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REVIEW

The fate of organoarsenic compounds in marine ecosystems

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Microbial degradation experiments were performed with each standard arsenical [arsenobetaine, trimethylarsine oxide, dimethylarsinic acid, methanearsonic acid, inorganic arsenic(V) and inorganic arsenic(III)]. As typical origins for marine micro-organisms, sediments, macro-algae, mollusc intestine and suspended substances were used. The results were from these experiments led us to the following conclusions: (1) there is an arsenic cycle which begins with the methylation of inorganic arsenic on the route to arsenobetaine and terminates with the complete degradation of arsenobetaine to inorganic arsenic; (2) all the organoarsenic compounds which are derived from inorganic arsenic in seawater, through the food chains, have the fate that they, at least in part, finally return to the original inorganic arsenic.

Keywords: Organoarsenic, marine ecosystems, arsenic cycle, micro-organisms, degradation

INTRODUCTION

Arsenic occurs ubiquitously in nature, including organisms. Its concentration is much higher in marine organisms than in terrestrial ones. Lunde reported the occurrence of both inorganic and organic arsenic compounds in marine organisms.[1] After that, many kinds of organoarsenic compounds were isolated or identified from marine organisms and nowadays it is well known that arsenic is accumulated in them mainly as the organic form. Edmonds, Francesconi and coworkers accomplished the first important work on the identification of the organoarsenic compounds, i.e. the isolation and identification of arsenobetaine from the muscle of western rock lobster.[2] This compound is, at present, considered as the final metabolite of arsenic in marine food chains and is accumulated in marine animals in a greater or lesser degree. We have also confirmed the ubiquitous occurrence of this compound in marine animals independently of their feeding habits and the trophic levels to which they belong.[3] As to the process of biosynthesis of organoarsenic compounds in food chains, it is generally accepted that inorganic arsenic taken from seawater is concentrated and converted to organoarsenic compounds by phytoplanktons or algae, further metabolized through the food chains and accumulated as arsenobetaine in marine animals.

Recently, we have been interested in arsenic circulation in marine ecosystems rather than the bioconversion of arsenic to arsenobetaine in food chains. The identification of arsenic compounds from various marine organisms was very important for an understanding of the conversion of arsenic through the food chains, however, it was insufficient for an entire understanding of arsenic circulation in marine ecosystems. This is because it does not answer the question of the fate of arsenobetaine or other organoarsenicals after the death of marine organisms which had accumulated them. We have tried to approach the fate of arsenobetaine or other organoarsenicals microbiologically. Degradation experiments were performed with synthetic arsenobetaine or other arsenic compounds and each of four kinds of typical origins for marine micro-organisms, namely sediments,[3–7] marine macro-algae,[8] marine mollusc intestine[9] and suspended substances.[10]

MATERIALS AND METHODS

Origin of marine micro-organisms

Sediments, two species of marine macro-algae (*Hizikia fusiforme* and *Monostroma nitidum*), mollusc intestine (*Liorophura japonica*) and

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suspended substances were collected from the coastal waters of Yoshimi, Shimonoseki, Japan. Suspended substances was collected from about 2 dm^3 of seawater by filtration with a membrane filter (0.22 μm).

Cultivation

Two culture media were used for the microbial degradation experiments:[3–10] a 1/5 ZoBell 2216E (pH 7.5) and an aqueous solution of inorganic salts at pH 7.5 containing no carbon sources. Except for suspended substances, about 1 g of each material collected as the origin for the micro-organisms was added to each of the two media (25 or 50 cm^3) containing each standard arsenic compound {arsenobetaine, trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA), disodium methanearsonate (MMA), disodium arsenate [arsenic(V)] and arsenic trioxide (arsenic(III)} (all with 8.4 mg As per 25 cm^3) and the mixtures were shaken at 25 °C in the dark for two to four months under an atmosphere of air. Suspended substances were added to the media together with the membrane filter. Some mixtures were covered with about 5 cm^3 of liquid paraffin for anaerobic culture. Mixtures autoclaved at 120 °C for 20 min served as controls. Filtered aliquots from the mixtures were withdrawn at intervals of several days and the arsenicals in them were analysed by high-performance liquid chromatography (HPLC).

