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Transformations of mercury species in the presence of Elbe river bacteria

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The influence of Elbe river bacteria isolated from suspended particulate matter (SPM) on dynamic species transformation of mercury was investigated. Experiments were carried out in the presence of bacteria (batch cultures) and in sterile tapwater as a control. For the methylation of inorganic mercury ions by bacteria several cofactors are under discussion. In this work, methylcobalamin, methyl iodide and *S*-adenosylmethionine were tested as biogenic methyl donors and trimethyl-lead chloride, trimethyltin chloride and dimethylarsenic acid as abiotic methyl donors. Transmethylation reactions as examples of abiotic methyl transfers have higher effectiveness in the formation of methylmercury (CH_3Hg^+) than methylation with biogenic compounds. This result was observed in batch cultures as well as in sterile water. SPM-bacteria inhibit methyl transfer to mercury(II) ions. This is not only due to passive adsorption processes of mercury(II) to bacterial cell walls; methylmercury is also decomposed very rapidly by SPM-bacteria and is immobilized as mercury(II) by the cells.

Keywords: Mercury, methylmercury, transmethylation, biomethylation, demethylation, species, bacteria

INTRODUCTION

The Elbe River in northern Germany has been one of the most contaminated rivers with regard to mercury for many years. Compared with the natural background level (0.4 mg Hg kg^{-1} dry weight)[1] in sediments and natural waters, average concentrations in the region of 30 mg Hg kg^{-1} (dw) and high concentrations of 157 mg Hg kg^{-1} (dw) have been found.[2] In natural waters and sediments mercury normally occurs as divalent inorganic mercury(II).[3] However, monomethylmercury, one of the most toxic mercury species, can also be determined in River Elbe sediments. Concentrations of 1–1.5 mg CH_3Hg^+ kg^{-1} (dw) are in absolute terms, and in relation to the total mercury content (8%), very high.[4] In the water column, mercury is principally bound to suspended particulate matter (SPM).[2] Therefore SPM seems essential for the transport of mercury both in the horizontal and in the vertical direction. In the horizontal direction the mercury freight in the river Elbe (in the non-tidal part) was calculated to be 25 t $year^{-1}$.[2,4] The importance of SPM for the vertical transport of mercury in the process of sedimentation is demonstrated by high concentrations of this element and its species in Elbe sediments.

SPM is densely populated with bacteria, which make up the most important part of the organic matrix of this material.[5] It is well known that bacteria are able to methylate inorganic mercury and to demethylate methylmercury.[6,7] However, knowledge of the role of SPM-bacteria on the actual mercury species during the process of sedimentation is very limited.

The aim of this work is to evaluate the methylation process of mercury by different methyl donors and to investigate the influence of SPM-bacteria on this species transformation. Several experiments were carried out in batch cultures with isolated SPM-bacteria and in sterile water as a control. For the methylation of heavy-metal cations, micro-organisms need methyl donors as cofactors. As models for biogenic methyldonors *S*-adenosylmethionine, methyl iodide and methylcobalamin were examined. For abiotic transmethylation reactions trimethyl-lead chloride, trimethyltin chloride and dimethylarsenic acid were used.

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MATERIALS AND METHODS

Bacterial cultures

Suspended particulate matter (SPM) was collected from a groyne field in the limnic part of the river Elbe, 50 km upstream from Hamburg, north Germany. In these groyne fields (with low flow rates) high concentrations of total mercury as well as methylmercury can be detected in sediment samples. SPM-bacteria were isolated according to a method described by Greiser.[5] Fractionated centrifugation was carried out to separate SPM from minerals and free bacterial cells. At 300–400 *g* SPM-flocules could be harvested selectively, whereas microfloccule up to 5 μm in diameter, small algae and free bacterial cells occur at 1000–4000 *g* in the sediment of the centrifuge tubes. The mixed bacterial cultures were obtained according to a slightly modified method described by Liu and Thomson.[8] Approximately 100 mg of the centrifuged SPM was inoculated into 150 cm^3 of growth medium in 250-cm^3 culture flasks with Kapsenberg caps on a rotary shaker at room temperature. When the stationary phase was reached, an inoculum (1 cm^3) was transferred twice into fresh growth medium to ensure young cultures with comparable metabolic activities. For all experiments an aqueous solution of standard nutrient broth (2.5 g dm^{-3}) was used as growth medium.

