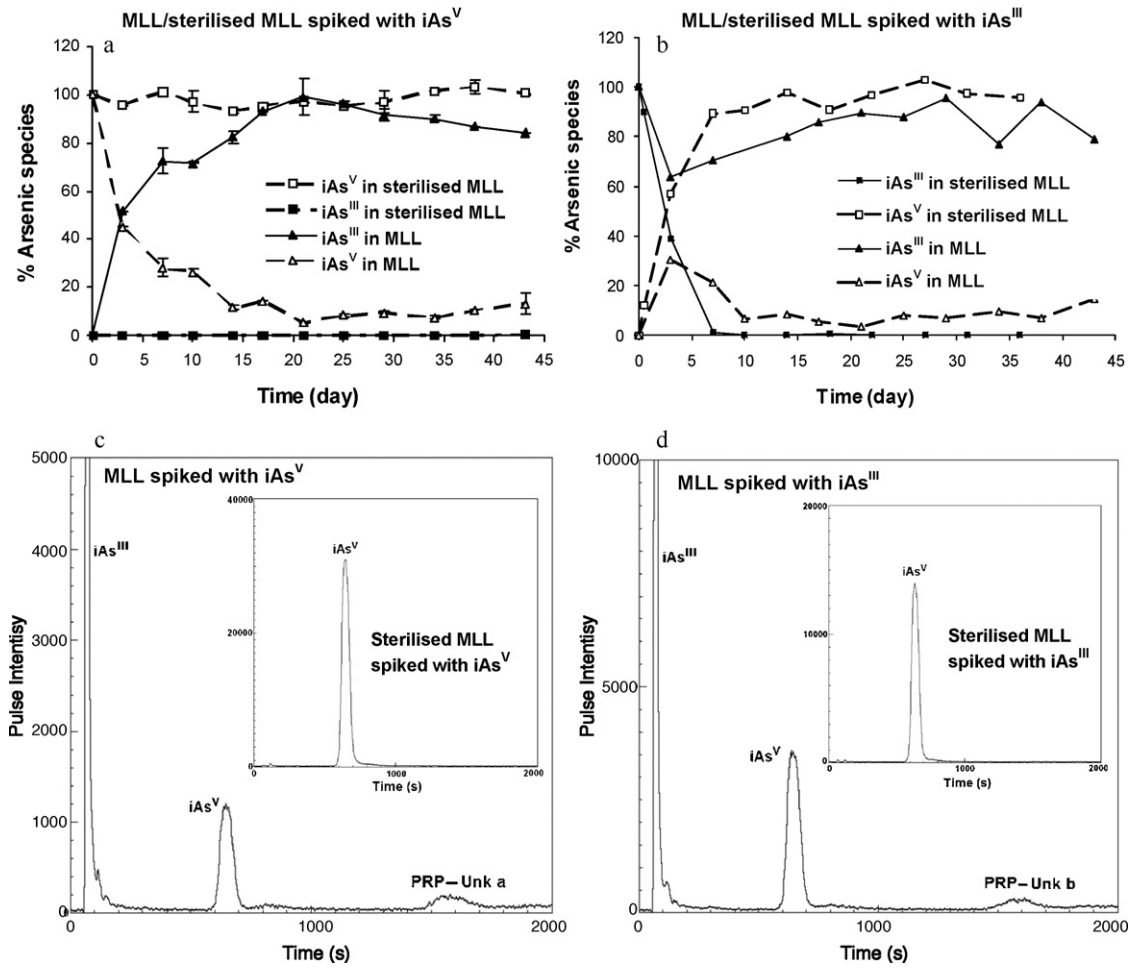

Fig. 1. Percentage of arsenic species (versus total spiked arsenic concentration) with respect to time in non-sterilised and sterilised MLL initially spiked with (a) iAs^{V} and (b) iAs^{III}. HPLC–ICPMS chromatograms of arsenic species in non-sterilised and sterilised MLL (insets) on the 38th day after spiking with (c) iAs^{V} and (d) iAs^{III}. Experimental conditions: ICPMS (DRC mode, selectively monitoring AsO at m/z 91) with chromatography method PRP-X 100. Results in profile (a) are means and SDs of duplicates.