Apparatus

Thin-layer chromatography (TLC) was performed on cellulose thin layers (Funakoshi Yakuhin Co. Ltd; Avicel SF, 0.1 mm). Dragendorff reagent and $SnCl_2$/KI reagent[11] were used for the detection of the spot. HPLC was carried out on a Model CCP 8000 series (Tosoh Co.) with a TSK gel ODS-120T column (4.6 mm × 250 mm) with a graphite furnace atomic absorption spectrometer serving as the arsenic specific detector. A 11.2 mmol dm^{-3} aqueous solution of sodium heptanesulphonate/acetonitrile/acetic acid (95 : 5 : 6, by vol.) was used as a solvent for the chromatography.[12] Besides the ODS-120T column, a Nucleosil 10 SB column (Wako-junyaku-kogyo Co., 4.6 mm × 250 mm) was also used with a 0.02 mmol dm^{-3} phosphate buffer as the mobile phase[13] as one of the methods to identify a metabolite. ^1H NMR and ^{13}C NMR spectra were measured on a Bruker AAM-400 NMR spectrometer in D_2O at 400 MHz and 100 MHz respectively with 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) as an internal standard. FAB mass spectra were performed with a JEOL JMS DX-300 mass spectrometer equipped with a fast atom bombardment ion source and xenon atoms at 6 keV. A combination of gas chromatographic separation with hydride generation, followed by a cold-tap technique and selected ion monitor mass spectrometric analysis, was also used for characterization of purified metabolites.

Purification and identification of the metabolites

Each medium containing the metabolites as microbial degradation products of arsenicals was centrifuged for 15 min at 3500 rpm and the supernatant was subjected to various column chromatographies to purify each metabolite.

In the degradation experiments so far, several metabolites have been derived and identified on HPLC. In addition, TLC and the physicochemical analyses described above were performed to confirm further the structure of each metabolite.

RESULTS AND DISCUSSION

Degradation pattern of arsenobetaine under aerobic conditions

Arsenobetaine has been degraded by micro-organisms from every origin investigated so far, i.e. sediments, macro-algae, mollusc intestine and suspended substances. The typical conversion pattern of arsenobetaine when the micro-organisms had a high activity is shown in Fig. 1. TMAO and DMA were derived from every micro-organism investigated. Further degradation was observed in sediments and suspended substances where a considerable amount of (or all) arsenobetaine was degraded to arsenic(V). Two practical conversion patterns are shown in Figs 2 and 3. Figure 2 shows the high degradation rates of arsenobetaine in the experiments performed with sediments and synthetic arsenobetaine, in which arsenobetaine was degraded to arsenic(V). On the other hand, relatively low degradation activity was shown by the intestinal micro-organisms of chitons *Liolophura japonica* in ZoBell medium (Fig. 3): only TMAO and

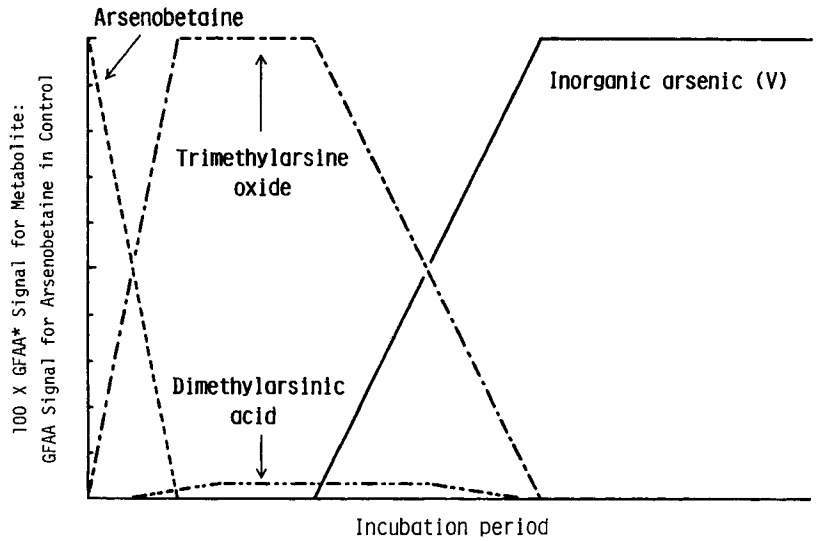

Figure 1 Schematic degradation pattern of arsenobetaine to inorganic arsenic *in vitro* by marine micro-organisms of high activity, arsenobetaine disappearing within 2–3 days. * GFFA, graphite furnace atomic absorption spectrometry.

