

DETERMINATION OF METHYL MERCURY IN A PILOT-SCALE ACTIVATED SLUDGE WASTEWATER TREATMENT PLANT

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ABSTRACT

The methylation of mercury has been investigated and documented mainly in sediments, fish and microorganisms, while limited number of relevant studies is available for wastewater. The procedure of mercury methylation can occur via biological pathway (by microorganisms) and via chemical or photochemical reactions.

Methylation of mercury occurs mainly under anaerobic conditions, but some studies have shown its existence also under aerobic conditions. The resulting concentration of methyl mercury, which is a highly toxic compound, depends on the specific rates of methylation/demethylation of mercury. The factors affecting these procedures are the availability of inorganic mercury, pH, organic matter concentration, microbial activity, redox potential and temperature. Bacteria which can methylate mercury are often present in wastewater, and, therefore, the formation of methyl mercury during wastewater treatment is possible.

The objective of the present investigation was the determination of methyl mercury in a pilot-scale activated sludge wastewater treatment plant supplied with synthetic wastewater enriched with mercury. For this purpose, a Liquid-Liquid Extraction / Simultaneous Derivatization - GC/MS method was developed and applied for the analysis of samples from the aeration tank, from the treatment plant effluent and from the sludge.

Methyl mercury was not detected in the samples (detection limit $0.07 \mu\text{g l}^{-1}$), leading to the conclusion that mercury is not methylated under the particular experimental conditions of the pilot-scale water treatment plant.

KEYWORDS: methyl mercury, wastewater, activated sludge, Gas chromatography – Mass spectrometry (GC/MS), Liquid-Liquid Extraction, derivatization

1. INTRODUCTION

The process of methylation of mercury has been investigated mainly in sediment, in fish and in microorganisms, and occurs at a large extent in sediments of lakes, rivers and oceans [1]. The process of methylation of mercury in natural ecosystems may follow the following pathways [2-3]:

- Biological activity by various species of microorganisms, mainly bacteria
- Chemical reactions through humic substances
- Photochemical processes.

The concentration of methyl mercury in a system depends on the relative rates of methylation and de-methylation of Hg. The particular processes are affected by the availability of inorganic mercury, pH, the amount of organic matter, the microbial activity [4], the redox potential and the temperature [4-5]. The methylation is enhanced by temperature increase. At neutral pH, the monomethylmercury is the dominant species [6]. According to Bisogni [4], the rate of methylation of Hg is described by the equation:

$$NSMR = \gamma * (Hg^{2+})n$$

where:

NSMR (Net Specific Methylation Rate): the actual methylation rate ($\mu\text{g (CH}_3)_2\text{Hg}$ or CH_3Hg^+ per gr of volatile suspended solids)

γ : coefficient determined from the rate of growth of microorganisms

(Hg^{2+}) : concentration of free Hg ions

n: pseudo-rate of the reaction

Methylation of mercury usually occurs in anaerobic conditions, but it has also been reported to occur also in aerobic conditions, with lower yields of methyl mercury [4, 7]. Microorganisms play an important role during the mercury methylation procedure. Increased growth and metabolism of microorganisms enhances methylation. Several species of bacteria that are capable of methylating mercury in aquatic and in terrestrial ecosystems are frequently detected also in wastewater, in considerable concentrations, e.g. *pseudomonas* spp. [4].

An activated sludge wastewater treatment plant could be considered as a simplified natural system where pH and redox potential remain unchanged. Therefore, while the temperature increase and the high concentration of suspended solids accelerate the methylation of mercury, the aerobic conditions support the growth of bacteria which demethylate the methyl mercury to inorganic mercury.

