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Biodegradation of arsenosugars in marine sediment

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In the marine environment, arsenic accumulates in seaweed and occurs mostly in the form of arsenoribofuranosides (often called arsenosugars). This study investigated the degradation pathways of arsenosugars from decaying seaweed in a mesocosm experiment. Brown seaweed (*Laminaria digitata*) was placed on top of a marine sediment soaked with seawater. Seawater and porewater samples from different depths were collected and analysed for arsenic species in order to identify the degradation products using high-performance liquid chomatography–inductively coupled plasma mass spectrometry. During the first 10 days most of the arsenic found in the seawater and the shallow sediment is in the form of the arsenosugars released from the seaweed. Dimethylarsenoylethanol (DMAE), dimethylarsinic acid (DMA(V)) and, later, monomethylarsonic acid (MMA(V)) and arsenite and arsenate were also formed. In the deeper anaerobic sediment, the arsenosugars disappear more quickly and DMAE is the main metabolite with 60–80% of the total arsenic for the first 60 days besides a constant DMA(V) contribution of 10–20% of total soluble arsenic. With the degradation of the soluble DMAE the solubility of arsenic decreases in the sediment. The final soluble degradation products (after 106 days) were arsenite, arsenate, MMA(V) and DMA(V). No arsenobetaine or arsenocholine were identified in the porewater.

KEYWORDS: seaweed; *Laminaria digitata*; anaerobic degradation; biotransformation; arsenosugars; arsenoribofuranosides; mesocosm; arsenic speciation

INTRODUCTION

Arsenic accumulates in marine algae (seaweed) in large amounts and is transformed into arsenoribofuranosides, which others refer to simply as arsenosugars. Figure 1 shows the most commonly occurring arsenosugars in seaweed. Since the 1920s, marine organisms have been known to contain substantial amounts of arsenic as a complex arsenic compound.[1] The transformation of inorganic arsenic in the environment has been widely studied, including the production of methylated species by micro-organisms,[2,3] of arsenobetaine (AsB) and arsenocholine (AsC) by marine animals,[4,5] of arsenosugars by seaweed[6] and of arsenolipids by seaweed[7] and marine animals.[8] Arsenic has been found in seaweed in amounts of more than 100 mg kg^{-1} (dry weight).[9] Neither the function of those organoarsenicals nor the origin of, in particular, AsB and the arsenosugars are known. The original school of thought emphasized that the origin of AsB might be from anaerobic degradation of the arsenosugars,[7] whereas recent findings oppose this proposed pathway.[10,11]

Despite the enormous amounts of arsenosugar in seaweed and of AsB in fish, only methylated compounds, such as dimethylarsinic acid (DMA(V)), monomethylarsonic acid (MMA(V)), trimethylarsine oxide (TMAO) and, recently, dimethyl arsenoyl acetate (DMAA), have been identified in the seawater or sediment, besides arsenite (As(III)) and arsenate (As(V)).[11] Howard and Comber[12] found that the aerobic degradation of phytoplankton in seawater results in inorganic arsenic, MMA(V), DMA(V) and 'hidden' species, which they suggested were either arsenosugars or the degradation products of arsenosugars; and recently, Ellwood and Maher[13] showed that arsenosugars (sugar-3 and sugar-4) do occur in anaerobic sediments.

Here, we report a study on the transformation of arsenosugars in sediment with the aim of identifying the degradation products from arsenosugars under different

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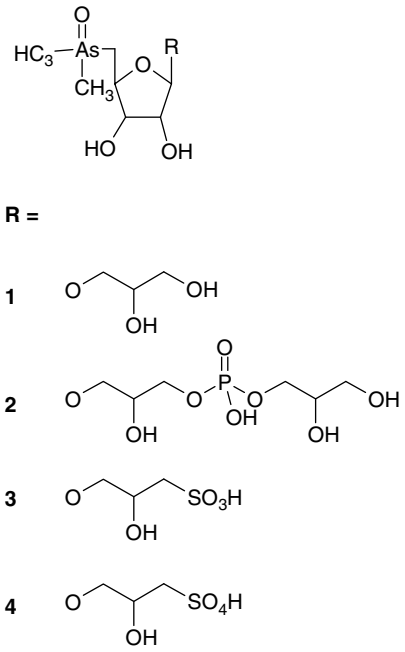

Figure 1. Structures of some arsenosugars found in seaweed.

microbial conditions, simulated by an artificial marine sediment core, in order to assess the mobility of the arsenic in the seawater–sediment interphase.