DMA appeared, arsenic(V) not being derived. Furthermore, in this case, little conversion was observed in the inorganic-salts medium.

The micro-organisms probably used the carboxymethyl moiety of arsenobetaine to satisfy their requirement for organic carbon, and converted arsenobetaine to TMAO. After this source of carbon had become exhausted, the methyl groups in TMAO became useful; they could have been cleaved from the arsenic compound, and become utilized by the micro-organisms with concomitant conversion of TMAO to DMA or arsenic(V).

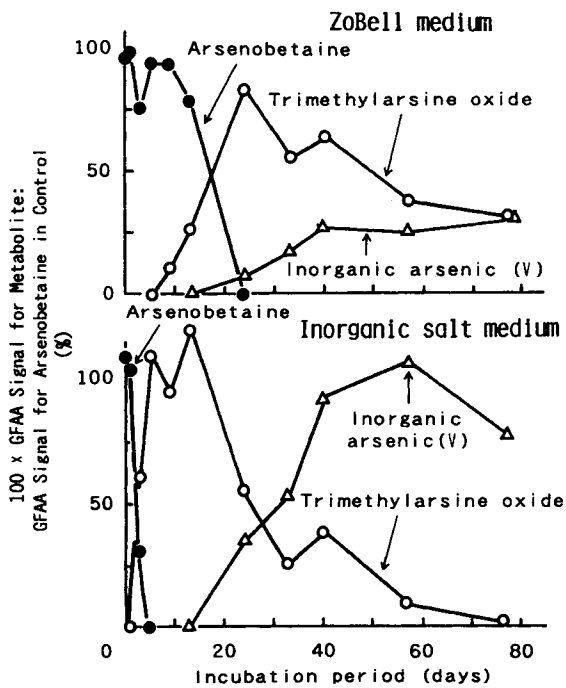

Figure 2 The conversion of arsenobetaine in an inorganic-salts medium and a ZoBell medium with sediments added during aerobic incubation at 25 °C.

The fact that arsenobetaine was degraded by micro-organisms from every origin suggests the ubiquitous occurrence of its microbial degradation in marine ecosystems. Furthermore, the complete degradation of arsenobetaine to inorganic arsenic occurred not only with the sediments but also with the suspended substances, indicating the occurrence of its degradation not only in the sediment but also in the water column. In other words, it is suggested that arsenobetaine derived from inorganic arsenic in seawater and accumulated in marine animals begins to degrade immediately after their death before their bodies or residues reach the bottom. If their residues themselves are transformed into suspended substances, their residence time in the water column may be so long that the arsenobetaine contained in them has an adequate time to suffer degradation to a considerable degree.

Besides degradation products such as TMAO, arsenocholine was also derived in the experiment with suspended substances. This reduction of arsenobetaine by micro-organisms was very interesting in terms of arsenic metabolism in marine ecosystems, and is now under investigation.

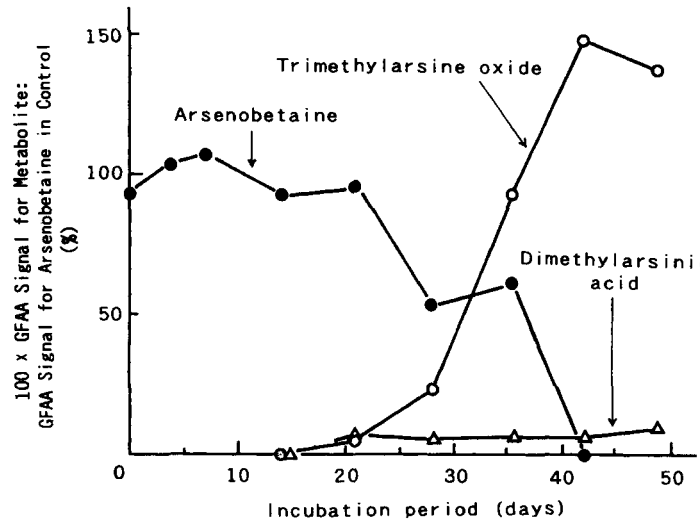

Figure 3 The conversion of arsenobetaine in a ZoBell medium with *Liolophura japonica* intestine added during aerobic incubation at 25 °C.

