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# Chemical kinetic isotope fractionation of mercury during abiotic methylation of Hg(II) by methylcobalamin in aqueous chloride media

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Mercury (Hg) is assumed to be predominantly methylated by microorganisms in the environment. However, the mechanisms and extent of abiotic methylation are poorly appreciated. The understanding of the mechanisms leading to abiotic methylation and demethylation in the aquatic environment is of special concern since methylmercury (MeHg) biomagnifies in the food web. Bioaccumulating organisms have also been found to preserve specific Hg isotopic signatures that provide direct insight into aquatic Hg transformations. In this study we investigated the influence of chloride on the magnitude of Hg isotope fractionation during abiotic methylation of inorganic Hg (Hg(II)) using methylcobalamin as methyl donor compound. Coupling of gas chromatography with multi-collector inductively coupled plasma mass spectrometry has allowed to determine simultaneously isotopic ratios of inorganic and methyl-Hg species. Kinetic experiments demonstrated that the presence of chloride not only slowed the chemical alkylation of Hg(II) by methylcobalamin, but also decreased the extent of the methylation, which it is especially significant under visible light conditions due to the enhancement of MeHg photodecomposition. Abiotic methylation of Hg(II) by methylcobalamin in the presence of chloride caused significant Hg mass-dependent isotope fractionation (MDF) for both Hg(II) substrate ( $\delta^{202}\text{Hg(II)}$  from  $-0.74\%$  to  $2.48\%$ ) and produced MeHg ( $\delta^{202}\text{MeHg}$  from  $-1.44\%$  to  $0.38\%$ ) both under dark and visible light conditions. The value of this MDF under such saline conditions was higher than that previously reported ( $\delta^{202}\text{MeHg}$  from  $-0.73\%$  to  $0.09\%$ ) in the absence of chloride and appeared mainly related to inorganic Hg speciation in solution, which is predominantly mercuric chloro-complexes (i.e.  $\text{HgCl}_4^{2-}$ ). Different isotopic signatures were observed for the different Hg species at the same time of reaction for either dark or visible light (450–650 nm wavelengths) conditions. However, no significant mass-independent fractionation (MIF) was induced under any conditions within the analytical uncertainties ( $-0.17 \pm 0.31 < \Delta^{201}\text{Hg} < 0.17 \pm 0.28\%$ ), suggesting that photo-induced demethylation does not always involve MIF. These results also suggest that methylation by methylcobalamin can be an experimental model to study Hg isotope fractionation extent during elementary reaction of methyl transfer in biotic systems.

## 1. Introduction

Methylmercury (MeHg) is the most hazardous compound among mercury (Hg) species due to its ability to bioaccumulate in aquatic biota, reaching higher levels than recommended values in top predators of both marine and freshwater environments. The net production of MeHg in aquatic ecosystems is closely dependent on the environmental conditions, such as presence of methylating microorganisms,

temperature, pH, organic matter, redox conditions, or salinity, which influence the equilibrium between methylation and demethylation pathways (Ullrich et al., 2001). Understanding bioaccumulation of MeHg in aquatic food chains requires differentiation between biotic and abiotic pathways that lead to its production and degradation. Microorganisms such as sulphate-reducing or iron-reducing bacteria are known to be widely involved both in the methylation of inorganic Hg (Hg(II)) and demethylation of MeHg (Compeau and Bartha, 1984; Compeau and Bartha, 1985) in aquatic ecosystems. On the other hand, chemical abiotic methylation, including methylation by compounds released to the environment by biotic processes, is supposed to be a relevant process which can occur in the water column with the presence of methyl donor compounds such as methylcobalamin,

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methyl iodide or methyltin compounds (Thayer, 1989; Celo et al., 2006). In this context, methylcobalamin (MeCo), a naturally occurring coenzyme of vitamin B12, plays an important role in the environment since it can be considered as one of the main potential methyl donors. It is responsible for the biological methylation of mercury leading to highly toxic compounds such as MeHg (Schneider and Stroinski, 1987; Thayer, 1989; Choi and Bartha, 1993).

Though Hg methylation by MeCo in the natural environment is usually a result of enzymatic or chemical transfer of a methyl carbanion to Hg(II) species in either aquated, ionic or complexed forms (Craig and Moreton, 1985), further studies have demonstrated that MeCo is also capable of transferring a methyl carbanion to the mercuric ion in absence of enzymes in aqueous abiotic systems (Bertilsson and Neujahr, 1971; De Simone et al., 1973; Celo et al., 2006; Chen et al., 2007). The transfer of the methyl group requires its activation which can be carried out by the metal ion itself or by a system of two metal ions (Wood and Fanchiang, 1979). Nevertheless, the key step in the process of methylation seems to be the cleavage of the Co—C bond which can be broken under different conditions to give a carbanion ( $\text{CH}_3^-$ ), a radical ( $\text{CH}_3\cdot$ ) or a carbonium ion ( $\text{CH}_3^+$ ) (Ridley et al., 1977; Craig and Morton, 1978; Schneider and Stroinski, 1987). Based on both structural and kinetic studies, different mechanisms such as electrophilic or nucleophilic attacks, free radical attack, oxidative cleavage or the so-called redox switch mechanism have been proposed for the methyl transfer from MeCo to metal ions (Wood and Fanchiang, 1979; Schneider and Stroinski, 1987). Thus, the transmethylation reactions involving MeCo as methylating agent can proceed by different mechanisms depending on the chemical properties of the metal compound. In the case of the chemical methylation of Hg, the heterolytic cleavage of the Co—C bond is likely to occur during electrophilic attack by mercuric ion producing MeHg and aquocobalamin as the reaction products (Bertilsson and Neujahr, 1971; De Simone et al., 1973).

Over the last decade advances in Hg stable isotope geochemistry have demonstrated meaningful variations in natural Hg isotope composition and highlighted the potential for tracing sources and quantifying Hg transformations (Bergquist and Blum, 2009). Significant variations in stable isotope ratios of Hg in natural samples has been reported in several studies (Bergquist and Blum, 2007; Biswas et al., 2008; Jackson et al., 2008; Smith et al., 2008; Perrot et al., 2010). Whereas most migration pathways and chemical transformations of Hg compounds in the environment only displayed mass-dependent fractionation of Hg isotopes (Kritee et al., 2007; Zheng et al., 2007; Kritee et al., 2008; Foucher et al., 2009; Kritee et al., 2009; Rodríguez-Gonzalez et al., 2009; Yang and Sturgeon, 2009), other biogeochemical processes can lead to both mass-dependent fractionation (MDF) of Hg isotopes and mass-independent fractionation (MIF) of the odd-mass isotopes (Bergquist and Blum, 2007; Jackson et al., 2008; Estrade et al., 2009b). Hence, new insights on isotopic data offer valuable information on the physicochemical mechanisms that distribute Hg species. However, the ability to meaningfully interpret and use Hg isotope data hinges upon precise determination of isotope ratios in representative samples and quantification of the magnitude of variations in isotopic ratios during individual biotic and abiotic transformations (Kritee et al., 2009). Recent developments in multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) have brought a new dimension to this field (Albarède et al., 2004; Foucher and Hintelmann, 2006). Additionally, the coupling of gas chromatography with MC-ICP-MS has enabled to determinate simultaneously isotopic ratios of the different Hg species as reported in previous studies (Krupp and Donard, 2005; Epov et al., 2008; Rodríguez-Gonzalez et al., 2009; Epov et al., 2010).

This work was aimed at assessing the extent of isotope fractionation of Hg species during abiotic methylation of Hg(II) by MeCo under seawater chloride concentration (0.5 M). Not only Hg speciation but also the magnitude and rate of methylation and

demethylation reactions were evaluated. Photodecomposition of MeHg, for which the rate seems to be dependent on the binding ligands (Zhang and Hsu-Kim, 2010), is supposed to enhance not only MDF but also MIF (Bergquist and Blum, 2007; Malinovsky et al., 2010). The visible light effect at wavelength of 450–650 nm on the incubations of Hg(II) with MeCo in chloride medium was also assessed. We also estimate MeCo as a good candidate to investigate the elementary methylation reaction taking place in biota through the carbanion transfer mechanism, similarly to other biogenic methylation agents.