EXPERIMENTAL

Chemicals and reagents

DMA(V) and sodium dichromate dihydrate ($Na_2Cr_2O_7 \cdot 2H_2O$) were obtained from Sigma chemicals, and MMA(V) was from Chem. Service MC, West Chester, USA. Sodium arsenate (Na_2HAsO_4) and sodium arsenite ($NaAsO_2$), reagent grade, were purchased from Merck. Arsenosugar **1**[14] (Fig. 1) and dimethylarsinoylethanol (DMAE)[15] were synthesized as reported previously. The remaining arsenosugars (**2**, **3** and **4**, Fig. 1) were isolated from natural sources.[16] Orthophosphoric acid (85%), concentrated nitric acid, ammonia solution (25%) and formic acid (98–100%) were all AnalaR® obtained from BDH Chemicals, and acetic acid (>99%) AnalaR® was from Fluka.

Owing to the lack of appropriate reference material, SRM NIST 2670 standard urine (elevated; NIST, Gaithersburg, USA) was used for quality control of the chromatography. Although this is not the appropriate material for checking the quality of the porewater and seawater analysis, the use of this material is beneficial. Although the arsenic species are not certified, a wealth of information about the arsenic species is available for this standard in the literature, with which our results can be compared. Furthermore, this urine has a chloride concentration similar to seawater samples.

Thioarsenate ($Na_3AsO_3S \cdot 7H_2O$) was synthesized by dissolving 1.20 g of NaOH (pellets) in 4 ml deionized water. 1.00 g of As_2O_3 was then added and stirred. 0.28 g sulfur was added to this solution and then boiled under reflux. Sodium thioarsenate was identified as a red solid using X-ray diffraction. (Purity was not established, but the diffraction pattern, besides the major thioarsenate signals, also contained those of arsenate and arsenite.)

Digestion for total arsenic determination

Approximately 0.2 g ± 0.1 mg of dried powdered seaweed was mixed with 4 ml of concentrated nitric acid–water (1 : 1) and digested in a microwave oven (CEM MDS-81D, maximum output power of 630 W). The temperature program used included four heating steps: at 190 W for 5 min, 315 W for 10 min, 315 W for 10 min (additional 3 ml nitric acid added), and 380 W for 20 min. At the end of each heating step the polytetrafluoroethylene (PTFE) bombs were cooled to room temperature and the cap was released to remove all generated acid vapour. At the end of the microwave digestion the sample solution was diluted to 25 ml with Milli-Q water. This solution was introduced directly to the inductively coupled plasma (ICP) mass spectrometer.

Extraction of water-soluble arsenic

Extracts from approximately 0.2 g of freeze-dried seaweed were obtained by adding 10 ml of methanol–water (1 : 1), vortexing the solution for 5 min, and centrifuging at 6000 rpm for 15 min at room temperature. This extraction was repeated five times. The supernatants were mixed and evaporated under reduced pressure. The residue was diluted with Milli-Q water before analysis by high-performance liquid chromatography (HPLC)–ICP mass spectrometry (MS). The HPLC parameters are given in Table 1.

Separation of arsenic species by HPLC

For the separation of the anionic arsenic species a strong anion-exchange column, PRP X 100 Hamilton (250 mm × 4.6 mm), was used. The buffer for this separation was a 30 mM phosphate buffer adjusted to pH 5.5 with ammonia. For the cationic species, a Supelcosil SCX (250 mm × 4.1 mm) column was used. A 20 mM pyridine buffer adjusted to pH 2.5 with formic acid was used as eluent for the cation-exchange column. The flow rates were 1 ml min^{-1} for the cation column and 1.2 ml min^{-1} for the anion column; the injected sample volume was 20 μl.

DETERMINATION OF ARSENIC BY ICP-MS

An ICP mass spectrometer (Spectromass 2000 from Spectro Analytical Instruments Kleve, Germany) was used as detector for the determination of total arsenic and arsenic species. The instrument was fitted with a water-cooled cyclonic spray-chamber and a Meinhard nebulizer. The operation parameters were controlled daily for optimum arsenic sensitivity and optimized when necessary. For the analysis of total arsenic