Bacterial growth was followed by optical density at 650 nm ($OD_{650\,nm}$) and simultaneously by determination of the protein content. According to Liu an approximation exists between bacterial numbers and turbidity at 650 nm. An optical density of 1.0 corresponds with approximately 10^9 cells cm^{-3}.[9] The protein content was measured according to Bradford. This method allows the determination of live and dead biomass as well. Therefore, it can be used to quantify microbial population in several matrices.[10]

Addition of mercury species and methyl donors

For the methylation experiments 1.75 μmol dm^{-3} Hg(II) as mercury(II) chloride and 17.5 μmol dm^{-3} methyl donor were added to 150 cm^3 of the enrichment cultures after bacterial growth had started. All experiments were carried out in triplicates.

Sterile tap water was treated in the same manner and was used as a control.

For the demethylation experiments 1.75 μmol dm^{-3} CH_3Hg^+ as methylmercury(II) chloride (CH_3HgCl) was added to 150 cm^3 of the bacterial cultures and to sterile tap-water (in triplicates).

Determination of total mercury

Determination of total mercury was carried out with cold-vapour atomic absorption spectrometry (CV AA) using amalgamation on gold. Bacterial cells isolated from 5 cm^3 of culture by centrifugation were digested with 1 cm^3 of a 20% w/v tetramethylammonium hydroxide solution (TMAH). This very effective tissue solubilizer is a suitable reagent for the rapid and complete digestion of bacterial cells.[11] Subsamples of the enrichment cultures were digested with TMAH solution in a 1:1 ratio. By addition of 200 μl bromine chloride solution (0.02 mol dm^{-3}) all available mercury species were transformed into inorganic mercury(II).[12] Excessive bromine chloride was destroyed by pre-reduction with 200 μl hydroxylamine hydrochloride solution (20% w/v) prior to CV AA analysis.

Determination of methylmercury

Determination of methylmercury was carried out using high-performance liquid chromatography (HPLC) with UV detection. The CH_3Hg^+ determination is based on charge neutralization chromatography, which has been developed for organic mercury compounds.[13–15] In this method 2-mercaptoethanol (5×10^{-4} mol dm^{-3}) is added to the eluent (H_2O/methanol, 80:20) in order to achieve an *in situ* complexation of organomercurials on the column during elution. The reaction can be described Eqn [1]:

$$RHgX + HS(CH_2)_2OH \rightarrow RHgS(CH_2)_2OH + HX \quad [1]$$

The resulting mercaptoethanol complex is retained on the reverse-phase column (LiChrospher RP-18, 150-3 (5 μm) glass cartridge) and separation and detection are possible. Although the UV-extinction maximum of organomercurials is below 210 nm,[16] detection at 230 nm is sufficient.[17] The measurements were performed with a Millipore–Waters system equipped with a Model 490 pump, a U6K injector and a 481 UV/VIS detector. Injected volumes were 20 μl.