## 2. Materials and methods

### 2.1. Reagents and standards

Stock solutions of Hg(II) and MeHg were prepared by diluting a standard reference material NIST SRM-3133 (Hg standard solution of  $10000 \text{ mg L}^{-1}$ ) or by dissolving methylmercury chloride (Strem Chemicals, USA) in methanol (Sigma Aldrich, France), respectively. Working standard solutions were prepared daily by appropriate dilution of the stock solutions in 1% HCl and were stored at  $4^\circ\text{C}$  until use. Stock solution of  $^{199}\text{HgCl}_2$  was prepared by dissolving  $^{199}\text{HgO}$  (Oak Ridge National Laboratory, USA) in HCl ( $12 \text{ mol L}^{-1}$ ).  $^{201}\text{MeHg}$  was synthesized from methylcobalamin and  $^{201}\text{HgO}$  obtained from Oak Ridge National Laboratory (USA) according to the procedure described elsewhere (Rodríguez Martin-Doimeadios et al., 2002). Hg species were derivatized using sodium tetraethyl- or tetrapropylborate solutions prepared daily by dissolving  $\text{NaBEt}_4$  or  $\text{NaBPr}_4$  (Merseburger Spezialchemikalien, Germany) in deionized water.

All the reagents (MeCo, acetic acid, sodium acetate, sodium chloride) used for the incubation experiments were ultrapure quality controlled for Hg concentration whereas organic solvents were HPLC grade. Trace metal grade acids ( $\text{HNO}_3$  and HCl) from Fisher Scientific (Illkirch, France) were used for the preparation of all the samples, standards and blanks. All solutions were prepared using ultrapure water ( $18 \text{ M}\Omega \text{ cm}$ , Millipore).

### 2.2. Experimental design

The abiotic methylation experiments were performed in 35 mL headspace Pyrex glass vials covered by PTFE/silicone septa (Supelco) and sealed with aluminium cap to prevent any evasion of Hg species. The vials were precleaned with 10%  $\text{HNO}_3$  and 10% HCl solutions and finally rinsed with ultrapure water. The incubations in the dark were conducted by covering the vial with aluminium foil whereas the photochemical incubations were carried out irradiating the samples with visible light (at 50 cm of  $123.5 \mu\text{W cm}^{-2}$  as total irradiance and 450–650 nm wavelength fluorescent tube). An experimental set-up of the experiments is presented in Fig. S-1. The optimal experimental conditions of pH, temperature and initial concentration and ratios of reagents were previously established in preliminary tests (see Supplementary Material for more information).

Solution of Hg(II) (prepared by diluting SRM NIST-3133 reference Hg standard solution) with initial concentration of  $3.5 \text{ mg L}^{-1}$  was incubated during 48 h with MeCo, which was added in a large excess (MeCo/Hg(II) molar ratio of 10). The incubation was performed at room temperature ( $\sim 20^\circ\text{C}$ ) and pH 4.0 controlled by 0.1 M acetate buffer. In chloride experiments, the salinity of the medium was adjusted close to marine conditions ( $\sim 0.5 \text{ M}$  of chloride) by adding sodium chloride. Kinetic experiments were conducted for over 48 h so that the aliquots of  $4 \mu\text{L}$  (for Hg species quantification analysis) and  $120 \mu\text{L}$  (for measurement of isotopic ratios) were subsampled at different incubation times (0, 0.15, 1, 2, 4, 8, 24 and 48 h). Sampling of aliquots was carried out with cleaned gas-tight syringes. The experimental vial initially contained 35 mL of reacting solution so that the reaction started with zero headspace. After each sampling step, the

volume of solution slightly decreased and gas phase concurrently increased. Thus, gas phase was also sampled after 4 h of reaction and analyzed for Hg concentration of eventual volatile Hg species generated. Nevertheless, the found total Hg(0) concentrations regarding Hg(0) present in both gas phase and reaction medium were lower than 10% of total Hg in any case.

### 2.3. Analytical procedures

#### 2.3.1. Quantification of Hg species

At the end of the incubation, Hg species were analyzed in the abiotic methylation experiments at different conditions (i.e. absence or presence of chloride and/or incubation in dark or visible light conditions). Thus, the aliquots of 4  $\mu\text{L}$  sampled from the reaction medium were immediately added to 5 mL of 0.1 M acetate buffer (pH=3.9) and extracted into 4 mL of iso-octane (2,2,4-trimethylpentane) after derivatization with 500  $\mu\text{L}$  of 2% NaBPr<sub>4</sub>. The organic phase was finally analyzed by gas chromatography (GC Trace, ThermoFisher) coupled with inductively coupled plasma mass spectrometry (ICPMS X2 series, ThermoFisher) (Rodriguez Martin-Doimeadios et al., 2002; Monperrus et al., 2004). In order to control accuracy of external calibration and inter-species transformations during analytical procedures, some samples were spiked with <sup>199</sup>Hg(II) and <sup>201</sup>MeHg before the derivatization step following an isotopic dilution technique described in a previous work (Monperrus et al., 2005). In some cases (<20%), Hg species mass balance was only slightly higher than 70%. These low recoveries have been attributed to the error in the volume of derivatized sample (4  $\mu\text{L}$ ), adsorption of Hg(II) complexes on the vials, and/or possible degassing of eventual Hg<sup>0</sup> generated by reduction of Hg(II) through the septum.

#### 2.3.2. Determination of species-specific Hg isotope fractionation

Simultaneous determination of Hg species isotopic ratios in the same sample was performed by coupling of gas chromatography (GC Focus, ThermoFisher) and multicollector ICP-MS (Nu Plasma HR, Nu Instrument). Device configuration, parameters and data treatment method for these simultaneous measurements of multiple species-specific isotope ratios of Hg were described in detail in previous publications (Epov et al., 2008, 2010) and also in the Supplementary Material (Table ST-1, Figs. S-2 and S-3).

Sampling derivatization prior to injection into GC-MC-ICPMS followed the same protocol used for Hg species quantification but hexane was used as organic solvent. The volume of the derivatized sample was higher than for quantification (120  $\mu\text{L}$ ) in order to have a transient signal suitable for precise and accurate isotopic ratios measurements (highest peak intensity from 0.5 to 5 V). NIST SRM-997 thallium was introduced simultaneously by nebulization for instrumental mass-bias correction using the exponential fractionation law.

Each sample was measured in triplicates and bracketed with NIST SRM-3133 (and STREM for MeHg species) to express results as delta notation ( $\delta$ ) using the following equations:

$$\delta^{\text{xxx}}\text{Hg}_{\text{sample}} = \left[ \left( \frac{{}^{\text{xxx}}/198\text{Hg}_{\text{sample}}}{{}^{\text{xxx}}/198\text{Hg}_{\text{NIST 3133}}} \right) - 1 \right] * 1000 \quad (1)$$

$$\delta^{\text{xxx}}\text{Hg}_{\text{sample-NIST3133}} = \left[ \left( \left( \frac{\delta^{\text{xxx}}\text{Hg}_{\text{STREM-NIST3133}}}{1000} \right) + 1 \right) * \left( \left( \frac{\delta^{\text{xxx}}\text{Hg}_{\text{sample-STREM}}}{1000} \right) + 1 \right) - 1 \right] * 1000 \quad (2)$$

In order to enhance the visual readability of the text and data tables, delta values of Hg species (i.e. Hg(II) and MeHg) were expressed

using a simplified nomenclature (i.e.  $\delta^{\text{xxx}}\text{Hg(II)}$  or  $\delta^{\text{xxx}}\text{MeHg}$ ) instead of the  $\delta^{\text{xxx}}\text{Hg}_{(\text{Hg species})}$  notation.