3. Results and discussion

3.1. iAs^{V} and iAs^{III} transformation

Fig. 1a and b illustrates the change in arsenic species with time in non-sterilised and sterilised MLLs spiked with iAs^{V} and iAs^{III}, respectively. Also shown are HPLC–ICPMS chromatograms (PRP-X 100) for each sample, 38 days after arsenic species addition (Fig. 1c and d). Upon adding iAs^{V} to the sterilised MLL (Fig. 1a), no change in its concentration with time was observed nor was the presence of iAs^{III}. When added to non-sterilised MLL, the iAs^{V} concentration had dropped to 45% of the initial value by day 3 with a concomitant increase in the iAs^{III} concentration. By day 22, the iAs^{V} concentration had decreased to approximately 5% the initial value with the iAs^{III} concentration reaching approximately 95% of the initial arsenic concentration. Differences in the sterilised and non-sterilised profiles indicate bacterial activity is responsible for reducing iAs^{V}.

The 38th day chromatogram for the iAs^{V}-spiked MLL (Fig. 1c) demonstrates only iAs^{V} was present in the sterilised MLL while iAs^{III} dominated the non-sterilised MLL with a comparatively smaller amount of iAs^{V} present. The figure also reveals a small unknown arsenic peak (PRP-Unk a, where 'PRP' represents the chromatography method PRP-X 100; RT: 1600 s) was present in the iAs^{V}-spiked MLL.

Fig. 1b shows a decrease in iAs^{III} concentration to 1% by day 7 with a parallel increase in iAs^{V} concentration under sterilised conditions, indicating nearly complete oxidation of iAs^{III} to iAs^{V} when there were no bacteria present. In the presence of bacteria, the iAs^{III} concentration dropped to approximately 65% of its initial value by day 3 with a corresponding increase in iAs^{V} (approximately 30%). Beyond day 3, the iAs^{III} concentration began to increase, reaching approximately 90% of its initial value by day 22. This was accompanied by a decrease in iAs^{V} to approximately 5% over the same time. The difference in the oxidation of iAs^{III} to iAs^{V} in the sterilised and non-sterilised MLLs is postulated to relate to the complex MLL matrix and the change in the matrix by sterilisation. The profiles in Fig. 1a and b indicate, under non-sterilised conditions, inorganic arsenic speciation was predominantly governed by bacterial activity, which rapidly depleted oxidants in MLL, eventually generating a reducing environment and reducing iAs^{V} to iAs^{III}.

Fig. 1d shows iAs^{V} was the only species in the sterilised sample, indicating a complete conversion of iAs^{III} to iAs^{V}. On the contrary, both iAs^{III} and iAs^{V} were present in the non-sterilised sample, indicating a partial conversion of iAs^{III} to iAs^{V}. The figure also shows the presence of a small unknown arsenic peak (PRP-Unk b; RT: 1600 s) similar to that observed in Fig. 1c. The unknown peaks appeared in the chromatograms from day 29 onward and their concentration remained small (<1% total arsenic) in both iAs^{V}- and iAs^{III}-spiked MLLs, indicating they were derived from the iAs^{V} or iAs^{III} and their formation rates were slow. PRP-Unk a and PRP-Unk b in iAs^{V}- and iAs^{III}-spiked MLLs were not investigated further due to their low concentrations.