Research regarding the possible methylation of mercury during activated sludge wastewater treatment, especially for full-scale plants, is limited [7-9]. Goldstone [7] investigated methyl mercury in a full scale wastewater treatment plant in Norwich, and found that a percentage <0,5% of the input Hg occurred as methyl mercury. In particular, in the mixed liquor (in 1986) he determined concentrations of total Hg 33-48,3 $\mu\text{g Hg l}^{-1}$ and concentrations of methyl mercury 0,07-0,24 $\mu\text{g MeHg l}^{-1}$ respectively. Research continued in the same treatment plant the next two years showed not detectable concentrations of methyl mercury (detection limit 10 ng l^{-1}). Bisogni *et al* [4] found that the percentage of input mercury chloride that was converted to methyl-mercury ranged from 0,1 to 15 %. In contrast, Wu *et al* [10] reported only traces of methyl mercury in the treatment plant effluent, and correlated the existence of methyl mercury to the existence of solids. The average concentration of methyl mercury determined by Gilmour [11], in a full- scale wastewater treatment plant was 0.104 g d^{-1} in the influent (for 19.3 g d^{-1} inorganic mercury), 0.269 g d^{-1} in the effluent (for 12.8 g d^{-1} inorganic mercury) and 0.125 g d^{-1} in sludge (for 144 g/d inorganic mercury). According to Gilmour, although the influent contained methyl mercury, Hg was methylated during the wastewater treatment at percentage <5% of the total incoming Hg. Also, demethylation of methyl mercury was observed during aeration of wastewater.

During collection and transport of wastewater in a treatment plant, bivalent Hg(II) is exposed to reductive conditions (due to poor oxygenation and existence of some species of bacteria), and therefore converted to elementary Hg⁽⁰⁾. Elementary mercury may be released into the atmosphere especially during aeration [6]. In the primary sedimentation tank, the mercury is highly associated to the suspended solids and removed along with the sludge. Goldstone [7]

determined that via this pathway, 30-60% of the incoming Hg in the treatment plant may be removed. In the aeration tank, a large number of microorganisms (bacteria, protozoa etc) transform Hg to organic forms, with dominant methyl mercury, and vice versa. The concentrations of the chemical species of mercury depends on the rates of methylation-demethylation, which in turn depend on microorganisms, temperature, redox potential, pH and suspended solids, as mentioned above. Therefore, although the effectiveness of mercury removal in the wastewater treatment plants is high, methyl mercury could be formed, which consists an environmental risk.

The objective of the present investigation was the determination of methyl mercury in a pilot-scale activated sludge wastewater treatment plant, which was supplied with synthetic wastewater enriched with mercury. The determination of organic mercury species in environmental samples is generally performed by gas chromatography (GC) techniques, which include extraction, derivatization, concentration, separation and detection. The most reliable detection technique is Mass Spectrometry (MS), which provides detailed data regarding compound identification. During this work, a Liquid-Liquid Extraction / Simultaneous Derivatization - GC/MS method was developed and applied for the analysis of wastewater samples from the aeration tank, from the treatment plant effluent and from the sludge.

2. MATERIALS AND METHODS

2.1. Experimental setup and sampling

A pilot scale activated sludge wastewater treatment plant was constructed as described in detail by Pavlogeorgatos [12-16] (*Figure 1*). The operational parameters of the pilot plant are presented in Table 1. The plant was supplied with synthetic wastewater, which contained 0.325 g glucose per liter of tap water. The nutrients were added with the introduction of 0.2 g thiophosphoric ammonia per liter of water. The synthetic wastewater that supplied the treatment plant was enriched with mercury at concentration levels 10, 100 and 500 $\mu\text{g l}^{-1}$. The increase of mercury concentration was performed at 3-day intervals. Sampling (in duplicate) was performed for each Hg concentration level, from the aeration tank, the treatment plant effluent and the sludge. Measurements of temperature, pH and dissolved oxygen were performed daily. Samples were also analyzed for COD for each investigated input concentration of Hg, as well as twice before the enrichment with mercury. Suspended solids were also determined twice before the enrichment with Hg and once for input concentration of Hg 500 $\mu\text{g l}^{-1}$ [13].