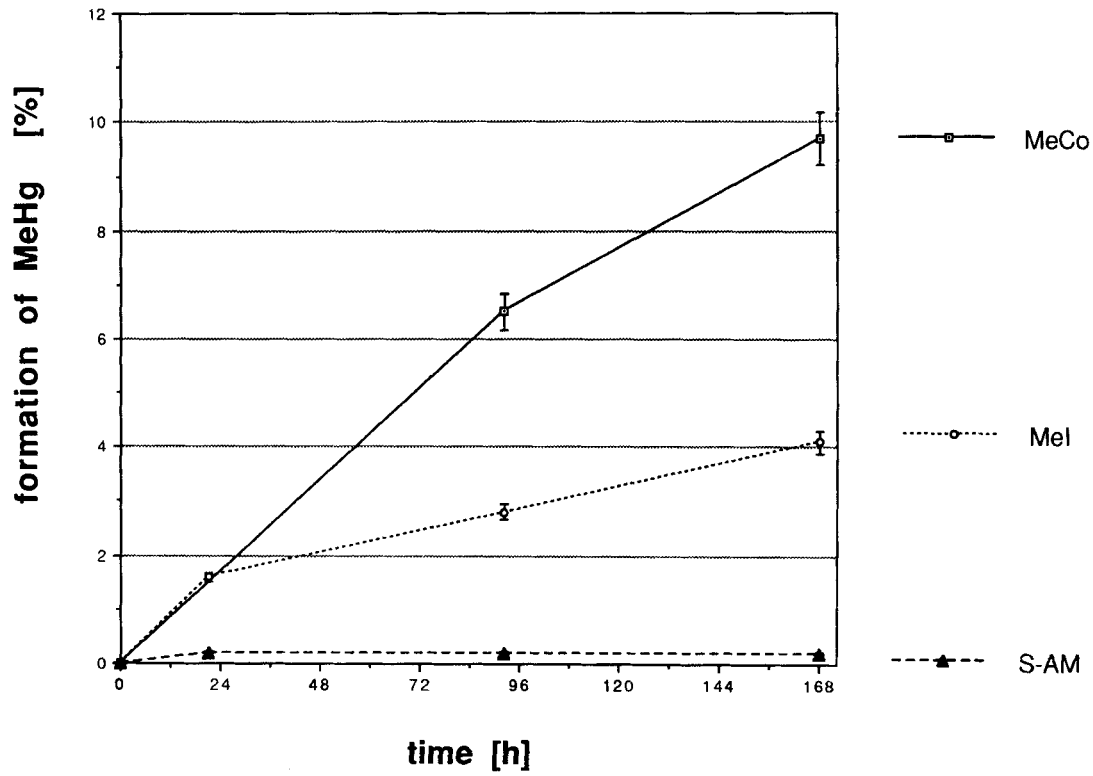

Figure 1 Methylation of mercury by biogenic methyldonors; initial concentration of Hg(II), 1.75 μmol dm^{-3}.

Bacterial cells isolated from 5 cm^3 of enrichment cultures were digested with TMAH, as described for the total mercury determinations. TMAH is non-oxidizing and therefore a suitable tissue solubilizer for species analysis.[18] Subsamples of the enrichment cultures were digested with TMAH solution in a 1:1 ratio. After the digestion was completed the extracts were acidified with 2 cm^3 of 6 mol dm^{-3} hydrochloric acid to obtain CH_3HgCl, which is relatively non-polar and has a high solubility in organic solvents. Methylmercury chloride was extracted from the aqueous phase three times with excess toluene (3×30 cm^3). Finally a back-extraction into an aqueous solution was necessary in order to obtain a sample in a solution which was miscible with the eluent of the HPLC. For that purpose the combined toluene extracts were carefully reduced to about 5 cm^3 at 50 °C and 300–400 Pa using a rotary evaporator. The organic phase was then back-extracted with 1 cm^3 of 10^{-4} mol dm^{-3} sodium thiosulphate solution. This final solution was miscible with the eluent of the HPLC and could be injected directly. The extraction procedure and the chromatographic conditions are described in detail by Wilken and Hintelmann.[17,19]

RESULTS

Mercury methylation with biogenic methyl donors

For the methylation of heavy-metal cations, micro-organisms need methyl donors. Methylcobalamin (MeCo), methyl iodide (MeI) and *S*-adenosylmethionine (S-AM) are the most important methylating coenzymes[20] and were used as biogenic methyl donors. MeCo was chosen as an example for a CH_3^- donor.[21] S-AM is a CH_3^+ donor and methylates exclusively in the presence of enzymes.[21] MeI is able to transfer a methyl group to a metal cation non-enzymatically in the course of a redox reaction.[22]

The addition of mercury(II) and the methyl donors mentioned has been described above. The results of the methylation experiments in sterile tap-water are shown in Fig. 1.