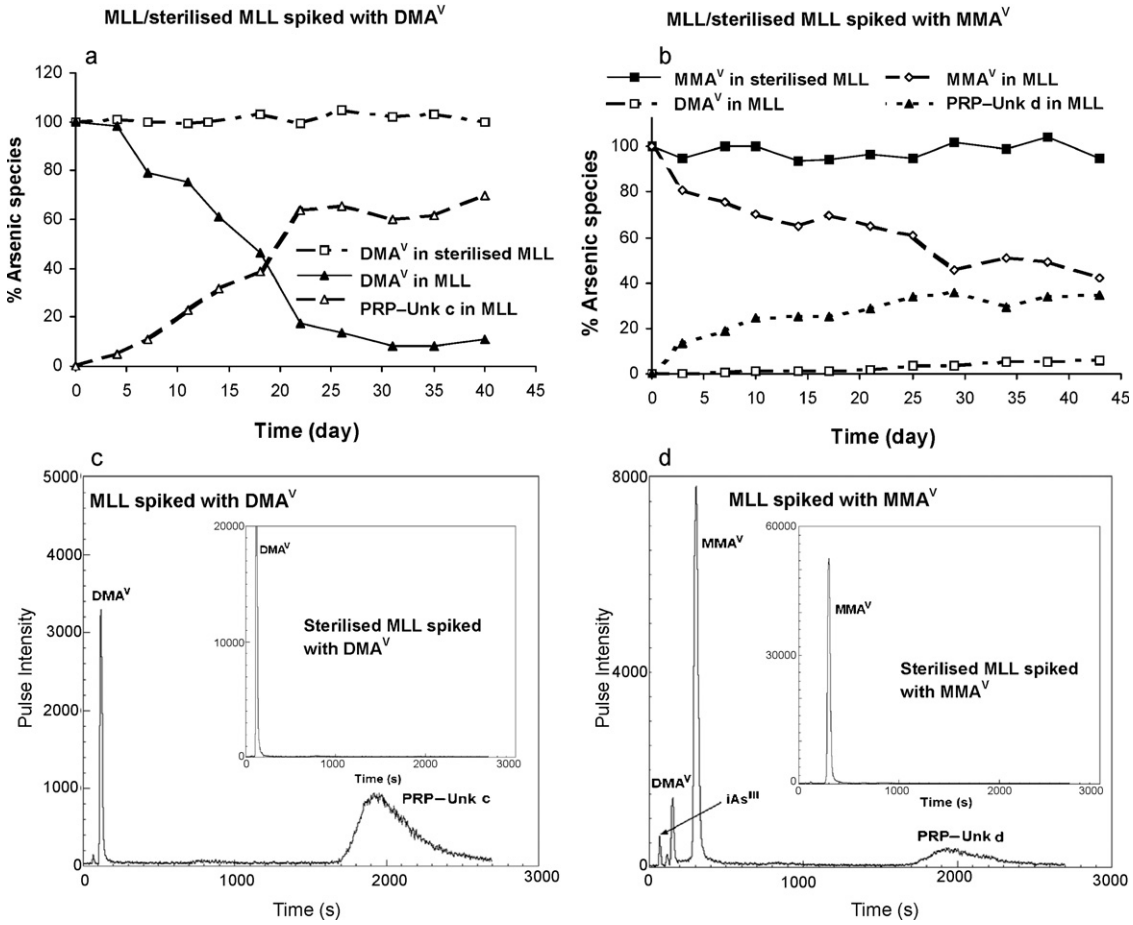

Fig. 2. Percentage of arsenic species (versus total spiked arsenic concentration) with respect to time in non-sterilised and sterilised MLL initially spiked with (a) DMA^V and (b) MMA^V. HPLC–ICPMS chromatograms of arsenic species in non-sterilised and sterilised MLL (insets) spiking with (c) DMA^V on the 40th day and (d) MMA^V on the 38th day. Experimental conditions: ICPMS (DRC mode, selectively monitoring AsO at m/z 91) with chromatography method PRP-X 100.

Fig. 1a and b indicate arsenic retained an approximate mass balance over days 1–43 in the iAs^V-spiked MLL (99%, SD = 4%) and iAs^{III}-spiked MLL (94%, SD = 5%), recognising the presence of the small amounts of PRP-Unk a and PRP-Unk b. MMA^V and DMA^V were not detected in both iAs^V and iAs^{III}-spiked MLLs, indicating methylation of iAs^{III} to MMA^V and DMA^V by the bacteria in MLL was insignificant over the period of study. This may be due to the relatively short reaction time or MMA^V and DMA^V were transformed to other species such as PRP-Unk a and PRP-Unk b.

3.2. Microbial DMA^V and MMA^V transformation

The transformation of DMA^V in MLL can be seen in Fig. 2a with the HPLC–ICPMS profiles (chromatography method PRP-X 100) on the 40th day after spiking given in Fig. 2c. Fig. 2a shows that under sterilised conditions there was no discernible change in the DMA^V concentration with time, which is similar to the earlier result for iAs^V-spiked MLL. Under non-sterilised conditions, the DMA^V concentration decreased to 18% of its initial concentration by day 22, after which its conversion distinctly slowed. There was a simultaneous increase in an unknown arsenic peak (PRP-Unk c; RT: 1900 s in Fig. 2c) whose concentration reached approximately 65% by day 22 and plateaued afterwards. The 40-day chromatograms demonstrate that under sterilised conditions only DMA^V was present while under non-sterilised conditions DMA^V and PRP-Unk c were the dominant species. Differences in the sterilised and non-sterilised profiles demonstrate bacterial activity is responsible for converting DMA^V to PRP-Unk c.