Deviation from the MDF line for the odd <sup>199</sup>Hg and <sup>201</sup>Hg isotopes is expressed using capital delta notation ( $\Delta$ ), as suggested by Blum and Bergquist (2007), according to the following equations:

$$\Delta^{199}\text{Hg} = \delta^{199}\text{Hg} - \delta^{202}\text{Hg} * 0.252 \quad (3)$$

$$\Delta^{201}\text{Hg} = \delta^{201}\text{Hg} - \delta^{202}\text{Hg} * 0.752 \quad (4)$$

Uncertainties for delta values were calculated using the interval of two standard deviations (2SD typical errors) for each sample measured in triplicate. Accuracy and precision of the method for the three weeks session analysis were given by measurements of secondary standards with different isotopic composition, i.e. RL24H ( $\delta^{202}\text{Hg} = 2.85 \pm 0.37\%$ , 2SD,  $n = 13$ ) and F65A ( $\delta^{202}\text{Hg} = -3.62 \pm 0.28\%$ , 2SD,  $n = 17$ ). The measured values were in the range of previously reported isotopic compositions (Estrade et al., 2009a; Epov et al., 2010). Additionally, in order to interpret carefully the experimental data-set, measurements of isotope <sup>199</sup>Hg were not taken into account in the following discussion because of accidental cross-contamination occurred for several samples (about 20% of the dataset) by isotopically-enriched standard of <sup>199</sup>Hg(II), which was used for species quantification by ID-GC-ICPMS. Hence, determination of possible MIF of Hg isotopes during our experiments were carried out only by the measurement of isotope <sup>201</sup>Hg expressed in the following section, tables and figures, as  $\delta^{201}\text{Hg}$  values since the isotope <sup>201</sup>Hg was free of contamination.

As mentioned above, some Hg<sup>0</sup> generation, even low, cannot be avoided, and that may fractionate Hg isotopes, leading to the enrichment of the remaining Hg(II) by heavier isotopes (Zheng and Hintelmann, 2010). Nevertheless, we observed only a global isotopic mass-balance (in  $\delta$ , ‰) lower than zero in few samples (<20%, see Table ST-2) which can be explained by adsorption of Hg(II) onto the vials favouring lighter isotope to remain in solution.

### 2.4. Complexation models and rate constants calculation

The software Visual MINTEQ (version 3.0, <http://www2.lwr.kth.se>) was used to calculate the aqueous speciation of Hg(II) and MeHg in the acetate buffer used for the abiotic methylation reactions (Table ST-3). The effect of chloride on Hg speciation was also computed for initial conditions and for the maximum yield of MeHg formation. The parameters used to calculate Hg speciation were pH, ionic strength, temperature, and concentrations of Hg species and ligands. The MINTEQ database derived from NIST database 46 (Smith et al., 2003) was modified by the addition of stability constants for some selected combinations of the Hg species and ligands. With regards to complexation between MeCo and Hg species, the nearest molecular model to mimic MeCo binding was found to be the binding of Hg species with acetamide. The MeCo structure presents six terminal acetamide groups so as to acetamide was the chosen model for the calculations of Hg(II) speciation. However, as there were no available stability constants for complexes formed between MeHg and acetamide, the MeHg complexation was modeled using the binding of MeHg with glycinate. Glycinate appears to be the closest group whose stability constants for MeHg complexes have been previously reported (Alderighi et al., 2003; Gans et al., 2008), though glycinate is not really present in the MeCo structure. Stability constants for the complexes of Hg(II) and MeHg with ligand considered for our study are listed in Table ST-4 (Supplementary Material).

The methylation and demethylation rate constants ( $k_m$  and  $k_d$ ) were calculated modeling the experimental data using Origin Pro 8.0 software (Origin Lab Corporation, Northampton, USA). For the experiments performed in the presence of chloride, Hg species

transformations were considered as pseudo first order reversible reactions for Hg(II) so that the MeHg net formation could be written as follow:

$$d[\text{MeHg}]/dt = k_m[\text{Hg(II)}] - k_d[\text{MeHg}] \quad (5)$$

Assuming that both Hg(II) and MeHg represent total concentrations of the Hg species and that initial total MeHg concentration was negligible, the former equation can be integrated obtaining the concentration of MeHg as a function of time:

$$[\text{MeHg}]/[\text{Hg(II)}]_0 = (k_m/k_m + k_d) \left( 1 - e^{-(k_m+k_d)t} \right) \quad (6)$$

Therefore, the kinetic rate constants can be calculated using a reversible reaction kinetic model by applying a non-linear fitting model (Box Lucas 1) as previously reported elsewhere (Rodriguez Martin-Doimeadios et al., 2004).

For the experiments without chloride addition, different kinetics models based on first-order reactions were used to calculate both methylation and demethylation rate constants for the incubations performed in either dark or light conditions as described in previous works (Jimenez-Moreno et al., 2010; Perrot et al., 2011).

### 3. Results and discussion

#### 3.1. Abiotic methylation and demethylation of Hg in aqueous chloride medium

##### 3.1.1. Effect of chloride on Hg speciation and complexation

**3.1.1.1. Inorganic mercury.** Apart from dissolved organic matter (DOM) which controls the speciation of Hg(II) in most aquatic environments, inorganic ligands such as hydroxide, chloride and sulfide play an important role in controlling the speciation of Hg in some aquatic systems. In the absence of DOM, chloride or any other ligands, it would be expected that the speciation of Hg(II) was controlled by free mercuric ion or mercury hydroxides. However, as our abiotic methylation experiments were performed in acetate buffer (pH = 4.0), two mercury acetate complexes,  $\text{Hg}(\text{CH}_3\text{COO})_4^{2-}$  and  $\text{Hg}(\text{CH}_3\text{COO})_3^-$ , were the dominant species in the reaction medium without any chloride addition. In the presence of chloride ion, Hg(II) tends to form  $\text{HgCl}^+$ ,  $\text{HgCl}_2$ ,  $\text{HgCl}_3^-$  and  $\text{HgCl}_4^{2-}$  species. At a relatively high concentration of chloride (~0.5 M), the speciation of Hg(II) in the initial conditions (Table ST-3) was represented by three chloride complexes with  $\text{HgCl}_4^{2-}$  being the most abundant (78.7%) followed by  $\text{HgCl}_3^-$  (17.9%) and  $\text{HgCl}_2$  (3.0%). Small contributions of some mercury acetate complexes were also found with 0.4% of  $\text{Hg}(\text{CH}_3\text{COO})_4^{2-}$  and 0.004% of  $\text{Hg}(\text{CH}_3\text{COO})_3^-$ . This species distribution would be consistent with the literature stating that, in absence of colloidal complexes, Hg(II) predominantly exists as  $\text{HgCl}_4^{2-}$  and  $\text{HgCl}_3^-$  in full-strength seawater (3.5% of salinity) with an average concentration of 0.56 M of chloride ion (Hahne and Kroontje, 1973; Ullrich et al., 2001). Additionally, the predominance of negatively charged mercury chloride complexes (i.e.  $\text{HgCl}_4^{2-}$  and  $\text{HgCl}_3^-$ ) was maintained along the alkylation reaction of Hg(II) with MeCo in both dark and visible light incubations.

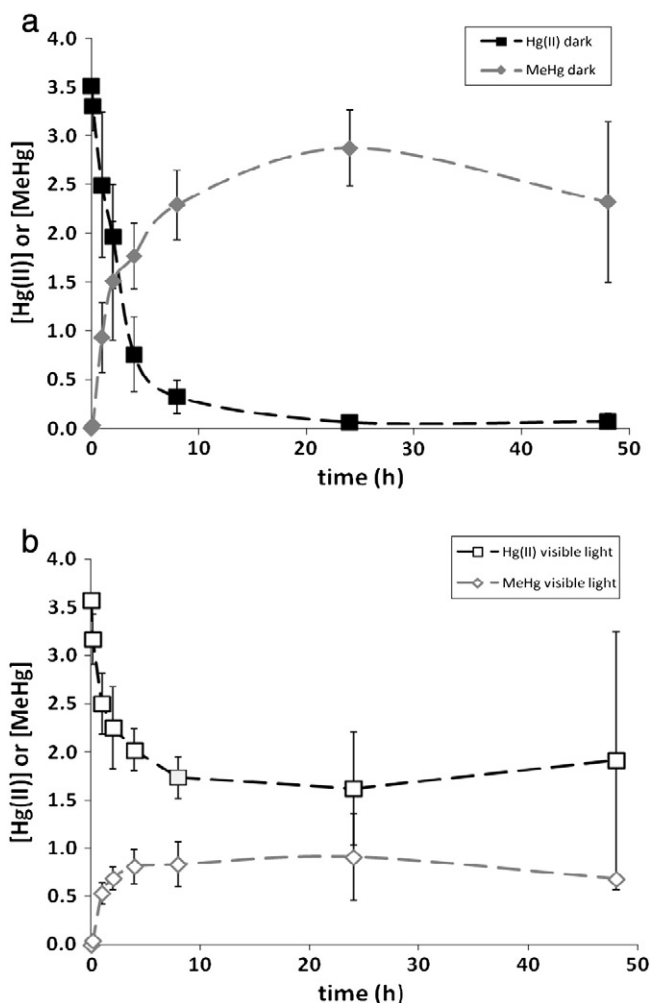
**3.1.1.2. Methylmercury.** Regarding the speciation of MeHg in seawater, this species is able to form two main complexes:  $\text{CH}_3\text{HgOH}$  and  $\text{CH}_3\text{HgCl}$ . It is well known that the ratio of hydroxide/chloride complexes depends on both chloride concentration and pH (Mason et al., 1996). At high chloride concentrations the speciation of MeHg would be pH-dependent, thus, methylmercury hydroxide is the main species for pH values higher than 6.0 whereas methylmercury chloride species is the main form at lower pH (Chen et al., 2003). Therefore, as our experiments