Table 1. HPLC parameters and retention times of the different arsenic species

HPLC column	Mobile phase	As species standard	Retention time (s)
Anion column Hamilton PRP X-100 250 mm × 4.6 mm, 5 μm	30 mM H_3PO_4 pH 5.5 (adjusted with NH_3) flow rate 1.2 ml min^{-1}	As(III)	164
		Sugar-1	185
		DMA(V)	201
		MMA(V)	360
		Sugar-2	432
		As(V)	494
		Sugar-3	912
		AsO_3S^{3-}	1070
Cation column Supelcosil SCX 250 mm × 4.1 mm, 5 μm	20 mM pyridine pH 2.5 (adjusted with HCOOH) Flow rate 1 ml min^{-1}	As(V)	180
		MMA(V)	217
		As(III)	219
		DMA(V)	257
		AsB	361
		Sugar-1	437
		DMAE	516
		TMAO	558
		AsC	758

concentration the instrument was fitted with an auto-sampler, and for species analysis the outlet of the HPLC column was connected with a 30 cm Teflon tube directly to the nebulizer.

Mesocosm experiment

Stormcased *Laminaria digitata* (brown algae) was collected from the beach in Stonehaven (Northeast Scotland). A stalk and broad fan-like blade were washed with water and chopped into small pieces of 1–2 cm. The marine sediment was collected from Aberdeen Harbour with a grab sampler. The sediment was dark, fine silt with a strong smell of hydrogen sulfide and this was thoroughly mixed with synthetic seawater. The synthetic seawater was added to the sediment to obtain a slurry, which was poured into a glass column (100 cm length × 4 cm diameter). Synthetic seawater was filled to above the sediment surface to prevent exposure of the sediment to oxygen and left overnight to settle by gravity. Chopped seaweed with an approximate weight of 250 g wet weight was added (Fig. 2). Synthetic seawater was carefully filled up to the top of the column to maintain a constant volume during the experiment. The chopped seaweed was left in the glass column to decay. A small flow rate of 10 mL day^{-1} increased the diffusion of the water-soluble degradation products into the different layers of the sediment, without transporting the organic material further. The column was kept in the dark at room temperature. Seawater and porewater were collected from sampling points at 0, 2, 12, 22, 32 and 42 cm. Samples of approximately 2 ml were taken daily until day 10, and then on a weekly basis for approximately 14 weeks. The porewater samples collected were kept in polypropylene tubes and stored in the freezer at −22 °C before analysis. All samples were filtered through 0.45 μm cellulose nitrate filters and diluted to appropriate concentrations prior to analysis. The arsenic species were analysed by HPLC–ICP-MS using anion-and cation-exchange chromatography.

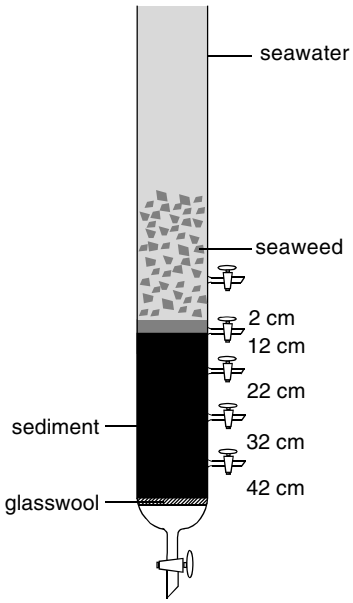

Figure 2. Mesocosm set-up: artificial sediment core with sampling ports for porewater extraction.

RESULTS

Total arsenic of the *L. digitata* was measured to be 94.2 ± 2.7 mg kg^{-1} (dry weight, $n = 3$). The extraction efficiency of

Table 2. Performance of HPLC–ICP-MS on NIST SRM 2670($As_{(total)}$ 480 ± 100 ng ml^{-1})

As(III)	DMA(V)	MMA(V)	As(V)	AsB	$As_{(total)}$	Reference
nd	51 ± 2	10 ± 1	355 ± 35	16 ± 1	431 ± 38	This study
<1	68 ± 4	15 ± 1	359 ± 22	34 ± 7	477	17
nd	49 ± 3	7 ± 1.3	443 ± 20	15 ± 3	514 ± 23	18
nd	49 ± 2	8.1 ± 0.7	403 ± 8	—	460 ± 10	19

the freeze-dried seaweed was, at 64.5% ($n = 3$), comparable to that found in earlier work, whereas the total arsenic level was slightly higher.[17] This may reflect the seasonal variability of *L. digitata* in the North Atlantic. The arsenic concentration of the sediment used from Aberdeen Harbour was approximately 13 ± 2 mg kg^{-1} ($n = 5$). The addition of 250 g of seaweed would increase the concentration of arsenic in the sediment by about 5 mg kg^{-1}, if the arsenic were immobilized during the decomposition procedure and homogenously distributed.