Conversion of arsenic compounds by micro-organisms occurring in the sediment under aerobic and anaerobic conditions

The conversion pattern of several arsenic compounds [TMAO, DMA, MMA, arsenic(V) and arsenic(III)] by the micro-organisms occurring in the sediments was investigated in both the media. Under aerobic conditions (Fig. 4), arsenobetaine was the only methylarsenical which was rapidly degraded, being converted to several metabolites, TMAO, DMA and more-degraded metabolites, successively. In contrast, the conversion of arsenicals other than arsenobetaine was observed only in three cases: the inorganic medium/DMA, the ZoBell/TMAO and the ZoBell/arsenic(III) mixtures. DMA in the inorganic salt medium was converted to more-degraded arsenicals, and arsenic(III) in the ZoBell mediun was converted to arsenic(V). With TMAO in the ZoBell medium, an unusual conversion pattern was observed, where DMA derived from TMAO was reconverted to the original TMAO after 40 days of incubation. This phenomenon, however, is not necessarily strange. The concept that arsenic metabolism by micro-organisms consists of degradations and syntheses of arsenicals is quite reasonable, although these methylations *in vitro* have not been observed in degradation experiments in our laboratory so far. The experiments in which arsenobetaine was used as a starting material may be unsuitable for a study to deal with the microbial methylation of arsenicals. This result *in vitro*, i.e. that the methylation of arsenicals is merely a minor phenomenon, might not be applicable in the field because there may be various arsenic metabolites able to be acted upon by various micro-organisms.

On the other hand, the conversion pattern of arsenicals observed under anaerobic conditions (Fig. 5) was the opposite that under aerobic conditions: either no (inorganic medium) or a little (ZoBell medium) arsenobetaine was converted to its metabolites, while all the methylarsenicals other than arsenobetaine were converted to less-methylated compounds. TMAO was converted to DMA, DMA to MMA and/or inorganic arsenic, and MMA to arsenic(V).

Thus, a clear difference in the conversion pattern of arsenicals was shown between aerobic and anaerobic conditions. Aerobic environments may be suitable for micro-organisms to act on arsenobetaine. With regard to other arsenicals with methyl groups, viz. TMAO, DMA and MMA, however, it was more difficult to solve the problem about in which environment it was more feasible for them to underto degradation. For example, TMAO derived from arsenobetaine was relatively rapidly converted to DMA under aerobic conditions, while TMAO added to the media as a starting material was not converted to its metabolite under the same conditions. On the whole, the methylarsenicals which were derived from arsenobetaine as metabolites under aerobic conditions and which were added to the media as

starting materials under anaerobic conditions were rapidly cleaved with the liberation of methyl groups, while those added to the media as starting materials under aerobic conditions were hard to cleave. As for the carboxymethyl moiety in arsenobtetaine, it may be reasonable for it to be utilized under aerobic conditions when one takes account of its possible utilization in an aerobic metabolic pathway such as the TCA (tricarboxylic acid) cycle. In spite of these complications, however, these results were very interesting for considering the degradation or conversion of arsenicals in marine ecosystems. In marine environments, when dead animals, their residues or faeces are decayed by micro-organisms, the arsenobetaine contained in them is possibly converted to less-methylated arsenicals mainly in water or on the bottom surface, but not in the anaerobic interior of the sediment.

Difference in conversion pattern of arsenicals between the inorganic-salts medium and the ZoBell medium

In each degradation experiment with two kinds of media, there were considerable differences in the conversion rate between these media. We could not explain the cause of this difference in the rate consistently: there is no definite tendency as to