In sterile tap-water both MeCO and MeI are

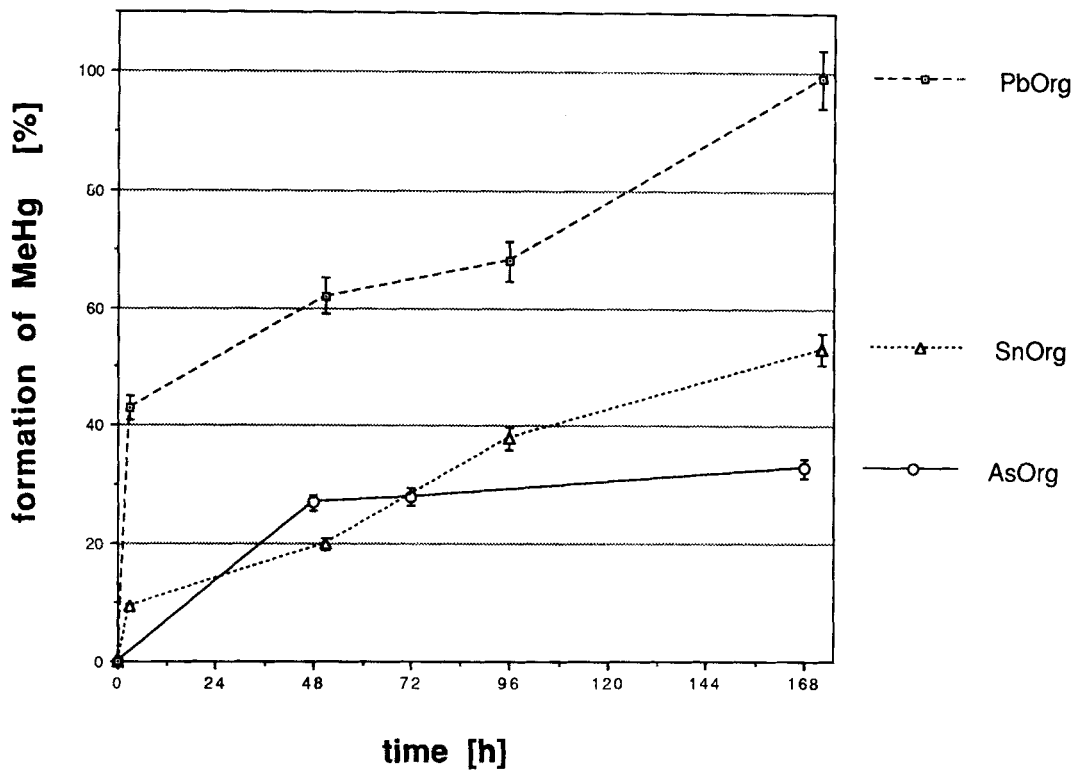

Figure 2 Transmethylation of mercury in sterile water; initial concentration of Hg(II), 1.75 μmol dm^{-3}.

able to methylate mercury(II) ions. Within one week the CH_3^- donor MeCo methylates 10% of the mercury ions present (i.e. 0.18 μmol dm^{-3}). In the presence of methyl iodide 4% of the mercury(II) ions were transferred into CH_3Hg^+. No methylation was observed when S-AM was used as the methyl donor. A turnover of 0.5% mercury(II) ions into CH_3Hg^+ would have been detectable in these experiments.

In the presence of bacteria (enrichment cultures), no methylmercury could be detected in any case. Therefore less than 0.5% of the present mercury(II) ions (i.e. 8.7 nmol dm^{-3}) were methylated by the biogenic methyl donors examined.

Mercury methylation by organometals: transmethylation

Transmethylation reactions can play an important role as abiotic methylators for mercury(II) ions in aqueous environments.[18] Jewett and Brinkman showed that both trimethyl-lead chloride and trimethyltin chloride are able to transfer methyl groups to mercury(II).[23,24] Chau found that methyl derivatives of lead(IV) compounds are able to transfer their methylgroups to tin(II) and tin(IV) salts. However, methylmercury does not donate its methyl group to either tin(II) or lead(II) salts.[25] Methylarsenic acids are also able to methylate tin(II) and tin(IV) salts in aqueous solutions but a transfer of their methyl groups to lead(II) was not detected.[25]

In addition to the high mercury concentrations (inorganic and organic mercury), the river Elbe is highly contaminated with organotin compounds and probably other organometals.[4,26,27] Therefore transmethylation reactions in sediments and in the water body may be responsible for the high methylmercury concentrations in Elbe sediments.

In the following experiments trimethyl-lead chloride, trimethyltin chloride and dimethylarsenic acid were examined for their methylation potential. Inorganic mercury (Hg(II): 1.75 μmol dm^{-3}) and the methyl donor (17.5 μmol dm^{-3}) were added to 150 cm^{-3} bacterial cultures and to sterile tap-water. The experiments were carried out in triplicates. The results for the formation of methylmercury in sterile water are shown in Fig. 2.