A standard mixture of As(III), As(V), MMA(V) and DMA(V)) and the arsenosugars and DMAE were separated by using either anion-exchange or cation-exchange chromatography and run before sample analysis by HPLC. The retention times are listed in Table 1. The concentrations of inorganic and methylated species were calibrated using standard solutions ([As]-(0, 10, 25 and 50 ng ml^{-1} mixed standard)). The concentrations of arsenosugars, DMAE and unknown degradation products were quantified using As(V) standard solution, since a species-independent calibration function was shown to exist for As(III), MMA(V), DMA(V) and As(V). The correlation coefficients of the calibration curves were better than $r^2 = 0.995$ for the concentration range 0–100 ng ml^{-1}. The performance of HPLC–ICP-MS was checked with NIST SRM 2670. The results were in agreement with studies published earlier (Table 2).

Chromatograms of the water-soluble arsenic species in *L. digitata* and an extract spiked with As(III), DMA(V), MMA(V) and As(V) are shown in Fig. 3. Three arsenosugars (sugar-1, sugar-2 and sugar-3) were identified in the seaweed extract by comparing the retention times with purified arsenosugars. The occurrence of these arsenosugars was confirmed in a later study using carbonate buffer and the parallel use of electrospray-MS and ICP-MS.[20] *L. digitata* contains sugar-3 (71.9%) as major extractable arsenic species (Fig. 3), in addition to the two minor arsenosugars sugar-2 (18.2%) and sugar-1 (7.6%).

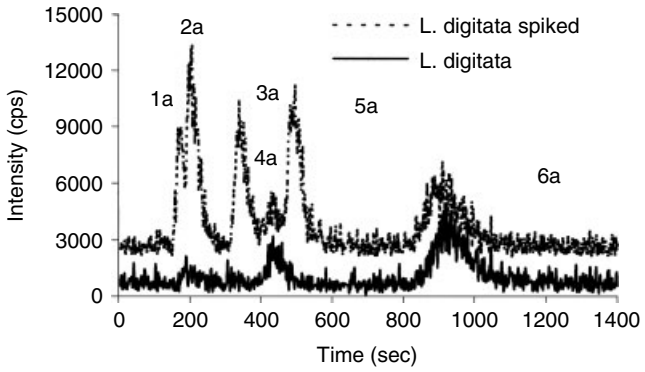

Figure 3. Anion-exchange HPLC–ICP-MS chromatogram of spiked *L. digitata* extract: As(III), Ia; DMA(V), 2a; MMA(V), 3a; As(V), 5a; sugar-2, 4a; sugar-3, 6a.

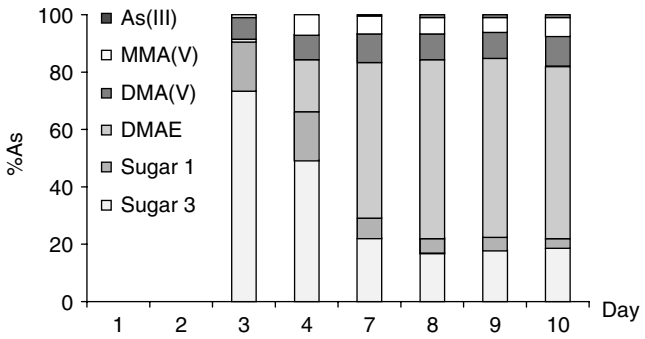

Figure 4. Arsenic species distribution in the seawater above the sediment in the first 10 days of seaweed degradation in the microcosm.

Mesocosm experiment

Seawater

Seaweed started to decompose immediately and release the arsenosugars into the seawater. While sugar-3 and sugar-1 were detected in the seawater (Fig. 4), sugar-2 was not present. It is apparent that the concentration of sugar-1 is higher than expected from the arsenic distribution in the seaweed. Furthermore, the concentration of sugar-1 did not decrease for the first few days, indicating that sugar-1 may be a degradation product of sugar-2 and sugar-3. After 7 days, most of the arsenosugars were degraded to DMAE. After day 8, species such as DMA(V), As(III) and As(V) started to occur in the seawater (Fig. 5). Surprisingly, As(III) began to occur at day 18, but it was finally removed from the seawater after day 23, possibly due to precipitation as sulfide. The presence of As(III) is probably due to the redox change in the seawater. The high load of decaying organic material will consume the dissolved oxygen in the water column and the oxygen diffusion from the air into the seawater was probably too low. After 38 days, MMA(V)