were performed at pH = 4.0, it could be expected that the methylmercury chloride complex would be the predominant species (67.9%) in the chloride experiments, as it was predicted by the speciation simulations performed with MINTEQA. A significant fraction of MeHg (more than 30%) was potentially complexed with MeCo, which is in a large excess in the reaction medium, probably by means of the coordination of acetamides via the terminal amines to the MeHg cation. But only a small contribution of MeHg acetate complex (0.03%) was predicted. It has been demonstrated that the addition of chloride is changing not only the aqueous-phase chemical speciation of Hg(II) but also the MeHg species distribution. For example, MeHg is almost totally (99.9%) in the form of its complex with MeCo in the medium without chloride whereas it becomes to be mostly in the form of  $\text{CH}_3\text{HgCl}$  in presence of chloride.

It is also important to note that the Hg(II) species differences in the presence of chloride did not inhibit the chemical alkylation of Hg(II) by MeCo. This observation can be related to the fact that the ability of Hg complexes to become methylated by MeCo is simply related to stability constants but it is dependent on the MeCo acid-base chemistry (Musante, 2008). The mechanism of the methyl group transfer as a carbanion from MeCo to mercuric ion is associated with the transition between the active ("base-on") and inactive ("base-off") forms of the MeCo. This corresponds to the transition from the six to the five coordination number of the cobalt, which is coordinated with benzimidazole group that is thought to occur in the range of pH between 1.5 and 4.8 (Craig and Morton, 1978; Chen et al., 2007; Musante, 2008). In addition, since acetic acid has been found to be responsible for Hg(II) abiotic methylation under certain conditions (Gardfeldt et al., 2003; Malinovsky and Vanhaecke, 2011), a control experiment was carried out (Hg(II) incubated at pH 4.0 with 0.1 M acetic acid and 0.5 M NaCl but without MeCo) and it did not show any detectable production of MeHg after 48 h. The methyl group of acetic acid is unlikely to be involved in the abiotic methylation of Hg in our conditions, which is in agreement with the observation of Malinovsky and Vanhaecke (2011), where only trace amounts of MeHg were produced in presence of excess acetic acid although their experimental conditions were slightly different (i.e. presence of  $\text{OH}^\bullet$  radicals). Thus, methylation pathway and species-specific isotope ratios in our experimental conditions are related to the reactivity with MeCo. Complementary UV-vis spectra of the reaction media revealed that no structural changes of MeCo were induced by the addition of chloride since it has the same pattern as the spectra without chloride. However, remarkable differences in the UV-visible spectra profile was observed for the MeCo in presence of other complexing agents such as cysteine (Fig. S-4). The last can be related to the conversion of MeCo to an inactive form that causes a total inhibition of abiotic methylation process under these conditions. No changes of MeCo solution red color were observed in presence of either acetate or chloride when Hg(II) was added. It is agreed with previous studies on MeCo chemistry (Chu and Gruenwedel, 1977).

##### 3.1.2. Effect of chloride on methylation reactions rate under dark conditions

Unlike studies suggesting that MeCo appears to be unreactive towards chloride complexes of Hg(II) in moderately or highly saline environments (Celo et al., 2006), our experiments showed that inorganic Hg(II) can be methylated by MeCo in the presence of chloride (Fig. 1a) which is consistent with other previous works (Chen et al., 2007; Musante, 2008). However, the increase in chloride concentration slowed considerably the methylation of Hg(II) by MeCo reaching the maximum methylation yield (~82%) after 24 h of incubation. Methylation in a high chloride concentration medium (0.5 M) is about 25-fold slower than in solution without sodium chloride where methylation was complete and almost instantaneous (Table 1). These results seem to be directly related to the different Hg(II) speciation presented in either chloride (where Hg-chloride



**Fig. 1.** Formation and degradation of Hg species during 48 h incubation of Hg(II) with MeCo in 0.5 M chloride medium. Concentrations ( $\text{mg L}^{-1}$ ) of Hg(II) and MeHg are represented as a function of time under (a) dark and (b) visible light conditions. Error bars (SD) are related to the long term reproducibility of four experiments within six months.

complexes are predominant) or non-chloride experiments (where Hg-acetate complexes are predominant) and it can be linked to the mechanism of the methylation reaction. As previously described, treating the process only as a chemical transfer, the chemical

alkylation of Hg involves not only the cleavage of a  $\text{CH}_3\text{—Co}$  bond but also the displacement of a ligand-Hg(II) bound by the methyl group. Therefore, it can be assumed that the bond strengths of the ligands complexing Hg(II) are the main determinants of the rates of methylation since the reaction mechanism is similar to the organic nucleophilic substitution but centred on Hg rather than on carbon (Craig and Moreton, 1985). Abiotic methylation reaction appears to be promoted via mercury acetate complexes as previously suggested by other authors (Craig and Moreton, 1985; Gardfeldt et al., 2003), and limited when the reactant is a chloro complex due to its inhibitory effect (Compeau and Bartha, 1983) probably because of higher stability of the Hg—Cl bond.

Another important consideration is that the presence of chloride substantially promoted the process of Hg demethylation. Without chloride demethylation of MeHg is negligible during the incubation period, while the abiotic demethylation of MeHg ( $k_d = 0.079 \pm 0.077$ ) significantly evolves after 24 h of incubation in presence of chloride (Table 1 and Fig. 1a). Both methylation and demethylation reactions are competitive processes, however, methylation seemed to be the kinetic predominant process since its rate constant is about 3-fold higher than the demethylation one. This observation agrees with previous works suggesting that both Hg abiotic and biotic demethylation is more effective in marine ecosystems than in freshwater ones (Compeau and Bartha, 1984; Hamasaki et al., 1995).

### 3.1.3. Effect of visible light on demethylation reaction

Photolytic decomposition can be considered one of the most significant MeHg decomposition mechanisms (Hammerschmidt and Fitzgerald, 2006; Lehnher and St Louis, 2009; Hammerschmidt and Fitzgerald, 2010). In our experiments performed at high chloride concentrations, visible light emission had a remarkable influence on MeHg demethylation rate since demethylation rate constant was about 9-fold higher than that for dark conditions (Table 1). However, no significant inhibition/activation was observed regarding the net rate of methylation since the kinetic constant in visible light conditions ( $0.216 \pm 0.021 \text{ h}^{-1}$ ) was comparable to that in dark conditions ( $0.255 \pm 0.048 \text{ h}^{-1}$ ). Thus, visible light emission had no significant effects on the methylation process but had a considerable influence on the extent of the reaction promoting demethylation. The net methylation yield decreased more than 3-fold, to reach a maximum yield of about 25% after 24 h of incubation as shown in Fig. 1b. Methylation appeared to be the predominant reaction under dark, whereas demethylation was the predominant process under visible light emission as evidenced by the kinetic rate constants exhibiting values more than 3-fold higher for demethylation reaction (Table 1). Therefore, the decrease of the magnitude of methylation in chloride medium

**Table 1**  
Percentages of Hg species as a function of time and kinetic rate constants ( $k$  notation ( $\text{h}^{-1}$ )) for different abiotic methylation incubations in chloride medium in comparison with experiments in absence of sodium chloride. Experiments carried out without NaCl showed formation of significant amounts of dimethylmercury between 2 and 48 h, as it has already been observed in a previous work (Filipelli and Baldi, 1993). Uncertainties in percentages of Hg species (expressed as standard deviation of the mean) represent the long term reproducibility of four experiments within six months. Uncertainties of rate constants are obtained from the standard deviation of the typical parameters of the Box Lucas models used for the non-linear fitting of experimental data with Origin.