In sterile tap-water trimethyl-lead chloride is the most effective methyl donor for mercury(II) ions. The formation of CH_3Hg^+ is very fast (40%

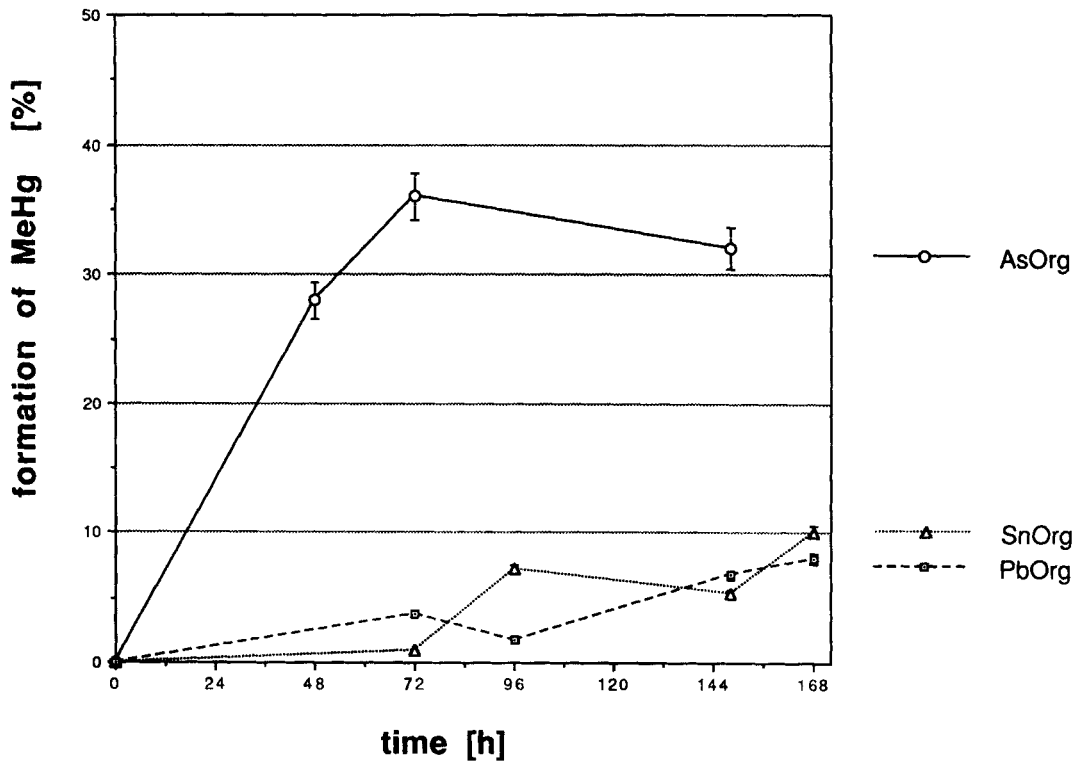

Figure 3 Transmethylation of mercury in the presence of bacteria; initial concentration of Hg(II), 1.75 μmol dm^{-3}.

turnover after three hours). After one week, 100% of the mercury(II) ions present were methylated. In the presence of trimethyltin chloride and dimethylarsenic acid, 45% (approx. 0.8 μmol dm^{-3}) and 37% (approx. 0.6 μmol dm^{-3}) methylmercury, respectively, could be detected in sterile water.

The results for the methylation experiments in the presence of SPM-bacteria are shown in Fig. 3.

In the presence of bacteria, the turnover mercury(II)→methylmercury was significantly lower than in sterile water, when trimethyllead chloride and trimethyltin chloride were used as methyl donors. After seven days' incubation, less than 10% of the mercury(II) ions present were methylated. The methylation activity of dimethylarsenic acid is much higher and is not affected by the presence of SPM-bacteria. The turnover mercury(II)→methylmercury in sterile water and in enrichment cultures was comparable. After six days, approximately 33% of the mercury(II) ions were methylated by the organoarsenic compound.

With the exception of dimethylarsenic acid, methylation activity of methyl donors (biogenic as well as abiotic) was degraded in the presence of SPM-bacteria. This can be explained by adsorption of mercury(II) ions and/or the applied methyl donors to bacterial cell walls, or by the active demethylation of methylmercury by microbial activity.