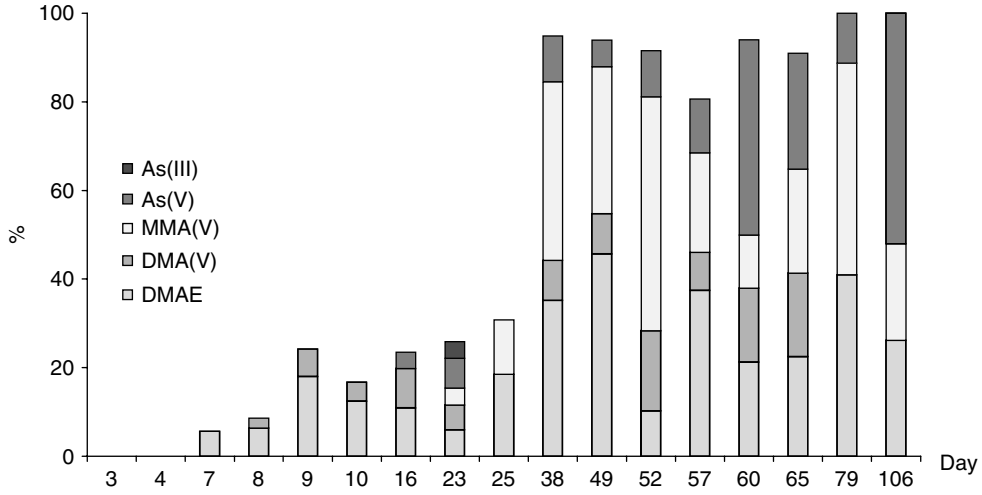

Figure 5. Proportion of degradation products from the initial arsenosugars in the seawater.

became the major intermediate degradation product, and this later demethylated to As(V). As(V) was the major final degradation product of the arsenosugars.

Porewater

The degradation of arsenosugars at 2, 12 and 22 cm depth could only be monitored for the first 10 days because of clogging of the sample ports at those depths. At depths of 2, 12 and 22 cm depth the behaviour of all arsenic species is very similar, which is represented by the data of the porewater at 12 cm (Fig. 6). Sugar-3 and sugar-1 were present in porewater only in the initial phase, whereas sugar-2 was never detected. After 7 days, only the degradation products DMAE, DMA(V), MMA(V) and As(III) were present in the porewater, of which DMAE was the major species at 80%. Figure 7 shows the identification of DMAE in a porewater sample in comparison with a mixed arsenic standard measured by using cation-exchange chromatography. DMA(V), MMA(V) and inorganic arsenic species appear in the sediment 4 days earlier than in the seawater. The concentration of DMAE increased dramatically with time and it became the major degradation product. A simultaneous increasing concentration of MMA(V) suggests that the demethylation of DMA(V) to MMA(V) is much faster in the shallow sediment than in seawater.

In the deep sediment, the behaviour of all species is similar at depths 32 and 42 cm (Fig. 8). No significant amounts of sugars were identified in the deep sediment. DMAE is the major degradation product, with 60–80% up to day 60. The concentrations of DMA(V) and MMA(V) were relatively constant throughout the experiment. However, As(V) increased steadily and became the major degradation product after 106 days, which points to demethylation of either DMAE or DMA(V). Surprisingly, the concentration of As(III) was low—perhaps As(III) was precipitated with sulfide as arsenic sulfide (AsS and As_2S_3) or formed the