Time (h)	Dark experiments				Visible light experiments			
	With NaCl		Without NaCl		With NaCl		Without NaCl	
	MeHg	Hg(II)	MeHg	Hg(II)	MeHg	Hg(II)	MeHg	Hg(II)
0.15	1 ± 1	94 ± 7	75 ± 8	5.9 ± 4.2	1 ± 1	91 ± 7	64 ± 32	14 ± 19
1	27 ± 10	71 ± 27	91 ± 21	0.8 ± 0.9	15 ± 3	71 ± 2	82 ± 15	1.2 ± 0.4
2	43 ± 17	56 ± 28	85 ± 12	0.7 ± 0.9	19 ± 3	64 ± 7	89 ± 22	1.2 ± 0.5
4	50 ± 5	22 ± 11	76 ± 14	0.7 ± 1.0	23 ± 5	58 ± 6	75 ± 10	1.1 ± 0.7
8	65 ± 10	9 ± 5	67 ± 11	0.6 ± 1.0	23 ± 2	50 ± 6	84 ± 13	0.8 ± 0.1
24	82 ± 11	2 ± 1	65 ± 15	2.0 ± 1.3	25 ± 1	46 ± 17	79 ± 8	1.1 ± 0.9
48	66 ± 23	2 ± 2	65 ± 7	2.0 ± 1.8	19 ± 1	55 ± 38	67 ± 24	2.3 ± 2.2
$k_{\text{methylation}}$	0.255 ± 0.048		6.246 ± 1.202		0.216 ± 0.021		6.073 ± 0.866	
$k_{\text{demethylation}}$	0.079 ± 0.077		-		0.683 ± 0.088		0.0061 ± 0.0002	
$R^2$	0.9673		0.9525		0.9929		0.9903	

promoted by the visible light emission can be directly related to the enhancement of the photo-activated degradation of MeHg.

Previous studies reported that photolytic degradation of MeHg is induced by the ultraviolet (UV) spectrum of sunlight (Lehnherr and St Louis, 2009; Zhang and Hsu-Kim, 2010). MeHg photodegradation by UV radiation is supposed to involve radical intermediates such as hydroxyl radicals which have been proposed as the reactive intermediates responsible for photodegradation (Chen et al., 2003; Malinovsky & Vanhaecke, 2011). However, our findings suggest that photochemical decomposition of MeHg can be also enhanced by visible light emission. It can be expected that the degradation rate of MeHg was slower than in samples exposed to the full solar spectrum as indicated by Lehnherr and St Louis (2009) since the total emission flux density of our visible light source of  $1.23 \text{ W m}^{-2}$  (Fig. S-4) was significantly lower and consequently less energetic than the maximum visible solar radiation flux density estimated at about  $500 \text{ W m}^{-2}$  assuming that about 40% of total solar radiation correspond to visible light region. Nevertheless, in natural aquatic systems the thickness of the water column as well as particulate and dissolved matter would significantly decrease the solar radiation intensity even at low depth, making visible light energy available for photochemical demethylation probably lower than  $500 \text{ W m}^{-2}$ . It should be noted that hydroxyl radicals could not be involved in the photodegradation of MeHg observed in our abiotic methylation experiments since formation of hydroxyl radicals from photolysis is not possible with visible light wavelengths (Dorfman and Adams, 1973). This observation suggests that different mechanisms may be responsible for abiotic photo-induced demethylation pathways, which is consistent with previous studies stating that not only hydroxyl radicals can induce MeHg degradation in surface waters (Zhang and Hsu-Kim, 2010). Furthermore, the MeHg photodecomposition appeared to be also dependant on the type of MeHg–ligand binding presented in water (Zhang and Hsu-Kim, 2010). Thus, the complexes formed between the MeHg and the MeCo, that are estimated to occur in high content (> 30%) in the reaction medium, are likely to be responsible for the activation of MeHg demethylation by the visible light radiation since MeCo chromophores are the only constituents in solution capable to absorb the incident visible radiation, especially within the wavelength range 450–650 nm.

It seems that Hg(II) reduction identified in sea and freshwaters can be also photochemically mediated (Whalin et al., 2007). It has been suggested that halides, such as chlorides or bromides, may also enhance the oxidation of Hg<sup>0</sup> (Mason et al., 1995; Lalonde et al., 2001; Hines and Brezonik, 2004). In our experiments, Hg(II) reduction to Hg<sup>0</sup> was considered negligible under visible light conditions without chloride which is in agreement with results previously reported by Gardfeldt et al. (2003). However, low quantities of Hg<sup>0</sup> seem to be produced in the presence of chloride as revealed by the mass balances for a closed

system in our experiments. No accurate determination of Hg<sup>0</sup> concentrations was possible because of the large variations observed due to the absence of equilibrium between both redox processes and the fugacity of gaseous Hg<sup>0</sup>.

### 3.2. Hg isotope fractionation during abiotic methylation in saline medium

#### 3.2.1. Mass-dependent fractionation

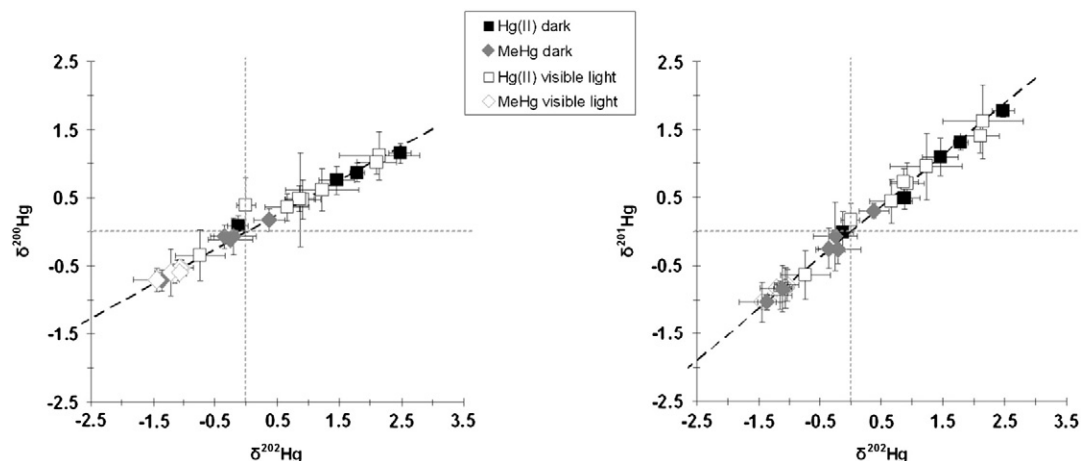
Significant mass-dependent isotope fractionation of both Hg species (i.e. Hg(II) and MeHg) was observed during the period of 48 h of Hg(II) incubation with MeCo in 0.5 M NaCl aqueous medium, at both dark and visible light conditions. According to  $\delta^{201}\text{Hg}$  values, no significant MIF of Hg was induced under these conditions (Table 2 and Fig. 2). The initial reactant Hg(II) at t0 had  $\delta^{202}\text{Hg(II)} = 0.00 \pm 0.15\%$ . The produced MeHg was preferentially enriched in the lighter isotopes ( $\delta^{202}\text{MeHg}$  from  $-1.44 \pm 0.38\%$  to  $0.38 \pm 0.25\%$ ) whereas the remaining Hg(II) was preferentially enriched in the heavier isotopes ( $\delta^{202}\text{Hg(II)}$  from  $-0.74 \pm 0.18\%$  to  $2.48 \pm 0.18\%$ ). However, as the evolution of the Hg species fraction during the 48 h of experiment discussed above was different under dark and visible light conditions, we observed significant different isotopic signatures of Hg species for these conditions at the same sampling time.