For MMA^V-spiked leachates (Fig. 2b and d), there was no change in MMA^V concentration in sterilised MLL, which is similar to the earlier findings for iAs^V- and DMA^V-spiked solutions. Fig. 2b illustrates under non-sterilised conditions the MMA^V concentration decreased continually to approximately 40% of its initial concentration by day 43. Concomitant with the decrease in MMA^V concentration, was a corresponding increase in an unknown arsenic peak (PRP-Unk d; RT: 1900 s in Fig. 2d) which reached approximately 35% by day 43. A slow increase in DMA^V concentration to approximately 5% (at day 43) was also evident in Fig. 2b. Differences in the sterilised and non-sterilised profiles show bacteria are also responsible for converting MMA^V to PRP-Unk d and DMA^V. The formation of DMA^V in the MMA^V-spiked non-sterilised leachate indicates that microbially assisted methylation of MMA^V to DMA^V has occurred. The percentage of iAs^{III} in the MMA^V-spiked MLL was negligible and the small iAs^{III} peak in Fig. 2d may have derived from the iAs^{III} in the initial MLL.

For DMA^V- and MMA^V-spiked non-sterilised leachates, there is an apparent discrepancy between the amount of arsenic added and the lower amount of arsenic detected. For example, approximately 80% of the total arsenic was recovered as DMA^V and PRP-Unk c in DMA^V-spiked MLL on day 40 (Fig. 2a) and a similar amount of arsenic was recovered as MMA^V, PRP-Unk d and DMA^V in MMA^V-spiked MLL on day 43 (Fig. 2b). This imbalance has been noted previously [26] and may be attributed to the formation of volatile arsenic species such as $(CH_3)_3As$ and $(CH_3)_2AsH$, which were reported in landfill biogases [24]. The incomplete elution of PRP-Unk c and PRP-Unk d and the increased background

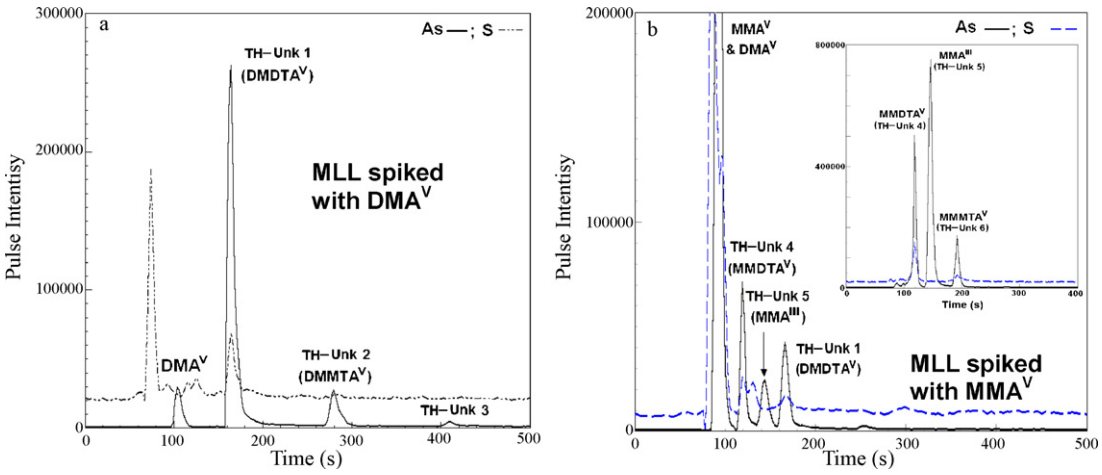

Fig. 3. HPLC–ICPMS chromatograms of arsenic species in: (a) 40-day DMA^V-spiked MLL and (b) 38-day MMA^V-spiked MLL (inset: the synthesised mixture containing MMDTA^V, MMA^{III} and MMMTA^V). Experimental conditions: ICPMS (DRC mode, selectively monitoring AsO at *m/z* 91 and SO at *m/z* 48) with chromatography method HyPURITY C18..

of the broad peak in Fig. 2c and d may also contribute to the low recoveries.