To survey the demethylation activity of Elbe river SPM-bacteria CH_3HgCl (1.75 μmol dm^{-3}) was added to 150 cm^3 of the bacterial cultures as described above. Sterile tap-water was used as a control to identify external influences (UV light, for example) on the methylmercury decomposition. The results of the demethylation experiments are shown in Fig. 4.

In the presence of SPM-bacteria the methylmercury concentration decreased rapidly. After 24 hours of incubation, 20% of the methylmercury disappeared. After one week, no methylmercury was detectable in the bacterial cultures. The total mercury content was constant over the same period. Therefore it can be excluded that the decrease of CH_3Hg^+ concentration is only due to sedimentation processes in which methylmercury is attached to sinking bacterial cells.

In the control samples (sterile water) the methylmercury concentration was constant over the same 12-day period. No external factors such as

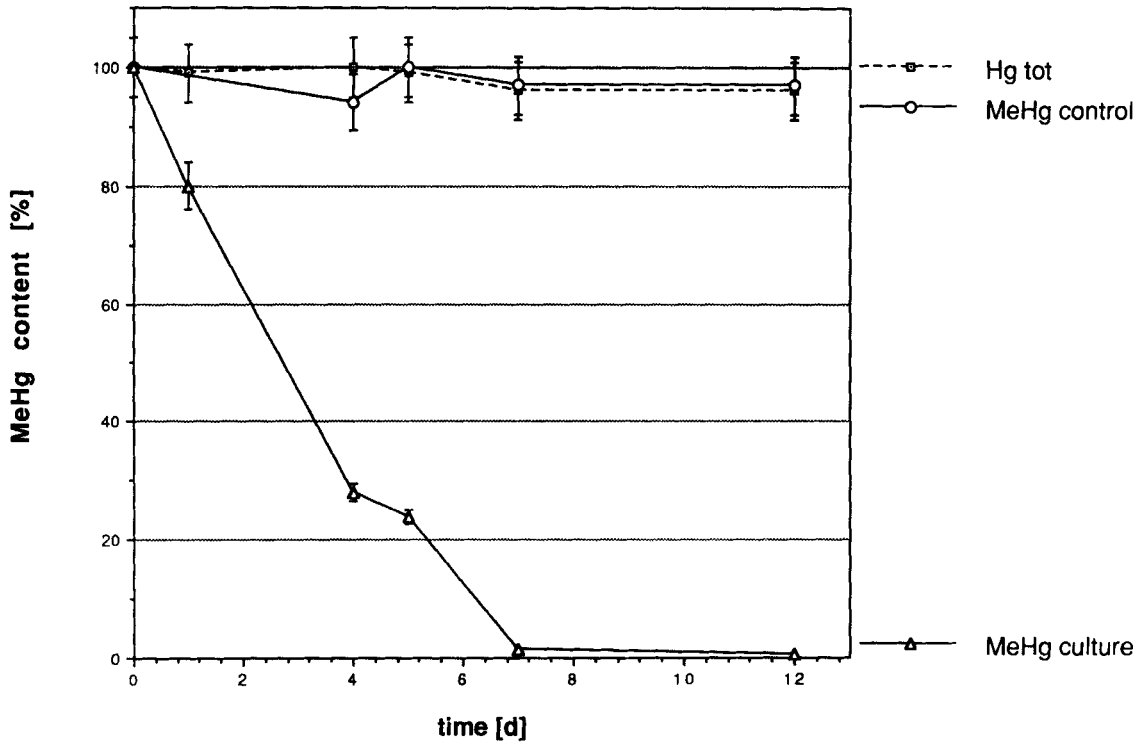

Figure 4 Methylmercury decomposition by SPM-bacteria; initial concentration of CH_3Hg^+, 1.75 μmol dm^{-3}.

UV light are responsible for the methylmercury decomposition in the bacterial cultures.

To determine the percentage of distribution and decomposition of methylmercury between the aqueous phase and bacterial cells, the following experiment was carried out. Methylmercury was added to enrichment cultures as described above. Over a period of 12 days, samples (5 cm^3 each) were taken and centrifuged at 1500 *g* for 20 min at 4 °C.