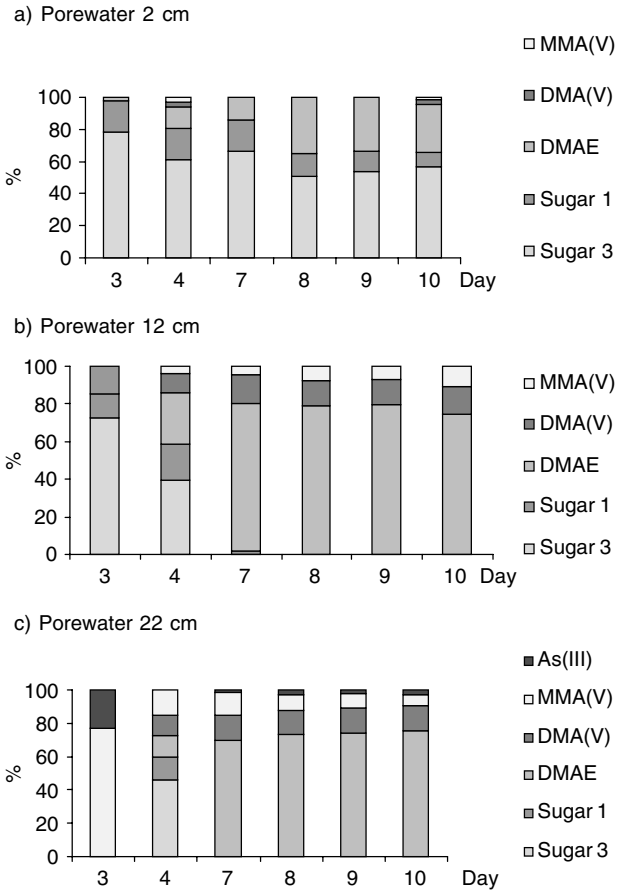

Figure 6. Arsenic distribution in the porewater of the sediment as a function of time and depth.

thioarsenite anion (AsS_2^-), which cannot be eluted from the strong anion-exchange column[21] used in our experimental set-up. Other organothioyl compounds, like those recently identified as metabolites of arsenosugars in sheep's urine,

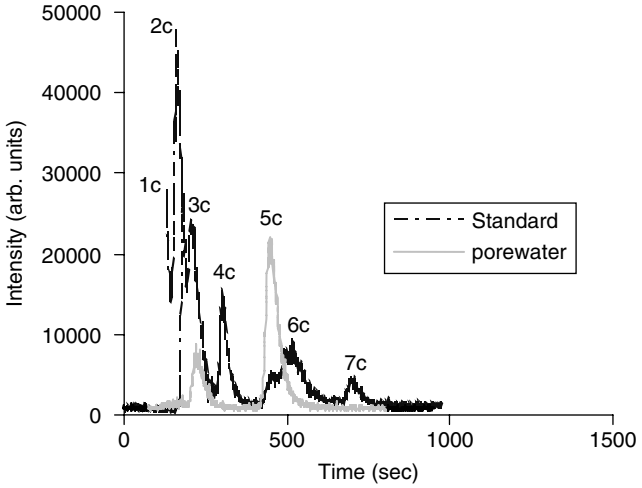

Figure 7. Chromatogram using cation-exchange chromatography of a standard mixture (As std) and a porewater sample showing the major compounds: DMA(V), 3c; DMAE, 5c. Standards were: As(V), 1c; As(III) and MMA(V), 2c; DMA(V), 3c; AsB, 4c; DMAE, 5c; TMAO, 6c; AsC, 7c.

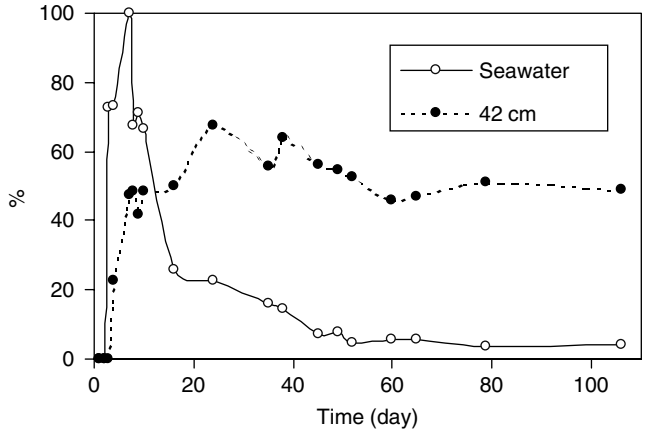

Figure 9. Total arsenic concentration in the water column and in the deep sediment during the experiment. The highest concentration measured in seawater was set to 100%.

would not elute from the column, and hence would not appear here[22]. However, a significant reduction of chromatographic recovery and an immobilization of arsenic is not obvious, since the concentration of arsenic in the porewater is remarkably constant during the experiment (Fig. 9).

As(V) is probably kinetically stabilized by complexing ligands such as humic acid[23], as it survives the reducing anaerobic environment deep in this core.