**3.2.1.1. Dark conditions.** As displayed in Table 1, after 48 h initial Hg(II) has been almost entirely converted to MeHg. Hence, assuming a closed system with no Hg loss from the incubation vial, MeHg at 48 h should have the same isotopic signature as Hg(II) at t0. Experimental data (Table 2) confirmed this since MeHg at 48 h ( $\delta^{202}\text{MeHg} = -0.20 \pm 0.37\%$ ) has the same isotopic composition as initial  $\delta^{202}\text{Hg(II)}$  within the analytical uncertainties. Note that after 24 h of incubation almost all Hg(II) has been converted to MeHg (only 2% of Hg(II) remained) with  $\delta^{202}\text{MeHg}$  ( $0.38 \pm 0.25\%$ ) being close to  $\delta^{202}\text{Hg(II)}$  initial value within the analytical uncertainties. On the other hand, between 1 h and 2 h of incubation (Hg(II) > 50%), the produced MeHg has significantly negative  $\delta^{202}\text{Hg}$  values (from  $-1.37 \pm 0.14$  to  $-1.11 \pm 0.15\%$ ). Similarly to a recent study (Malinovsky and Vanhaecke, 2011), we did not observe MIF of Hg species during methylation of Hg(II) with MeCo under dark conditions. However, higher magnitude of MDF for both Hg(II) and MeHg ( $\delta^{202}\text{Hg(II)}$  from  $-0.12\%$  to  $2.48\%$  and  $\delta^{202}\text{MeHg}$  from  $-1.37\%$  to  $-0.20\%$ ) was observed in comparison with values reported by Malinovsky and Vanhaecke (2011) ( $\delta^{202}\text{Hg(II)}$  from 0.05% to 0.98% and  $\delta^{202}\text{MeHg}$  from  $-0.73\%$  to  $-0.29\%$ ). It could be explained by higher methylation yields promoted by our experimental conditions (pH, MeCo/Hg(II) ratio and presence of sodium chloride). Influence of the chloride medium and the methylation yield on the isotope fractionation of Hg will be discussed below (Section 3.2.2).

**Table 2**

$\delta^{\text{xxx}}\text{Hg}$  (‰) of Hg species (Hg(II), MeHg) at different times and for different fractions (f) during the incubation in 0.5 M chloride medium of Hg(II) with MeCo.

	time(h)	f Hg(II)	SD	f MeHg	SD	$\delta^{202}\text{Hg(II)}$	2SD	$\delta^{201}\text{Hg(II)}$	2SD	$\delta^{200}\text{Hg(II)}$	2SD	$\delta^{202}\text{MeHg}$	2SD	$\delta^{201}\text{MeHg}$	2SD	$\delta^{200}\text{MeHg}$	2SD
Dark conditions	0	1.00	0.07	0.00	–	0.00	0.15	0.17	0.24	0.39	0.40	–	–	–	–	–	–
	0.15	0.95	–	0.01	0.01	–0.12	0.17	–0.01	0.29	0.09	0.15	–	–	–	–	–	–
	1	0.71	0.27	0.27	0.10	1.79	0.21	1.30	0.10	0.87	0.13	–1.37	0.14	–1.03	0.12	–0.71	0.16
	2	0.56	0.28	0.43	0.17	1.46	0.12	1.09	0.27	0.75	0.21	–1.11	0.15	–0.83	0.34	–0.56	0.15
	4	0.22	0.11	0.50	0.05	2.48	0.29	1.77	0.09	1.15	0.15	–0.25	0.36	–0.07	0.50	–0.12	0.08
	8	0.09	0.05	0.65	0.10	0.88	0.18	0.49	0.17	0.47	0.69	–0.35	0.17	–0.25	0.29	–0.07	0.18
	24	0.02	0.01	0.86	0.11	–	–	–	–	–	–	–0.38	0.25	0.30	0.11	–0.18	0.17
	48	0.02	0.02	0.66	0.23	–	–	–	–	–	–	–0.20	0.37	–0.26	0.21	–0.07	0.27
	Visible light conditions	0	1.00	–	0.00	–	0.00	0.15	0.17	0.24	0.39	0.40	–	–	–	–	–
0.15		0.91	0.07	0.01	0.01	–0.74	0.18	–0.63	0.14	–0.34	0.16	–	–	–	–	–	–
1		0.71	0.02	0.15	0.03	1.23	0.59	0.95	0.48	0.61	0.31	–1.44	0.38	–1.03	0.29	–0.70	0.17
2		0.64	0.07	0.19	0.03	2.15	0.65	1.61	0.54	1.11	0.35	–1.04	0.20	–0.78	0.23	–0.52	0.10
4		0.58	0.06	0.23	0.05	2.10	0.31	1.40	0.26	1.02	0.18	–1.15	0.20	–0.93	0.20	–0.61	0.14
8		0.50	0.06	0.23	0.02	0.92	0.28	0.71	0.30	0.47	0.29	–1.07	0.12	–0.82	0.07	–0.52	0.13
24		0.46	0.17	0.25	0.00	0.87	0.23	0.73	0.19	0.48	0.19	–1.21	0.26	–0.82	0.07	–0.59	0.34
48		0.55	0.38	0.19	0.00	0.66	0.35	0.45	0.32	0.36	0.20	–1.07	0.07	–0.83	0.30	–0.59	0.09



**Fig. 2.** Tri-isotopic plots ( $\delta^{xxx}\text{Hg}$  vs  $\delta^{202}\text{Hg}$  (‰)) of isotopes 200 (left) and 201 (right) for Hg species at different times during abiotic methylation of Hg(II) by MeCo in 0.5 M NaCl and 0.1 M acetate buffer (pH 4) medium under dark conditions (filled symbols) and visible light conditions (open symbols). Black dashed lines represent the theoretical mass-dependent fractionation line. Uncertainty bars are 2SD of triplicate analyses. MeHg species (diamonds) are mainly enriched in lighter isotopes (up and right part of the graphs); Hg(II) species (squares) are mainly enriched in heavier isotopes (down and left part of the graphs).

**3.2.1.2. Visible light conditions.**  $\delta^{202}\text{Hg}$  values of produced MeHg under visible light conditions remain significantly negative after 1 h of experiment (from  $-1.07 \pm 0.07\%$  to  $-1.44 \pm 0.38\%$ ), as well as the remaining Hg(II) had positive  $\delta^{202}\text{Hg}$  between  $0.66 \pm 0.35\%$  and  $2.15 \pm 0.60\%$ . The net methylation during the 48 h experiment under visible light conditions was significantly lower than under dark conditions, leading to a maximum of  $25 \pm 1\%$  MeHg which was due to more effective demethylation (see Sections 3.1.2 and 3.1.3). Thus, interpretation of the isotopic ratios under these conditions is likely to be dependent on the different demethylation extent when comparing with dark conditions. The quantity of produced MeHg versus the quantity of degraded MeHg under visible light conditions would give rapid equilibrium between the isotope ratios of both Hg species. That is not the case under dark conditions where the production of MeHg is not followed by significant demethylation yields and it is almost full after 48 h of experiment.

