A Dissertation for the Degree of Doctor of Philosophy

The Mechanism of Abiotic Methylation and Demethylation of Mercury in Aquatic Environment

수체 내 수은의 이화학적 메틸화 및 디메틸화 반응의 기작 연구

August, 2014

By

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ABSTRACT

The Mechanism of Abiotic Methylation and Demethylation of Mercury in Aquatic Environment

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Methylmercury (MeHg) is among the most widespread contaminants that pose severe health risks to humans and wildlife. In determination the levels of MeHg in aquatic environments, methylation of inorganic mercury (Hg(II)) to MeHg and demethylation of MeHg are the two most important processes in the cycling of MeHg. So, the knowledge of the efficiency of these different pathways of Hg methylation and demethylation is one of the key steps to predict MeHg concentrations in the different environmental compartments and to estimate the Hg bio-accessibility to the organisms.

However, the factors that influence the competing methylation and demethylation reactions are yet insufficiently understood and little to no attempt has been made to determine end products, especially abiotic processes. The relative importance of each reaction and the resulting net effect will probably depend on the environmental conditions. Therefore, this study investigated the possible photochemical processes and mechanism of Hg demethylation and methylation in water with simulating various environmental conditions. The main objectives of this study were (1) to investigate the influence of several environmental factors and other water constituents on photo-decomposition of MeHg (*Study 1*), (2) to understand the mechanism of MeHg demethylation process in seawater by assessing the production of dissolved gaseous mercury (DGM) generated from MeHg photo-degradation (*Study 2*), and (3) to assess the possibility of various methyl donors such as acetate, malonate, dimethylsulfoxide, and litter-derived DOM for photochemical methylation of Hg(II) in aquatic systems (*Study 3*).

For *Study 1*, photo-initiated decomposition of MeHg was investigated under UVA irradiation in the presence of natural water constituents including nitrate (NO_3^-), ferric (Fe^{3+}), and bicarbonate (HCO_3^-) ions, and DOM such as humic and fulvic acid (HA and FA). MeHg degradation followed the pseudo-first-order kinetics; the rate constant increased with increasing UVA intensity ranged from 0.3 to 3.0 mW cm^{-2}. In the presence of NO_3^-, Fe^{3+}, and FA, the decomposition rate of MeHg increased significantly due to photosensitization by reactive species such as

hydroxyl radical ($OH^{\bullet}$). However, the presence of HA and HCO_3^- ions lowered the degradation rate through a radical scavenging effect. Increasing the pH of the solution increased the degradation rate constant by enhancing the generation of $OH^{\bullet}$. Therefore, $OH^{\bullet}$ play an important role in the photo-decomposition of MeHg in water, and natural constituents in water can affect the photo-decomposition of MeHg by changing radical production and inhibition.

For *Study 2*, the photo-induced formation of dissolved gaseous mercury (DGM, Hg^0) from MeHg removal was investigated. This study examined the effect of various environmental factors (i.e., light wavelength and intensity and MeHg concentration), and primary water constituents on the abiotic photo-degradation of MeHg, especially under different salinity. Photo-degradation rates of MeHg were positively correlated with the UV light intensity, implying that the attenuation of UV radiation had a significant effect on MeHg photo-degradation in water. However, a high dissolved organic carbon (DOC) concentration and salinity inhibited MeHg photo-degradation. DGM was always produced during the photo-degradation of MeHg. Photo-degradation rates of MeHg and DGM production decreased with increasing salinity, suggesting that the presence of chloride ions inhibited MeHg photo-degradation. Therefore, this study imply that MeHg in freshwater could be more rapidly demethylated than that in seawater and MeHg flowing into the lake or river would be almost removed by photo-demethylation. However, MeHg flowing to

seawater would be hardly removed, which could have more chance for bioaccumulation in seawater.