In addition, unknown anionic peaks appeared in the porewater of the deep sediment at days 60 and 65, exactly at the time at which DMAE started to degrade further. It seems that these unknown species are intermediates, since they disappear again at day 79 in the porewater of the deep sediment. Since no available standard matched the retention times of the unknowns, thioarsenate was synthesized and separated by the anion exchange method (Fig. 10). The potassium thioarsenate (K_3AsO_3S), which was characterized by X-ray diffractometry, showed three peaks: two of them co-elute with As(III) and As(V) the major peak elutes at 1070 s, which is around the same time as the small unknown peak U2 found in the porewater on days 60 and 65 (Fig. 10). Therefore, it might be possible that the unidentified peaks are inorganic thioarsenicals. However, a thorough electrospray analysis is required to confirm this.

DISCUSSION

Seaweed contains mainly arsenosugars; whether these are generated by the algae or by bacteria that may live on the

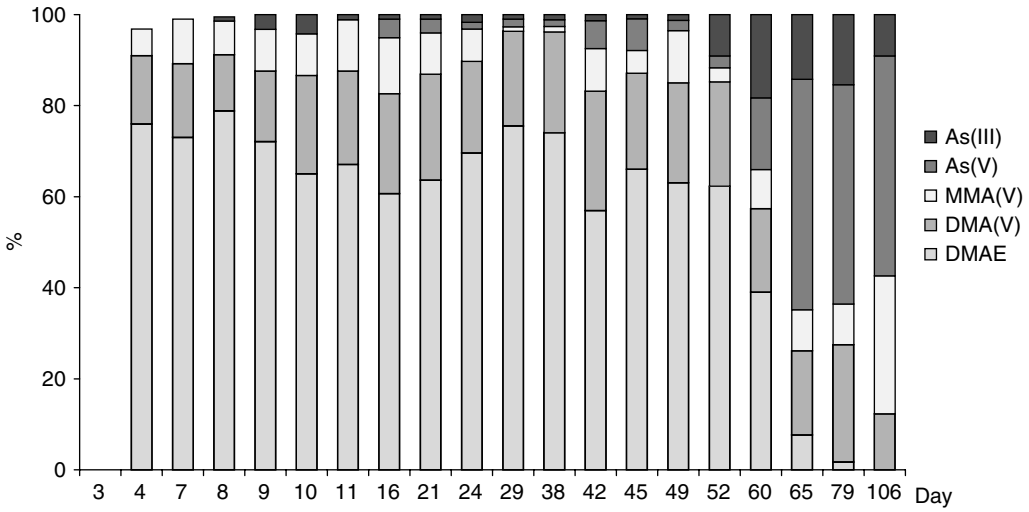

Figure 8. Arsenic distribution in the deep sediment (at 42 cm).

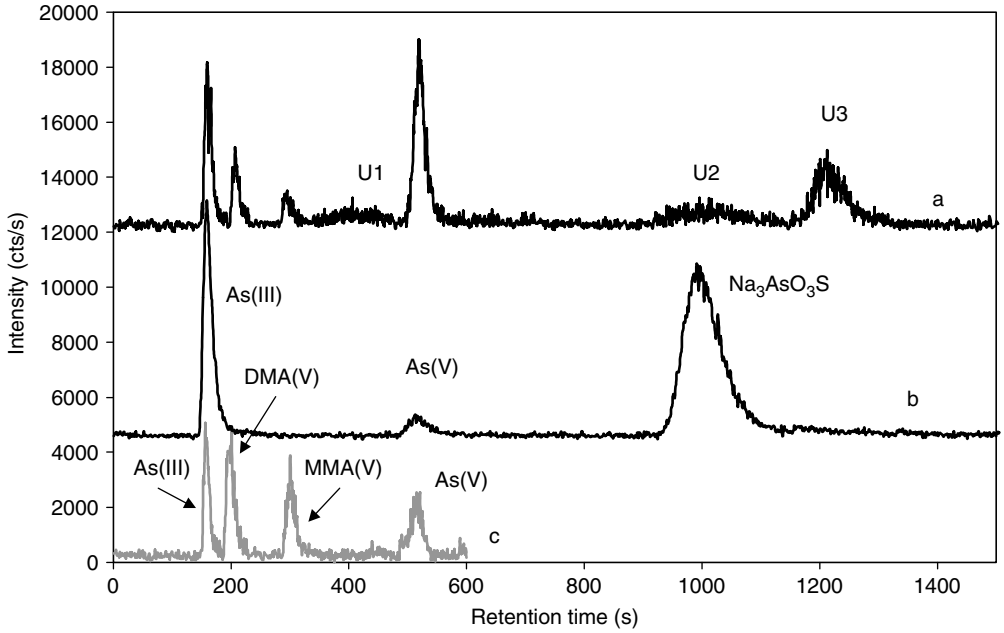

Figure 10. Chromatograms of (a) a porewater sample (at day 60 at 42 cm depth), compared with the (b) synthesized thioarsenate standard containing impurities of As(III) and As(V) and (c) a standard mixture of As(III), DMA(V), MMA(V), As(V). U1, U2 and U3 are unidentified arsenic species in the porewater. LC-method, Nr.1: PRP-X100 anion-exchange column, 1.2 ml min^{-1} and pH 5.5.