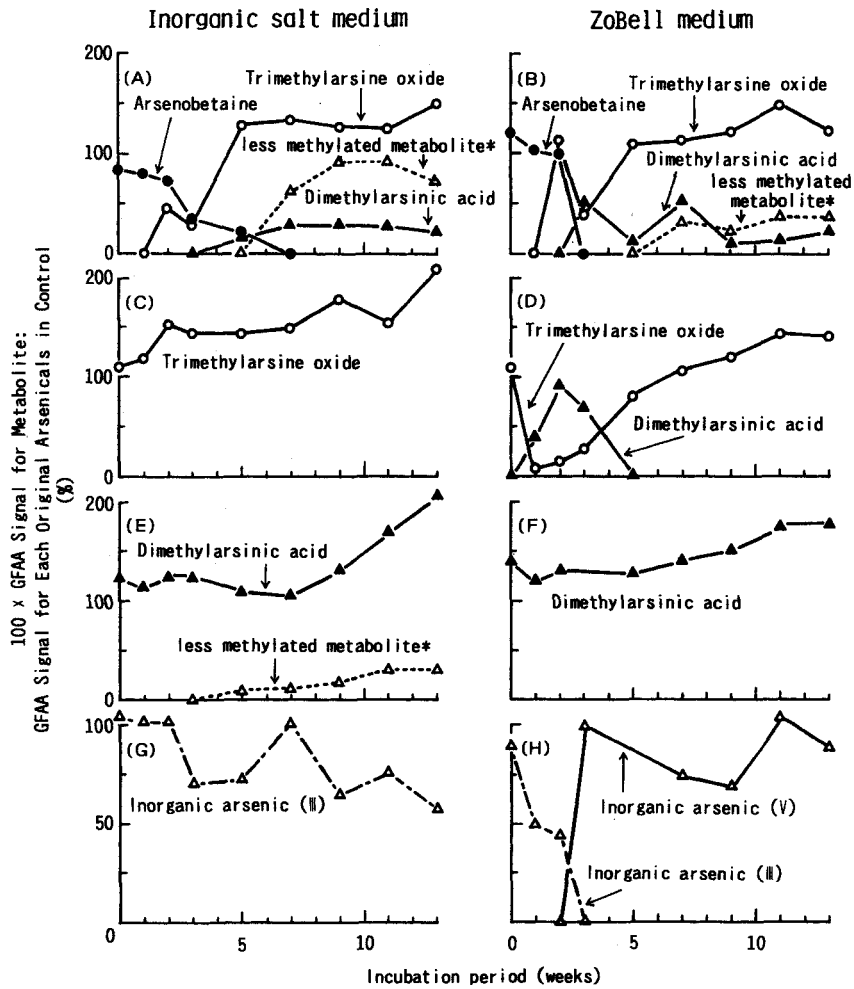

Figure 4 The conversion of arsenobetaine, trimethylarsine oxide, dimethylarsinic acid and arsenic(III) in an inorganic-salts medium and a ZoBell medium with sediments added during aerobic incubation at 25 °C. Methanearsonic acid and arsenic(V) were not converted in either medium. * Methanearsonic acid, arsenic(III) and/or arsenic(V).