For *Study 3*, the photochemical methylation of Hg(II) using various methyl donors such as acetate, malonate, dimethylsulfoxide (DMSO), and litter-derived dissolved organic matter (LDOM) was examined. The methylation reaction via acetate was followed the pseudo-first-order kinetics for Hg(II), and the methylation ability of acetate decreased with the solution pH increased. In the Hg(II) methylation by LDOM, LDOM leaded to the production of new MeHg under not only UV irradiation but dark condition. Especially, from the results of the production new MeHg by LDOM in the microbial free and dark condition, this work suggests the possibility that the abiotic chemical reaction such as a non-dependence upon light occurs in the natural aquatic environment. In addition, for the MeHg formation of Hg(II) by DMSO in seawater, abiotic methylation reaction appeared to be promoted via Hg-DMSO complexes, and limited when the reactant is a chloro complex (i.e., seawater) due to its inhibitory effect probably because of higher stability 0of the Hg-Cl bond. Therefore, this study emphasized the importance of possible abiotic methylation by a non-dependence upon light in aquatic systems, while the abiotic chemical reactions for methylation are mostly caused by a dependence upon light up to date.

In conclusion, this thesis achieved MeHg methylation and demethylation through photochemical reaction in aquatic systems. From the results of this thesis,

the site-specific environmental factors i.e. environmental conditions of spatial and temporal variations can be effect on the relative importance of each reaction and the resulting net effect in the aquatic environment. In other words, the reduction of MeHg accumulation possibility in aquatic food chain will be mainly affected by the enhancement of demethylation processes with increasing of UV radiation at the surface waters. Ultimately, the results of this thesis could be a significant contribution to understand the possible photochemical processes and mechanism of Hg demethylation and methylation in water and to estimate the factors that influence the competing methylation and demethylation reactions.

Keywords: methylmercury, fate and transport, photo-decomposition, dissolved organic matters, hydroxyl radical

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List of Abbreviations

CVAFS	Cold Vapor Atomic Fluorescence Spectrometer
$CH_3^{\bullet}$	Methyl radical
DGM	Dissolved Gaseous Mercury
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EtHg	Ethylmercury
FA	Fulvic acid
$Fe(OH)^{2+}$	Ferrous hydroxide ion
Fe^{3+}	Ferric ion
HA	Humic acid
HCO_3^-	Bicarbonate ion
Hg	Mercury
Hg(0)	Elemental mercury
Hg(II)	Divalent mercury
Hg_2^{2+}	Dimeric mercury ion
HMWOC	High-molecular-weight Organic Compound
LMWOC	Low-molecular-weight Organic Compound
LOI	Loss On Ignition
MeHg	Methylmercury
NO_2^-	Nitrile ion
NO_3^-	Nitrate ion
NOM	Natural Organic Matter

$^1O_2$	Singlet oxygen
OC	Organic Carbon
$OH^{\bullet}$	Hydroxyl radical
OM	Organic Matter
$OOCH_3^{\bullet}$	Peroxomethyl radical
r^2	Determination coefficient
$RO_2^{\bullet}$	Organic peroxy radical
ROS	Reactive Oxygen Species
SRB	Sulfate-reducing Bacteria
SRHA	Standard Suwanee River humic acid
SRFA	Standard Suwanee River Fulvic Acid
SRM	Standard Reference Matter
THg	Total mercury
US EPA	United States Environmental Protection Agency
UV	Ultra Violet

Chapter 1. Introduction

1.1 Backgrounds

The amount of mercury in the environment is much higher than the global background level as a result of the anthropogenic activities during the 20th century (Eckley and Hintelmann, 2006). Mercury has been used in several industrial or agricultural applications for ages and mercury species are stable and persistent in the natural systems. In this sense, several harmful situations to the environment and human health have been associated to mercury and its compounds. Methylmercury (MeHg) poisoning in Minamata (Japan), the organic mercury poisoning in Iraq, the MeHg exposure in the Amazon (Brasil) and the elemental mercury spill in Catamarca (Peru), are examples of real situations that involved mercury species (Gochfeld, 2003).