Methylmercury and total mercury were determined both in the aqueous phase and in the centrifuged bacterial cells. The results are shown in Fig. 5.

After 24 hours of incubation, approximately 53% of the added methylmercury ions were attached to the cells. Figure 5 shows that the methylmercury concentration decreased both in the aqueous phase and in the biomass. The total mercury concentration in the cells was constant whereas a slight decrease was detectable in the aqueous phase.

DISCUSSION

In the present work the formation of highly toxic methylmercury from inorganic mercury and several methyl donors was examined. In the presence of biogenic methyl donors, only methylcobalamin as a carbanion donor and methyl iodide transfer methyl-groups to mercury(II) ions. However, the formation of monomethylmercury can only be observed in the absence of bacteria isolated from SPM. *S*-Adenosylmethionine does not methylate inorganic mercury in any case.

Besides biogenic methyl donors, organometalic compounds can transfer methyl groups to mercury in the course of a transmethylation. Significant turnovers of mercury(II) to methylmercury could be observed in the presence of trimethyl-lead chloride, trimethyltin chloride and dimethylarsenic acid. The most effective methyl donor in sterile and cell-free water is trimethyllead.

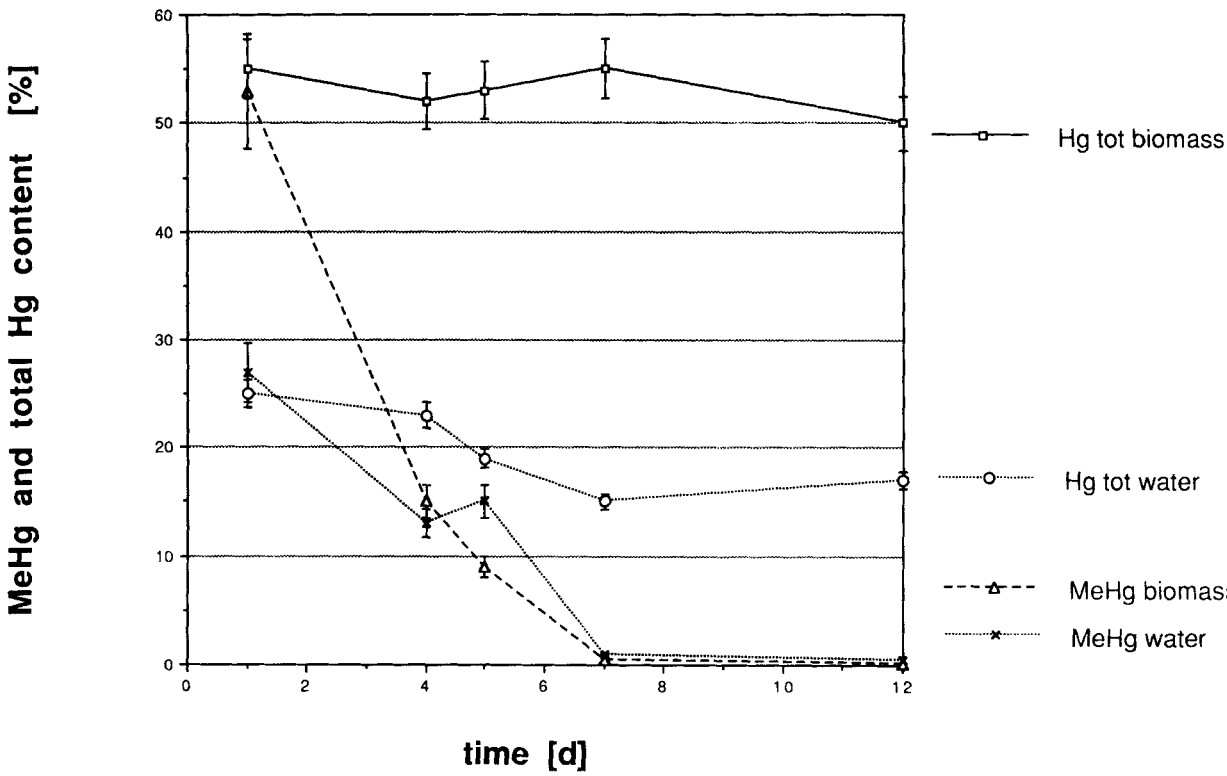

Figure 5 Methylmercury decomposition in the bacterial and aqueous-phase compartment; initial concentration of CH_3Hg^+, 1.75 μmol dm^{-3}.