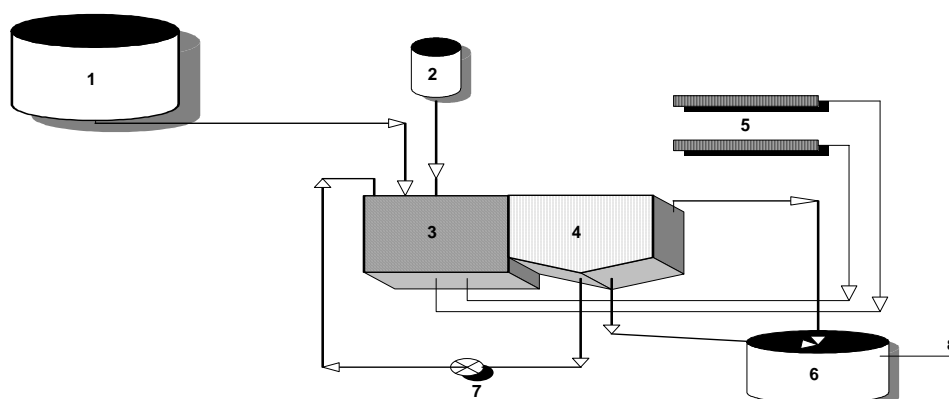


Figure 1. Scheme of the activated sludge pilot plant [12]

1. Mixture tank of glucose and macro-nutrients
2. Mercury- containing feed tank
3. Aeration tank
4. Precipitation tank
5. Air compressors
6. Treated wastes tank
7. Return sludge pump
8. Outlet of the plant

Table 1. Operational parameters of the activated sludge pilot plant [12-13]

Parameter	Value	Parameter	Value
V_{AT^*}	80 l	S	20 mg l ⁻¹
$V_{PT^{**}}$	40 t	Eo	93%
Θ	5 h	Concentration of fertilizer	0,2 g l ⁻¹
Θ_c	7 d	Q	340 l d ⁻¹
X	3,000 mg l ⁻¹	Q/Qr	1
So	300 mg l ⁻¹	A_{AT^*}	257 cm ²

AT*: Aeration Tank

PT**: Precipitation Tank

2.2. Reagents-Standard solutions

The ultrapure water used was type 1, from Milli RO5/MilliQ 185 columns (Millipore). The following high-purity reagents were also used: NaBe₄ (Merck) solution 1%, NaCl (Merck), sodium acetate (Merck), acetic acid (Merck) and hexane (BDH). A stock solution 10 mg MeHg l⁻¹ was prepared in hexane from a certified MeHg standard (solid, CH₃HgCl), Aldrich Chem Co, by dilution of the appropriate amount. This stock solution was used for the preparation of standard solutions of known concentrations for the GC-MS system calibration. A stock solution 1000 mg l⁻¹ Hg (HgNO₃) (Merck) was also used for the preparation of standard calibration solutions for the determination of Hg.

2.3. Sample preparation

Following shaking of the plastic bottle containing the standard or sample, to 200 ml of sample 30 gr NaCl plus 2.72 gr CH₃COONa were added. Some drops of CH₃COOH or NaOH were also added for pH adjustment to 4.5. The solution was placed in a magnetic stirrer for 5 min to obtain proper dilution and then it was transferred into a separatory funnel, where 20 ml hexane and 2 ml NaBe₄ 1% were added. Liquid-liquid extraction with simultaneous derivatization (ethylation) was performed for 20 min and then the solution was left undisturbed for approximately 40 min, for phase separation. Finally, the solution was centrifuged for 10 min at 2,000 rpm and the supernatant was concentrated (x10) under a gentle stream of nitrogen (purity 99.999%). 1 µl of the final extract was injected into the GC-MS.

2.4. Equipment-Analytical conditions

The determination of MeEthHg and DiEthHg was performed by use of a HP 5890 Series II gas chromatograph (GC) with a Hewlett Packard 5971 Mass Selective Detector (MSD). The column used was fused silica capillary DB-624 30 m x 0.32 mm i.d. x 1.8 µm, the injection technique was split/splitless and the carrier gas was helium (purity 99.999%). The analytical conditions are presented in Table 2 and a GC-MS chromatogram in Fig 2.