### 3.2.2. Mass-independent fractionation

Several studies inferred Hg isotope fractionation for both abiotic (Bergquist and Blum, 2007; Malinovsky et al., 2010; Malinovsky and Vanhaecke, 2011) and biotic demethylation pathways (Kritee et al., 2009), and the MIF was reported in all previous studies on Hg abiotic photodemethylation. In this study, despite significant uncertainties ( $-0.17 \pm 0.31 < \Delta^{201}\text{Hg} < 0.17 \pm 0.28\%$ ), we did not discover significant MIF. Several parameters can be responsible for MIF caused by magnetic isotope effect (Buchachenko et al., 2007; Epov, 2011b): (i) involvement of radical pairs; (ii) presence/absence of light; (iii) the arrangement of the ligands around the metal ion; (iv) the nature (strength) of the ligands surrounding the metal ion. Radical pairs are not involved in the described reactions, thus this mechanism will not produce MIF. For the mechanism involving ligands, the following explanation can be given to the MIF absence in our experimental conditions: during the reaction of Hg(II) chloride complexes, the ground electron state of the reagent ( $\text{HgCl}_2$ ,  $\text{HgCl}_3^-$ ,  $\text{HgCl}_4^{2-}$  as main calculated species in the experimental conditions) and the product ( $\text{CH}_3\text{HgCl}$ ,  $\text{CH}_3\text{CONHCH}_3\text{Hg}$ ) is the singlet one. The MIF can take place if the intermediate products can change their state from the singlet to triplet one. It is possible in the presence of light for  $\text{HgCl}_2$  (easier) and  $\text{HgCl}_3^-$  species and is much more difficult for  $\text{HgCl}_4^{2-}$  species (Epov, 2011a). As tetrachloro-Hg species does not have free  $6s^0$  orbital where electron can move from  $5d$ -orbitals due to light excitation and hyperfine coupling between magnetic nucleus and electrons, thus only MDF will be produced (see Supplementary Material for detailed mechanisms). Because the complexation model predicted about 80% of

$\text{HgCl}_4^{2-}$  under our experimental conditions (see paragraph 3.1.1.), no significant MIF is expected. Similar explanation can be given for the demethylation of  $\text{CH}_3\text{HgCl}$  or  $\text{CH}_3\text{CONHCH}_3\text{Hg}$  because of the high concentration of ligands in our experimental media, and that main Hg species may coordinate by these ligands through the free Hg  $sp$ -orbital. Hence, Hg in MeHg species would undergo electron spin evolution from singlet to triplet during demethylation but just participate in the chemical reaction.

Also, previous studies reported MIF during photodemethylation associated with UV light (natural or not). The most likely mechanism leading to Hg MIF during photochemical demethylation reactions is formation of the intermediate radical pair which can change the electron state from singlet to triplet one (Buchachenko et al., 2007; Bergquist and Blum, 2009). Our light radiation conditions (visible spectrum) allow catalysis of MeHg demethylation (Hammerschmidt and Fitzgerald, 2006), but probably cannot provide enough energetic excitation to allow photolytic demethylation involving radicals (Dorfman and Adams, 1973; Chen et al., 2003; Lehnher and St Louis, 2009). Therefore, the magnetic isotope effect (MIE) is unlikely to affect the odd Hg isotopes. Thus, within the uncertainties associated to our data, we can suggest that not all the Hg photochemical demethylation pathways lead to mass-independent fractionation.

### 3.2.3. Implications for Hg isotope mass-dependent fractionation

**3.2.3.1. Influence of simultaneous methylation and demethylation.** Isotope fractionation of iron in aqueous media has been shown to be dependent on the Fe-Cl complexes (Hill et al., 2009; Hill et al., 2010), underlying the assumption that ligands present in aqueous environment are potentially important drivers of elements isotope fractionation. As it was shown in a preliminary work (Perrot et al., 2011), extent of Hg isotope MDF during Hg(II) methylation by MeCo in 0.1 M acetic acid (pH = 4.0) in the dark was higher in the presence of chloride (0.5 M). Experiments carried out under visible light conditions showed the same pattern, with higher extent of MDF for 0.5 M chloride medium (Table ST-3). Hence, our results demonstrate that abiotic methylation of Hg by MeCo, both under dark or visible light, leads to higher MDF extent of Hg species under saline conditions. The presence of chloride (0.5 M) also induces larger Hg isotope fractionation when comparing with hydroxyl radicals, photochemical methylation, or presence of others potential methyl donors molecules such as monomethyltin, dimethylsulfoxide or acetic acid (Malinovsky and Vanhaecke, 2011). Both kinetic effect of methylation and demethylation reactions and equilibrium isotope effect of the strong



Hg–chloro complexes can be involved in the fractionation extent observed, but precision of the results does not allow to discriminate between these two processes (Young et al., 2002).

Under both dark and visible light conditions, we observed a shift in the evolution of the isotopic composition of Hg(II) as a function of time (Fig. 3).  $\delta^{202}\text{Hg}(\text{II})$  is increasing during 4 h of incubation under dark conditions (up to  $2.48 \pm 0.18\%$ ) (Fig. 3a) and during 2 h under visible light conditions ( $2.15 \pm 0.65\%$ ) (Fig. 3b), whereas after 8 h  $\delta^{202}\text{Hg}(\text{II})$  is significantly lower ( $0.88 \pm 0.25\%$  and  $0.92 \pm 0.28\%$ , respectively). According to the kinetic constants and the fractions of Hg species, the magnitude of both methylation and demethylation pathways are responsible for this shift of isotopic composition. During first 4 h under dark conditions, methylation is the main process affecting Hg isotope fractionation, while demethylation starts to be significant after this time. On the other hand, after 2 h of incubation the demethylation rate constant under visible light conditions was 9-fold higher than under dark conditions. Thus, the demethylation process becomes more significant. Since demethylation

reaction generates kinetic MDF of Hg isotopes towards the enrichment of the formed Hg(II) in lighter isotopes (Malinovsky et al., 2010), the overall  $\delta^{202}\text{Hg}(\text{II})$  starts to decrease when demethylation begins to be significant. Under dark conditions, MeHg concentrations is still increasing during first 24 h period (Fig. 1a), exhibiting that methylation remains the dominant process till this time. This observation leads up to the hypothesis that either methylation or demethylation was the dominant reaction in our closed system and influenced substantially the reported MDF.

### 3.2.3.2. Kinetic fractionation factors for methylation and demethylation.

In order to evaluate the potential differences of fractionation magnitude between methylation and demethylation, we calculated chemical kinetic isotope fractionation factors ( $\alpha_{202/198}$ ), which are commonly used for the determination of the magnitude discrimination between two isotopes of an element during transformation pathway (Young et al., 2002; Scott et al., 2004; Elsner et al., 2005). The calculation was made to compare the values with previously reported  $\alpha_{202/198}$  in experimental studies of specific Hg transformations pathways such as methylation and demethylation (Kritee et al., 2009; Rodriguez-Gonzalez et al., 2009; Malinovsky and Vanhaecke, 2011), (photo)reduction (Bergquist and Blum, 2007; Kritee et al., 2007; Kritee et al., 2008; Zheng and Hintelmann, 2009) or evaporation (Estrade et al., 2009b). According to the theory, as our experiments were carried out in a closed system, the fractionation factors at equilibrium can be calculated using the following equation:

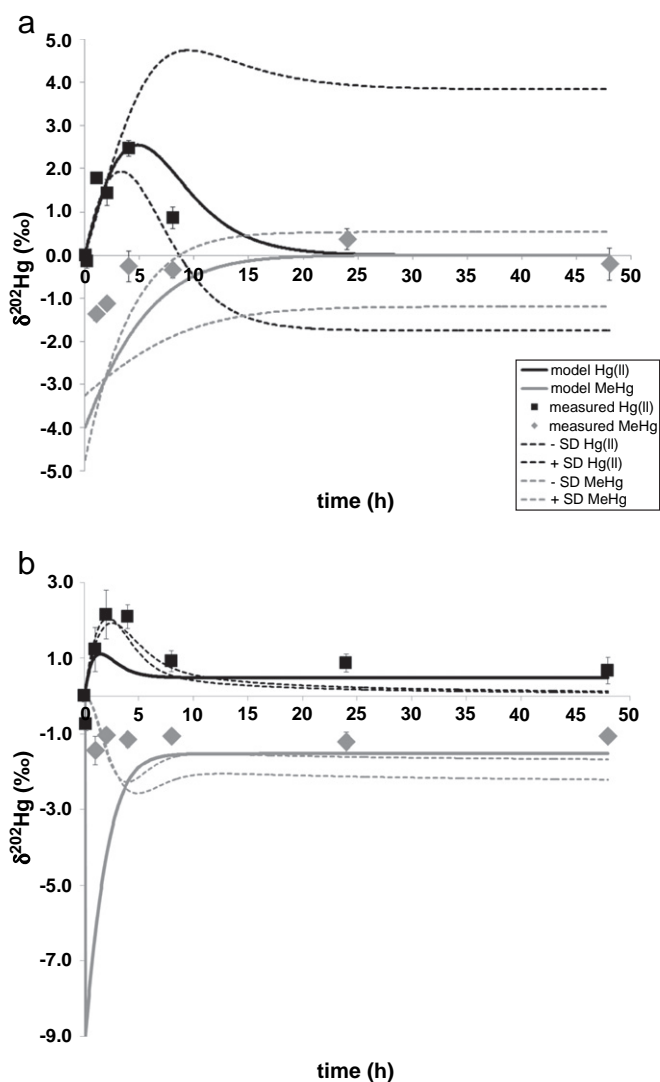
$$1000 * \ln(\alpha_{202/198}(\text{react}-\text{prod})) = \delta^{202}\text{Hg}_{\text{react}} - \delta^{202}\text{Hg}_{\text{prod}} \quad (7)$$