With the exception of dimethylarsenic acid, the methylation activity of the methyl donors examined, biogenic as well as abiotic, is degraded in the presence of SPM-bacteria. At least three explanations for this fact are possible:

(1) Mercury(II) ions are attached to bacterial cell walls and are not available for methylation. Beveridge and Fyfe identified carboxyl groups as very effective potential binding sites for metal cations using *B. subtilis* as test organisms.[28]
(2) The methylating agents themselves are bound to cell walls and are not available for methylation.
(3) SPM-bacteria are not only passive inhibitors for the mercury methylation; it is also possible that the methylmercury produced is demethylated by microbial activity.

The results from the demethylation experiments show that SPM-bacteria are able to decompose methylmercury under aerobic conditions in batch cultures. The cleavage of the mercury–carbon bond can be detected in the biomass and in the water phase. However, the total mercury in the bacteria cells remains constant. Therefore the combination of demethylation of organometallic mercury species followed by immobilization of inorganic mercury can be regarded as an example of microbial detoxification. The loss of total mercury detected in the water phase may be due to the formation of volatile mercury species [mercury (0) and dimethylmercury]. Under the conditions examined, elemental mercury seems to be the more probable transformation product.[4]

The transmethylation of mercury(II) to methylmercury in the presence of dimethylarsenic acid seems to have a maximum after 72 h. This can be explained by an inhibition of the microbial demethylation activity. Probably because of the toxicity of dimethylarsenic acid, SPM-bacteria need an adaption period of 72 h. After this period the microbial decomposition of methylmercury begins. However, in the presence of the presumably more toxic trimethyl-lead chloride and trimethyltin chloride this phenomenon was not observed.

A fluvial system, especially a tidal one, is highly

complex and cannot be treated experimentally as a whole. Therefore simplifying laboratory experiments can be necessary and helpful in investigating single processes in selected areas or compartments of the ecosystem. The conception of the experiment should include environmental conditions as far as possible and should exclude disturbances which always occur in field experiments.

Bacteria isolated from the Elbe ecosystem have been selected as the compartment of interest because of their importance for the transport and transformation of mercury and its species in fluvial systems. The population resulting from cultivation of isolated bacteria in nutrient media is certainly not identical with the original one. High concentrations of both nutrients and pollutants do not usually represent natural conditions. Although the applied mercury concentrations were significantly lower than necessary to inhibit bacterial growth in the enrichment cultures, it should be taken into consideration that mercury-resistant strains are probably predominant in the laboratory experiments. Therefore it is certainly not permissible to make quantitative assessments of mercury transformations in the Elbe ecosystem on the basis of batch cultures. Nevertheless it seems clear that an active microbial methylation of inorganic mercury under aerobic conditions in the water phase can be excluded. In all experiments where the formation of methylmercury could be observed, the turnover decreased drastically in the presence of SPM-bacteria. The exceptional reaction with dimethylarsenic acid as methyl donor has been discussed previously. On the other hand, none of the experiments shows the microbial formation of methylmercury, whereas the decomposition of that species is carried out rapidly in batch cultures. Therefore it seems clear that bacteria attached to suspended material of the Elbe river are not responsible for elevated methylmercury concentrations in Elbe sediments. The demethylation is the preferred transformation reaction mediated by bacteria in the water phase. Consequently, microbial formation of methylmercury during the process of sedimentation of SPM flocules is unlikely.

Besides methylation of mercury by sulphate-reducing bacteria (SRB) or methanogenic bacteria, the transmethylation may play an important role for the genesis of monomethylmercury in sediments. The reaction of ionic mercury with organometals like trimethyltin- or trimethyl-lead compounds is very effective with respect to the formation of methylmercury. Especially for areas where high concentrations of organotin and organolead species can be detected, these completely abiotic reactions can be the decisive factor for the genesis of methylmercury. Knowledge about compounds of organotin and especially of organolead in Elbe sediments is still very limited but there are distinct indications for 'hot spots' of organotin pollution in the Mulde river-mouth and elsewhere.[26,27]

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