While PRP-Unk c and PRP-Unk d have similar peak shapes and retention times it is unclear at this time whether they comprise the same species. Comparing non-sterilised MLLs in Fig. 2 with Fig. 1, it is apparent organic arsenic species (DMA^V and MMA^V) were more readily converted to unknown arsenic species than inorganic species (iAs^V and iAs^{III}). Between DMA^V and MMA^V, the effect was more prevalent for DMA^V, indicating an additional methyl group in the arsenic species increases its capacity for producing these unknown arsenic species. This is discussed further in Section 3.5.

3.3. Identifying the unknown arsenic species

To identify PRP-Unk c and PRP-Unk d from the DMA^V- and MMA^V-spiked experiments, the samples used to generate Fig. 2c and d were also analysed by HPLC–ICPMS using the chromatography method HyPURITY C18 (Fig. 3a and b). Fig. 3a indicates PRP-Unk c was further separated into TH-Unk 1 (RT: 165 s), TH-Unk 2 (RT: 280 s) and TH-Unk 3 (RT: 410 s), in which TH-Unk 1 and TH-Unk 2 were previously identified as thiol-organoarsenic DMDTA^V ($(CH_3)_2As(=S)(SH)$) and DMMTA^V ($(CH_3)_2As(=S)(OH)$) [26].

Fig. 3b demonstrates PRP-Unk d was further separated into TH-Unk 1 (RT: 165 s) and two other species, TH-Unk 4 (RT: 120 s) and TH-Unk 5 (RT: 145 s). By comparing retention times, molecular ion and S/As ratios of TH-Unk 1, TH-Unk 4 and TH-Unk 5 to the synthesised DMDTA^V, MMDTA^V and MMA^{III}, TH-Unk 1 is consistent with DMDTA^V and TH-Unk 4 and TH-Unk 5 are anticipated to be MMDTA^V ($CH_3As(=S)(SH)(OH)$) and MMA^{III} ($CH_3As(OH)_2$), respectively. Details pertaining to TH-Unk 4 and TH-Unk 5 identification can be found in Supplementary materials. These results demonstrate DMDTA^V, DMMTA^V and MMDTA^V were transformed from DMA^V and MMA^V, suggesting there is a potential for DMA^V and MMA^V in landfill leachate to be converted into thiol-organoarsenic species.

Observing MMA^{III} and DMA^V in the MMA^V-spiked MLL demonstrates bacteria in MLL are capable of reducing a fraction of MMA^V to MMA^{III} and methylating to DMA^V, which is consistent with Challenger's biomethylation of MMA^V [11,12]. Nevertheless, further methylation of DMA^V to trimethylarsine oxide (TMAO) was not observed in the DMA^V-spiked MLL [26], suggesting transformation of DMA^V to thiol-organoarsenic species is preferred over TMAO formation in MLL.

3.4. Microbial transformation of arsenic species in LB medium

To confirm the effect of bacteria on arsenic speciation, the transformation of iAs^V, iAs^{III}, MMA^V and DMA^V was also investigated in LB medium with and without bacteria from MLL. The findings from the control samples; LB media containing sterilised MLL (10%, v/v) and individually spiked with iAs^V, iAs^{III}, MMA^V and DMA^V (data not shown); were similar to the results for sterilised MLL as shown in the insets of Figs. 1c and d and 2c and d. That is iAs^V, MMA^V and DMA^V remained unchanged, while iAs^{III} was oxidised to iAs^V in the control samples.