2.5. Recovery tests and detection limit of the method

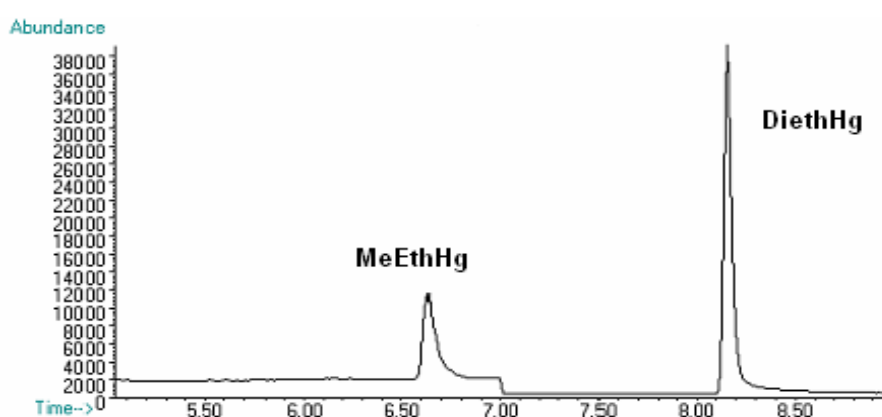
Recovery tests for MeHg were conducted in biomass substrate from the activated sludge pilot plant described above in samples from the aeration tank of the plant and from the sludge, after spiking with 2 mg MeHg l⁻¹ (six replicates). The recovery of MeHg was 98% (RSD 2,5%) for the samples from the aeration tank and 90% (RSD 3,8%) for the sludge samples. The recovery of DiEthHg was 85% (RSD 6%). The detection limit of the method described, estimated based on signal-to-noise ratio 2/1 (S/N =2/1), was 0.07 µg MeHg l⁻¹. For mercury, the detection limit was 2 µg Hg l⁻¹.

3. RESULTS AND DISCUSSION

The analytical technique utilized during the present work (GC-MS) is one of the most reliable techniques for the determination of methyl mercury, and the recovery tests have shown very satisfactory results. Moreover, the high temperature during the pilot-plant experiment as well as the high concentration of suspended solids are factors which should enhance the methylation of mercury [5, 7, 9]. Taking into account these facts, the formation of methyl mercury would be expected.

Table 2. Analytical conditions of GC-MS for the determination of MeEthHg and DiEthHg

GC analytical conditions	
Carrier gas flow:	1.25 ml min ⁻¹
Split ratio:	1:25
Oven temperature program:	Initial temperature 60 °C (2 min) Rate of increase 20 °C min ⁻¹ Final temperature 200 °C
Injector temperature:	200 °C
MS analytical conditions	
Solvent delay:	5 min
MS transfer line temperature:	280 °C
EMV:	2200
SIM Mode	Ions 217, 202, 244, 252, 231

Figure 2. GC-MS Chromatogram of a standard solution Me-EthHg and DiethHg 2.5 mg l⁻¹

However, in all samples analyzed, the concentration of methyl mercury was not detectable (detection limit 0.07 µg MeHg l⁻¹, S/N = 2/1). The sample where the highest concentration of total mercury was determined was the sludge sample after input of 500 µg Hg l⁻¹ in the treatment plant. In the particular sample, the total mercury concentration was 17,8 mg Hg l⁻¹ (Figure 3). In average, the 82,8 ± 7,4% of the input dissolved Hg into the plant was adsorbed to the particular matter of the aeration tank, a fact explaining the high removal capacity of the plant for Hg. However, this percentage decreases somewhat in the effluent (the corresponding percentage adsorbed to the particulate matter of the effluent was 76,22 ± 14,5%).

During this investigation it was confirmed that the reduction of the incoming Hg and its subsequent volatilization is one of the major mechanisms of its removal. As soon as the microorganisms of the aeration tank become acclimatized to the presence of Hg, this mechanism becomes the second removal mechanism, the first being the adsorption of Hg onto the flocs.