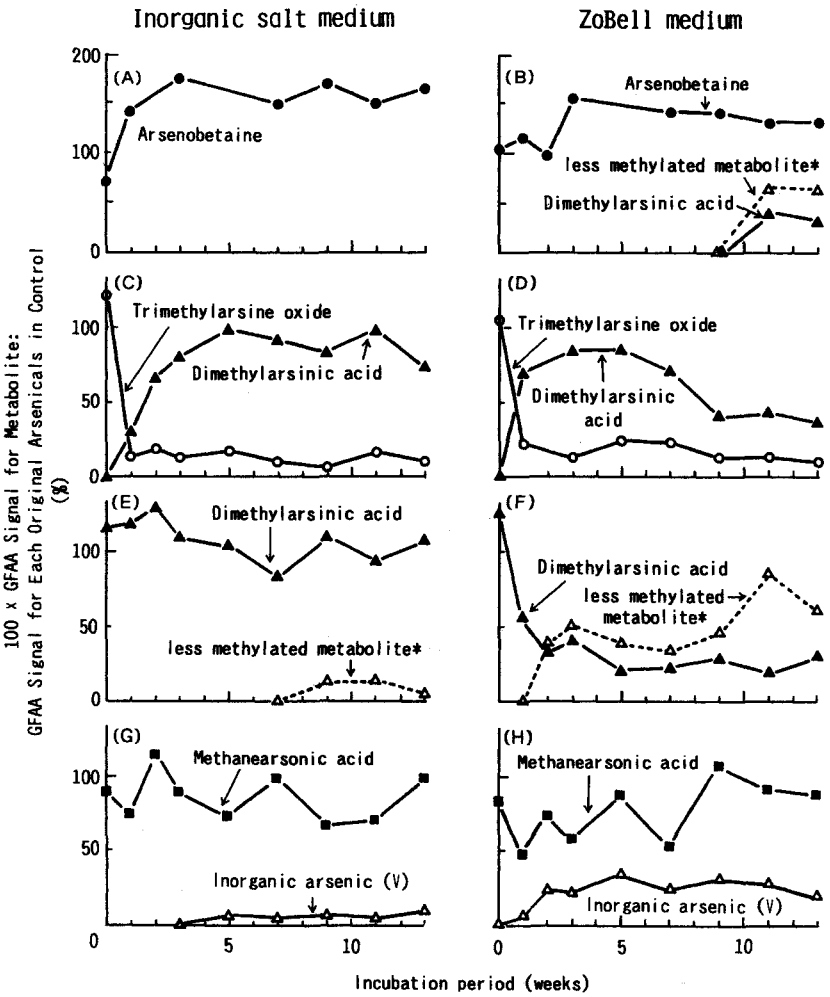

Figure 5 The conversion of arsenobetaine, trimethylarsine oxide, dimethylarsinic acid and methanearsonic acid in an inorganic salt medium and a ZoBell medium with sediments added during anaerobic incubation at 25 °C. * Methanearsonic acid, arsenic(III) and/or arsenic(V).

which medium was more suitable for the conversion of arsenicals. At the present stage, we can conclude only that the conversion rates of organoarsenicals depend on the flora or the number of micro-organisms introduced by addition of these materials, rather than the presence of abundant carbon sources.

Degradation of arsenobetaine by 'arsenobetaine-decomposing bacteria' isolated from coastal sediment

'Arsenobetaine-decomposing bacteria' were isolated from the coastal sediment. Two bacterial strains were isolated from the inorganic medium and several from the ZoBell medium added with the sediment by the culture enrichment method. The two strains from the inorganic medium which contained no organic carbon except arsenobetaine were identified as members of the *Vibrio–Aeromonas* group by means of biochemical reactions and morphological characteristics. The conversion of arsenobetaine by the two strains provisionally called 'arsenobetaine-decomposing bacteria' was investigated.[14] Arsenobetaine was degraded by them only to DMA under aerobic conditions (Fig. 6), little conversion being observed under anaerobic conditions. This result was apparently inconsistent with those from the experiments performed with the sediment itself, in which arsenobetaine was degraded to inorganic arsenic. This contradiction led us to a conclusion that a specific bacterium or

micro-organism alone may not degrade arsenobetaine to inorganic arsenic, but various micro-organisms, including the isolated bacteria, may participate in this degradation.

Arsenic circulation in marine ecosystems and the fate of organoarsenic compounds

It seems to be a ubiquitous phenomenon that arsenobetaine is degraded to TMAO or DMA, or even to inorganic arsenic, this being concluded from the following facts:

(1) Microorganisms occurring in every origin investigated degraded arsenobetaine to some degree; especially those occurring in the sediment and suspended substances completely degraded it to arsenic(V).

(2) The two strains of bacteria isolated from sediment having arsenobetaine-decomposing activity were of the *Vibrio–Aeromonas* group, being not unusual members but very common marine bacteria. If the proposed degradation pathway of arsenobetaine viz.

Arsenobetaine→TMAO→DMA→MMA→

inorganic arsenic

is assumed in marine ecosystems, and linked to the hypothesis that arsenobetaine is derived from seawater through food chains, the following hypothesis is necessarily called to mind. An arsenic cycle occurs in marine ecosystems, which begins with the methylation of inorganic arsenic on the route to arsenobetaine and terminates with complete degradation of arsenobetaine to inorganic arsenic, both in the sediment and the water column (Fig. 7).

The biosynthesis of arsenobetaine, the final metabolite of arsenic in this cycle, is not always necessary when the fate of organoarsenic compounds is considered. Although arsenosugar or arsenocholine, for example, may circulate along this cycle or others, they probably also suffer degradation to inorganic arsenic by the micro-organisms without the conversion to arsenobetaine. Thus, all the organoarsenic compounds