The results of arsenic speciation in LB media containing non-sterilised MLL illustrate the presence of DMDTA^V in both iAs^V- and iAs^{III}-spiked samples with its concentration being approximately 250 $\mu g\,L^{-1}$ at day 40 (Fig. 4a and b). This concentration was markedly higher than the DMDTA^V initially derived from MLL (approximately 3 $\mu g\,L^{-1}$), with the concentration difference due to its transformation from iAs^{III} and iAs^V. In addition to the formation of DMDTA^V and MMDTA^V, another unknown species, TH-Unk 6 (RT: 190 s), was formed in LB medium inoculated with bacteria and spiked with MMA^V (Fig. 4c). TH-Unk 6 is likely to be MMMTA^V ($CH_3As(=S)(OH)_2$) by comparing its retention time with that of the in-house synthesised MMMTA^V (Fig. 3b inset). MMMTA^V was not observed in MLL spiked with MMA^V (Fig. 3b), suggesting the transformation of arsenic species is related to both bacterial activity and the medium constituents. The formation of DMDTA^V in Fig. 4c aligned with the methylation of MMA^V to DMA^V and the transformation DMA^V to DMDTA^V in the leachate system. In contrast to the small fraction of MMA^V converted to MMDTA^V, MMA^{III} and DMDTA^V, and the majority of DMA^V converted to DMDTA^V and DMMTA^V in MMA^V- and DMA^V-spiked MLLs (Fig. 3a and b), it is apparent a nearly complete transformation of MMA^V and DMA^V was achieved in LB over the same period (Fig. 4c and d). The conversion of iAs^V and iAs^{III} to DMDTA^V and the enhanced transformation of MMA^V and DMA^V to thiol-organoarsenic species are due to increased nutrition available in LB medium compared to MLL.

3.5. Mechanism for thiol-organoarsenic species formation in MLL

To elucidate the role bacteria played in forming thiol-organoarsenic species, the presence or generation of hydrogen sulfide in MLL was investigated. Fig. 5 illustrates HS^- was absent in sterilised MLL, but was apparent in both non-sterilised MLL and LB medium inoculated with bacteria from MLL, indicating HS^- was generated from microbial activity. This finding is consistent with