The enrichment of the pilot plant with Hg resulted in significant reduction of the removal capacity for organic matter. For concentrations of Hg lower than 100 µg l⁻¹, the average decrease of the organic matter removal capacity was 10 %, while for Hg concentrations higher than 100 µg l⁻¹, it was 15%.

In the relevant literature, contradictory results have been published regarding the methylation or not of the mercury in wastewater treatment plants. In some cases methyl mercury was not detected, whereas in other studies 0.5% - 15% of the total mercury has been reported to be methylated.

A possible reason for the absence of methyl mercury from the analyzed wastewater samples is that the conditions were aerobic, a fact that does not favor the methylation procedure. In

addition, since the ratio methylation/demethylation is directly affected by the numbers and species of microorganisms [1, 7], it can be assumed that under the particular conditions the demethylation procedure was predominant.

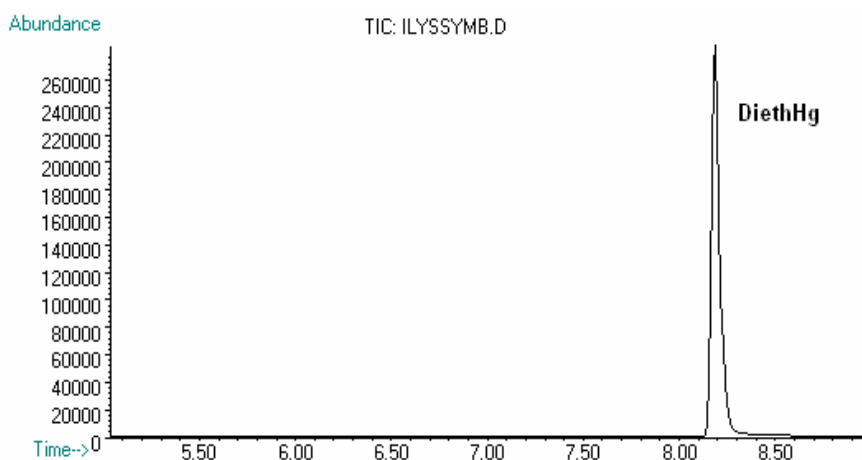


Figure 3. GC-MS chromatogram in the sludge sample with the highest Hg concentration

The determination of methyl mercury, especially in wastewater samples, shows analytical difficulties, mainly poor sensitivity of the methods. Up to now, research on analytical methods optimization is in progress. The existing analytical methods are time consuming, expensive and need high scientific expertise. Moreover, according to the existing regulations in Europe and in the USA, the determination of methyl mercury in wastewater treatment plants is not obligatory. Therefore, the references regarding the formation of methyl mercury during wastewater treatment are still very scarce, especially for full-scale plants [8-9, 17-20]. An issue of concern, given the elevated concentrations of total Hg in municipal wastewater, is the environmental and health risk posed by the possible existence of methyl mercury at concentrations lower than the detection limits obtained with the currently available analytical techniques.

4. CONCLUSIONS

A simultaneous extraction-derivatization method was developed for the determination of MeHg in wastewater, and applied for the examination of Hg methylation in a pilot-plant activated sludge wastewater treatment plant supplied with synthetic wastewater enriched with Hg. Under these particular conditions, methylation of Hg did not occur, although the high temperature during the pilot-plant experiment and the high concentration of suspended solids are factors which should enhance the methylation of mercury. The fact that the conditions were aerobic, as well as the speciation of microorganisms in the particular case could be the reason that Hg was not methylated during the present experiment.

During this investigation it was also confirmed that the reduction of the incoming Hg and its subsequent volatilization is one of the major mechanisms of its removal, while after the acclimatization of the microorganisms of the aeration tank, this mechanism becomes the second removal mechanism, the first being the adsorption of Hg onto the flocs. Another observation was that the enrichment of the pilot plant with Hg resulted in significant reduction of the removal capacity for organic matter.

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