seaweed or in the water column is still under debate.[24–26] Three arsenosugars, sugar-3, sugar-2 and sugar-1, were released from *L. digitata* into seawater. Sugar-2 proved to be rather unstable in seawater. On the one hand, this was to be expected, since sugar-2 has shown a fast degradation in a soil environment, when soil was amended with *L. digitata*.[27] On the other hand, however, it is in contrast to non-sterile anaerobic conditions in which sugar-2 was stable for more than 2 weeks.[28] Sugar-1 may benefit from the degradation of sugar-2 and later also from sugar-3. Sugar-3 seems more stable and, therefore, is able to diffuse deeper into the sediment before it degrades to sugar-1. The carbon–carbon bond between C_3 and C_4 can be cleaved to give DMAE, which can be further dealkylated to DMA(V). Before demethylation to inorganic arsenic occurs, a significant amount of MMA(V) is generated. The demethylation takes place after the cleavage of the ribofuranoside; therefore, it is much more likely to find DMA(V) than MMA(V) or mono-methylated ribofuranosides during the first days after the start of the decay process. It is not yet possible to detect mono-methylated ribofuranosides in the marine environment. These results are in contrast to the degradation of arsenosugars in arable soil, in which neither MMA(V) nor DMAE were identified. The experiments showed that DMAE is one of the major degradation products and that it is also rather stable in shallow and in deeper sediments. These findings confirm results from Edmonds and Francesconi,[7] who detected DMAE as a degradation product in anaerobic sediment in laboratory experiments. No DMAE has ever been detected in a natural marine environment. This is very surprising, since it seems that it is rather stable in an anaerobic environment. The concentration of arsenic in the porewater is, however, constant and does not decrease with depth as expected. This might be due to the high amount of organoarsenicals such as DMAE, which are highly soluble and cannot easily be reduced. A transformation of DMAE to dimethylarsinothioyl ethanol might be likely in the presence of free sulfide.[22] This means arsenic is highly mobile and arsenic from a massive decay of seaweed would be able to diffuse in the deeper anaerobic zones of a sediment without immobilization. Although sugar-3 and sugar-4 have been identified in marine sediments,[13] DMAE has never been identified to occur in seawater or porewater. Methylation to AsB, AsC, TMAO or tetramethylarsonium (TetraMA) was not observed during the entire experiment. Edmonds and Francesconi thought that arsenosugars are transformed to AsB via microbial degradation, while a trimethylarsonylribofuranoside is the starting material for the synthesis of AsC.[29] Since AsB is the most abundant arsenic species in the marine environment, it is quite unlikely that the small amounts of trimethylarsonylribofuranosides in seaweeds are the origin of AsB.[30] Our results confirm those obtained by Edmonds and Francesconi,[6] who did not identify any AsC or AsB when arsenosugars were decaying in anaerobic sediment; only DMAE was identified as a metabolite.[31] This indicates that AsB and AsC, which are abundant in the marine environment, are not directly generated by anaerobic microbial degradation of arsenosugars in the sediment, as has often been suggested in the past. The occurrence of As(V) in the anaerobic porewater is surprising and has to be assessed critically. It should be

stated here that As(V) was only identified based on retention time comparison with a standard. It cannot be ruled out that unidentified organoarsenicals or organothioarsenic species might have the same retention time. However, it should be pointed out that the anaerobic porewater was never exposed to any air that could alter the arsenic speciation.

CONCLUSIONS

When seaweed decays in the interphase between seawater and sediment, arsenosugars are released into the seawater, where they show a reasonable stability, so that arsenic is rather mobile and it is unlikely that it contributes to a buildup of arsenic in sediment. When however these arsenosugars diffuse into the porewater of the sediment, or when decaying seaweed becomes covered by sediment, arsenic is not immobilized under anaerobic conditions due to the high level of rather soluble organoarsenicals.

Acknowledgements

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