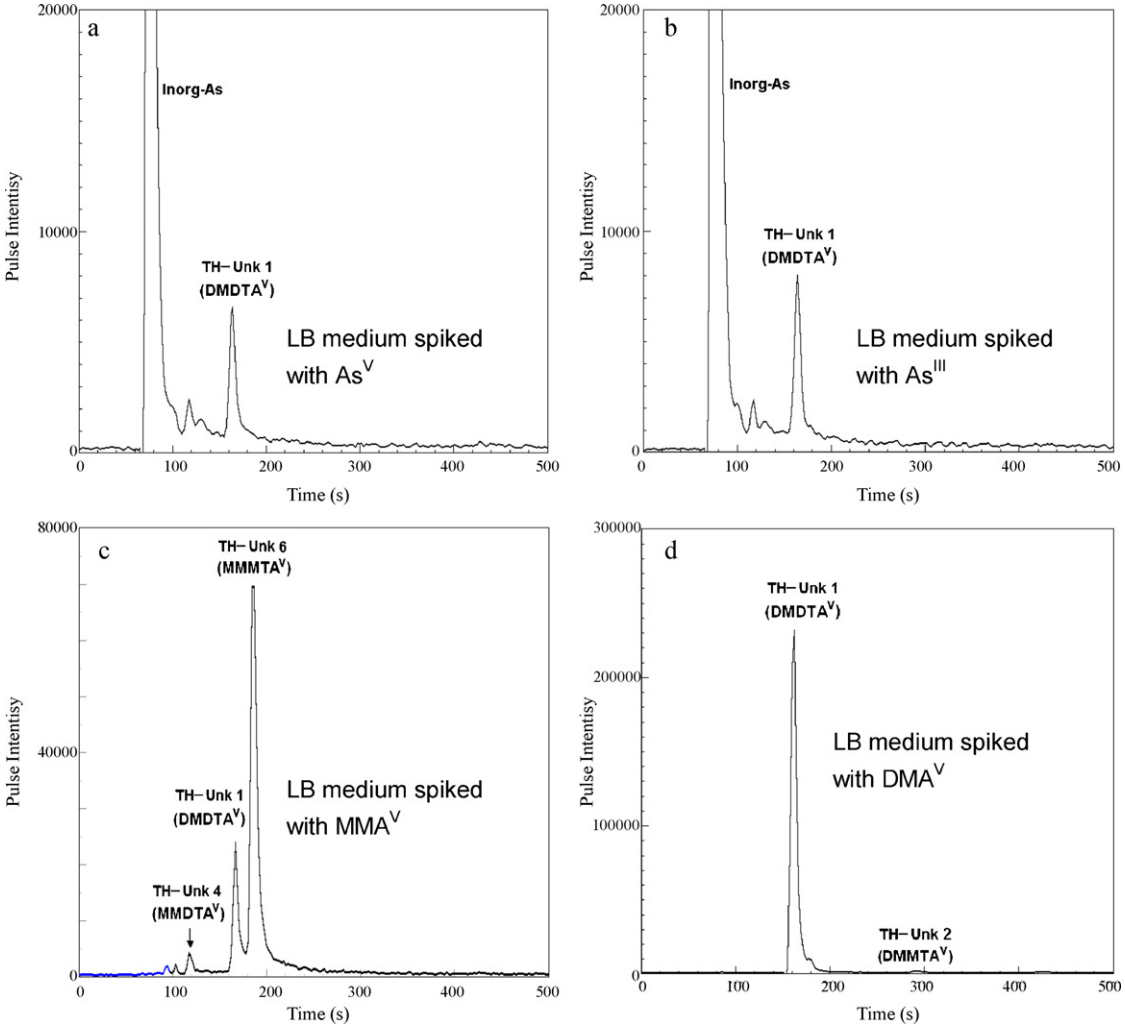

Fig. 4. HPLC–ICPMS chromatograms of arsenic species in LB medium containing MLL on day 40 after spiking with (a) iAs^V; (b) iAs^{III}; (c) MMA^V; and (d) DMA^V. Experimental conditions: ICPMS (DRC mode, selectively monitoring AsO at m/z 91) with chromatography method HyPURITY C18.

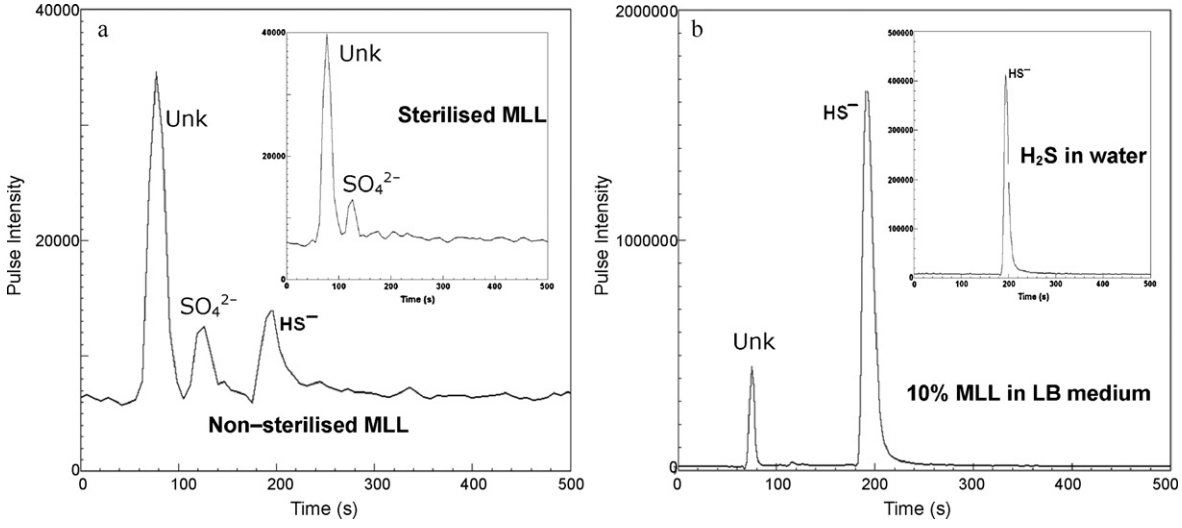

Fig. 5. HPLC–ICPMS chromatograms of hydrogen sulfide in: (a) non-sterilised MLL (inset – sterilised MLL); (b) LB medium on day 16 after inoculating with bacteria from MLL (10%, v/v) (inset – hydrogen sulfide (HS^-) in water). Experimental conditions: ICPMS (DRC mode, selectively monitoring SO at m/z 48) with chromatography method HyPURITY C18.