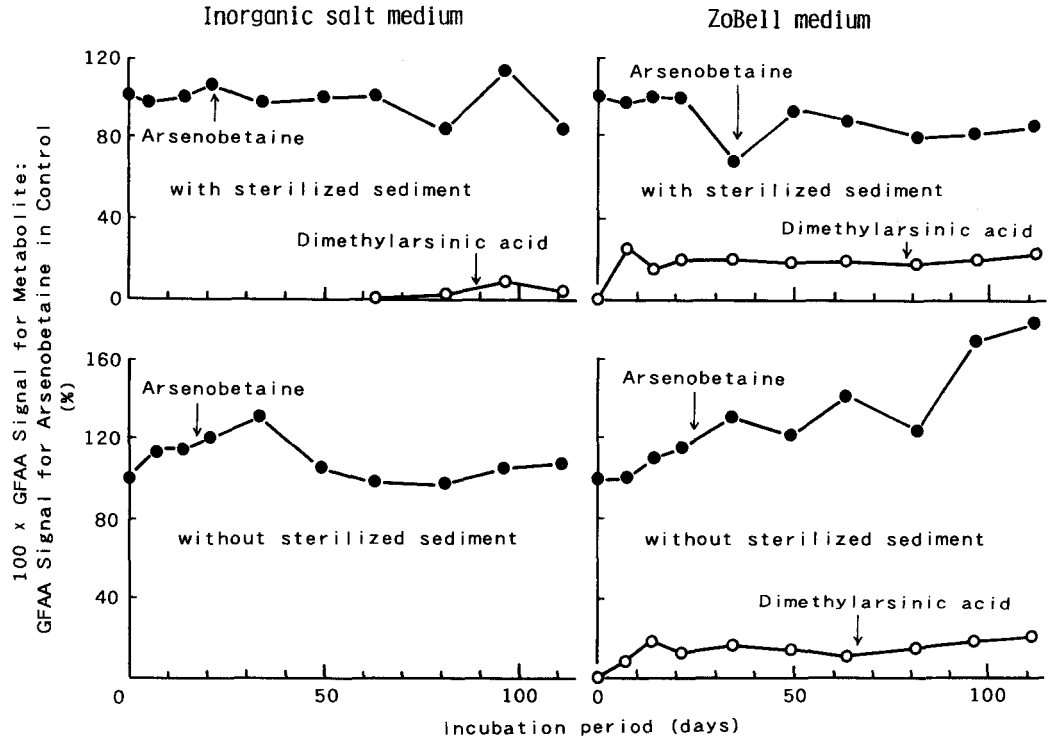

Figure 6 The conversion of arsenobetaine in an inorganic-salts medium and a ZoBell medium inoculated with 'arsenobetaine-decomposing bacteria' during aerobic incubation at 25 °C.

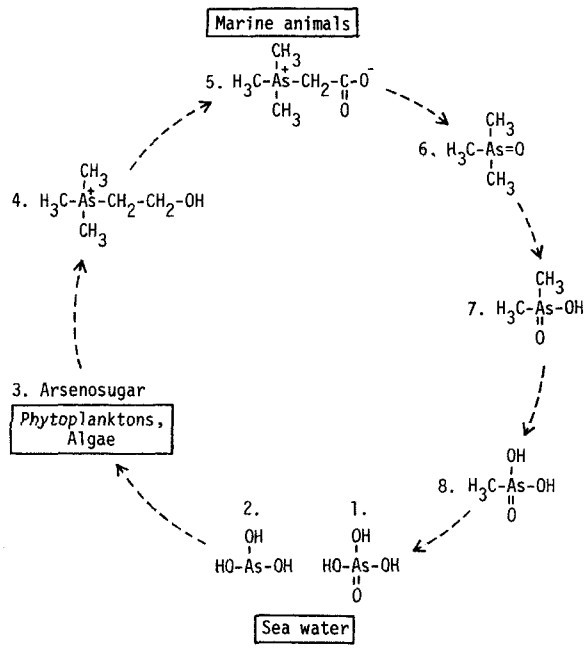

Figure 7 A tentative arsenic cycle in marine ecosystems, which begins with the methylation of inorganic arsenic in seawater on the route to arsenobetaine and terminates with the degradation of arsenobetaine to inorganic arsenic. 1, Inorganic arsenic(V); 2, inorganic arsenic(III); 4, arsenocholine; 5, arsenobetaine; 6, trimethylarsine oxide; 7, dimethylarsinic acid; 8, methanearsonic acid.

derived from inorganic arsenic in seawater may have the fate that they, at least in part, finally return to the original form, inorganic arsenic.

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