where  $\delta^{202}\text{Hg}_{\text{react}}$  is the isotopic composition of the reactant,  $\delta^{202}\text{Hg}_{\text{prod}}$  is the isotopic composition of the product, and  $\alpha_{202/198}(\text{react}-\text{prod})$  is the fractionation factor between the reactant and the product for a given reaction. Nevertheless, the use of this model implies that the right part of Eq. (7) is constant, which is not the case for our study. On the other hand, estimation of kinetic fractionation factors via Rayleigh distillation equation is supposed to be done for irreversible and unidirectional reaction (Scott et al., 2004; Hoefs, 2009) (i.e. methylation or demethylation). Since our study was conducted in a closed system and we demonstrated that both methylation and demethylation occurred during the experiment, the theoretical calculation of kinetic fractionation factors of the methylation and demethylation reactions is consequently difficult to resolve. We propose here that a double Rayleigh system may be employed, where possible chemical equilibrium fractionation is avoided and only kinetic fractionation factors for methylation ( $\alpha_m$ ) and demethylation ( $\alpha_d$ ) are estimated. If we assume that  $\alpha_m$  and  $\alpha_d$  are constant within the time-course of the experiment, then the observed isotopic composition of both Hg(II) and MeHg at a given time will be dependent on their respective concentrations. Thus, Hg species isotopic composition is greatly influenced by the rate of the simultaneous methylation and demethylation, as shown by the following equation that describes Hg(II) isotopic composition at time  $t$ :

$$\begin{aligned} \delta^{202}\text{Hg}(\text{II})_t = & \left( \delta^{202}\text{Hg}(\text{II})_{t-dt} * [\text{Hg}(\text{II})]_{t-dt} \right. \\ & + \left( [\text{MeHg}]_{t-dt} * k_d * \left( \delta^{202}\text{MeHg}_{t-dt} - \alpha_d \right) \right. \\ & \left. \left. - [\text{Hg}(\text{II})]_{t-dt} * k_m * \left( \delta^{202}\text{Hg}(\text{II})_{t-dt} - \alpha_m \right) \right) * dt \right) / [\text{Hg}(\text{II})]_t \end{aligned} \quad (8)$$

where  $\delta^{202}\text{Hg}(\text{II})$  and  $\delta^{202}\text{MeHg}$  at  $t_0$  is 0 (NIST3133).

According to the relative uncertainties on the rate constants ( $k_m$  and  $k_d$ ), Fig. 3a and 3b show how the double Rayleigh distillation model, giving evolution of Hg species isotopic composition as a function of time, is dependent on these rate constants. Fixed alpha values



**Fig. 3.** Experimentally measured and theoretically modeled  $\delta^{202}\text{Hg}$  versus time for both Hg(II) and MeHg during simultaneous methylation and demethylation under (a) dark and (b) visible light conditions. Modeled lines agree with a double Rayleigh distillation system (one for each reaction). Solid lines represent the evolution of Hg(II) and MeHg when mean of rate constants ( $k_m$  and  $k_d$ ) are used to build up the model. Dashed lines represent the evolution of species  $\delta^{202}\text{Hg}$  when lower ( $-SD$ ) or higher ( $+SD$ ) values of  $k_m$  and  $k_d$  are used to build up the model. Constant fractionation factors are defined: (a)  $\alpha_m = 1.0040$  and  $\alpha_d = 1.0040$ ; (b)  $\alpha_m = 1.0090$  and  $\alpha_d = 1.0070$ .

( $\alpha_m$  and  $\alpha_d$  equal to 1.0040 and 1.0040 under dark conditions, 1.0090 and 1.0070 under visible light conditions) have been chosen to display this phenomena. Under both conditions, our experimental data fit reasonably well with the double Rayleigh model, although it can be easily seen that a little change on  $k$  values affects dramatically the evolution of Hg(II) and MeHg isotopic compositions. An important finding is that, according to the model and our experimental data, fractionation factors of both methylation and demethylation reactions are significantly higher under visible light conditions than in the dark. Also, methylation and demethylation seem to have comparable fractionation factors in the dark, while under visible light conditions methylation have higher fractionation factor than demethylation.

We conclude that, although our experimental conditions may not represent natural conditions, competitive transformations in aquatic environments (such as methylation/demethylation and/or oxidation/reduction) and their associated reaction rates will greatly affect Hg isotope composition. Thus, local water chemistry and Hg species concentrations would both contribute to generate specific Hg stable isotope fractionation. Additionally, equilibrium exchange isotope effect in aquatic media (i.e. Hg-chloro species equilibration in solution for this study) may also contribute to Hg isotope fractionation (Wiederhold et al., 2010; Jiskra et al., 2012).

## 4. Conclusions

### 4.1. Importance of chloride and visible light for both yield and isotope fractionation of methylation and demethylation pathways

The presence of chloride levels (0.5 M) equivalent to seawater content has been already demonstrated to influence the methylation of Hg by methylating organisms such as sulphate reducing bacteria (Compeau and Bartha, 1984; Compeau and Bartha, 1987), as Hg-chloride complexes, similar to Hg-sulfide complexes, control the bio-availability and uptake of Hg by bacteria (Benoit et al., 1999; Ullrich et al., 2001). The role of chloride for abiotic methylation of Hg by MeCo has also been previously reported (Celo et al., 2006; Chen et al., 2007; Musante, 2008), despite of the fact that authors disagree with the ability of MeCo to methylate Hg at higher chloride levels. On the other hand, previous studies suggested that MeHg binding ligands (organic compounds) had significant influence on both rate (Zhang and Hsu-Kim, 2010) and Hg isotope fractionation (Bergquist and Blum, 2007) during photodemethylation.

We demonstrated that, though the presence of high chloride level reduces the yield of net Hg abiotic methylation by MeCo (because of higher demethylation extent), this pathway should not be neglected in a global budget of MeHg production/degradation in aquatic ecosystems, particularly in marine environments. At the same time, Hg chloro-complexes have a strong influence on the isotope fractionation of Hg due to methylation of Hg(II) by MeCo. This increases the magnitude of the MDF which may be due to the substantial contribution of the activation energy of Hg-chloro complexes to chemical kinetic isotope fractionation. Furthermore, the visible light emission had a significant influence on demethylation reaction enhancing MeHg demethylation rate and Hg isotope fractionation. Such photo-catalyzed demethylation can be associated with the activation of MeHg-methylcobalamin complexes by visible light. We then report for the first time, within the uncertainties inherent to the analysis, that photo-induced demethylation does not necessarily involve MIF of odd Hg isotopes.

### 4.2. Abiotic methylation of Hg by MeCo as a model to predict isotope fractionation by biogenic methylation

For the abiotic methylation reaction by MeCo under dark conditions, we obtained a fractionation factor (about 1.0040) in the same

order of magnitude as those previously reported for the Hg methylation induced by two different sulphate-reducing bacteria strains in the dark under fermentative ( $1.0026 \pm 0.0004$ ) or sulphate-reducing conditions ( $1.0031 \pm 0.0011$ ) (Rodriguez-Gonzalez et al., 2009; Perrot et al., in preparation). From this observation, we suggest that the reaction step involving a methyl transfer in both biotic and abiotic methylation may produce similar extent of Hg isotope fractionation. On the other hand, recent works on biotic Hg demethylation and reduction inferred lower fractionation factors (Kritee et al., 2007; Kritee et al., 2008; Kritee et al., 2009). Nevertheless, the observed Hg isotope fractionation during transformation mediated by microorganisms may involve several and complex pathways (Kritee et al., 2009). Even if additional experimental studies are obviously required to better constrain such chemical and biochemical influences on Hg isotopic composition, our results suggest that the abiotic methylation by MeCo is a simple chemical approach to evaluate the role of the methylation reaction in the environment.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.chemgeo.2012.08.029>.

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