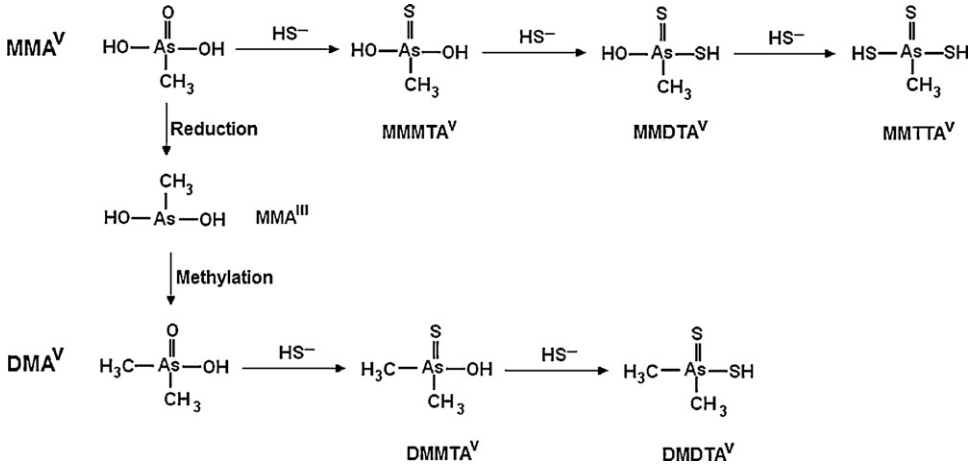

Fig. 6. Proposed pathways for the transformation of MMA^V and DMA^V to thiol-organoarsenic species in MLL.

vanLoon and Duffy [27] in that sulfate can be reduced to HS^- in a reducing environment. The microbial generation of HS^- also suggests the presence of sulfate reducing bacteria in MLL.

The presence of HS^- not only explains the previous findings of microbial reduction of iAs^V and MMA^V in MLL [12,28], but is also anticipated to be responsible for the microbial transformation of DMA^V and MMA^V to thiol-organoarsenic species in MLL. One hypothesis is that the HS^- involved microbial transformation is similar to the chemical reaction between DMA^V or MMA^V and H_2S, which were used to synthesise $DMMTA^V$, $DMDTA^V$, $MMMTA^V$ and $MMDTA^V$ [16,26,29–31]. A second hypothesis is that thiol-organoarsenic species are formed through intermediates dimethylmonothioarsinous acid ($DMMTA^{III}$) and monomethylmonothioarsonous acid ($MMMTA^{III}$), which is similar to the proposed pathways for the formation of DMMTAV and DMDTAV in rat and red blood cells by Suzuki et al. [17,32] and Naranmandura and Suzuki [21].

As $DMDTA^V$ was the dominant arsenic species in both the original and the DMA^V-spiked MLLs [26], it appears microbial sulfate reduction generated a sufficient quantity of sulfide to continuously convert DMA^V to $DMDTA^V$. Besides sulfide, microbial processes may also generate other compounds, such as GSH and thiols [33], which may participate in thiol-organoarsenic species formation [12,34–36].

Incorporating microbial formation of thiol-organoarsenic species into microbial generation of HS^- in MLL, the pathways for transformation of MMA^V and DMA^V in MLL are proposed in Fig. 6. The figure shows that arsenic species in disposed waste can transform into thiol-organoarsenic over time in a landfill environment. In the instance of MMA^V in MLL, besides methylation to DMA^V and further formation of $DMDTA^V$, it is progressively transformed to $MMDTA^V$ through an intermediate $MMMTA^V$, which is supported by its observation in LB medium. Although monomethyltrithioarsonic acid ($MMTTA^V$) was not observed in this study, the proposed mechanism suggests its involvement. The difference in the transformation pathways also explains the different transformation rates of MMA^V and DMA^V in MLL.

4. Conclusion

Bacteria present in municipal landfill leachate play crucial roles in transforming arsenic species, including the reduction of iAs^V and MMA^V, methylation of MMA^V and conversion of DMA^V and MMA^V to their thiolated analogues. The transformation of DMA^V and MMA^V to thiol-organoarsenic species in MLL involves interactions between arsenic species and hydrogen sulfide generated by bacteria in MLL. The microbial transformation of MMA^V and DMA^V into their thiol-organoarsenic species by bacteria in MLL has significant implications for arsenic speciation and toxicology as there is little available knowledge regarding the ecotoxicological significance of thiol-organoarsenic compounds in the environment. The microbially transformed toxic thiol-organoarsenic species have the potential to penetrate into groundwater and consequently increase the risk of arsenic harm to human health.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.01.093.

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