Mercury properties are well known and have been reported in numerous works (Segade et al., 2010 and references cited therein). Mercury can be found in three oxidation states: 0 (elemental), 1+ (mercurous), and 2+ (mercuric) (Segade et al., 2010 and references cited therein), such as in Hg^0, Hg_2^{2+}, and Hg^{2+}; however, the last form is the most common in aquatic environments. This mercury form has strong tendency to form extremely stable coordination complexes and organometallic

compounds (Segade et al., 2010 and references cited therein). Several complexes might be formed between mercury and different ligands. These can be sulfur, namely thiol groups and sulfides, nitrogen (e.g. R-NH_2), phosphorous or carbon (Segade et al., 2010 and references cited therein). In relation to oxygen ligands, mercury has low affinity to them (Segade et al., 2010 and references cited therein).

Due to mercury affinity to ligands containing sulfur, low molecular weight thiols, (i.e., sulfhydryl containing molecules) such as cysteine, are emerging as important factors in the transport and distribution of mercury throughout the body (Rooney, 2007) due to the phenomenon of "Molecular Mimicry" (Bridges and Zalups, 2005), whereby the bonding of metal ions to nucleophilic groups on certain biomolecules results in the formation of organo-metal complexes that can behave or serve as a structural and/or functional homolog of other endogenous biomolecules or of the molecule to which the metal ion has bonded. When observed with mercury, this phenomenon might cause significant injuries.

1.2 Organomercury Compounds

Organomercury compounds are the most toxic mercury species, not belonging to this group of compounds the mercury complexes formed with organic matter originally present in the aquatic systems. The organomercury compounds can be divided into two groups: one in which mercury atom is linked to an organic radical (RHgX), and another group to that mercury is linked to two organic radicals (R_2Hg) (Segade et al., 2010 and references cited therein). The compounds that belong to the first group are soluble in water, dissociating in the $R\text{-}Hg^+$ cation and X^- anion, being the most common the Cl^-, OH^-, NO_3^- and SO_4^{2-} anions (Segade et al., 2010 and references cited therein). Depending on anion nature, the compounds obtained will have different properties. Poorly coordinating anions, such as ClO_4^-, NO_3^-, PF_6^- and BF_4^- anions confer an ionic character to RHg^+X^- salt and are correspondingly more hydrophilic, while Cl^-, Br^- and I^- anions confer on a linear covalent character (C-Hg-X), being these MeHg halides among the more lipophilic MeHg species (Segade et al., 2010 and references cited therein). The second group includes compounds such as dimethylmercury and diphenylmercury (Segade et al., 2010 and references cited therein). These compounds are volatile, non polar and have low solubility in water, not being affected by air, and weak acids and bases (Segade et al., 2010 and references cited therein). These properties might be due to their covalent bonds. Both groups of organomercury compounds (RHgX and R_2Hg) are broad-spectrum

biocidal agents acting via diverse mechanisms in biological systems. Organomercurials are supposed to induce membrane associated oxidative stress in living organisms through different mechanisms, including the enhancement of the lipid peroxidation and intracellular generation of reactive oxygen species (ROS) (Milaeva, 2006).

MeHg is the most common organomercury compound found in aquatic environments. It is also one of the most hazardous mercury species, due to its high stability in combination with its lipid solubility, leading to a high ability to penetrate membranes in living organisms (Segade et al., 2010 and references cited therein). MeHg is of particular public health concern due to its bio-accumulation and bio-magnification within the aquatic food web (Orihel et al., 2007; Coelho et al., 2008). In terms of the bio-magnification factor that corresponds to the concentration increase for each trophic transfer it is about two- to five-fold for various aquatic ecosystems and to all typical trophic levels, being an order of magnitude higher than the one for inorganic mercury (Meili, 1997).

Although most of mercury emitted to the environment is in inorganic form, nowadays it is well-known that inorganic mercury can be naturally methylated in the environmental ecosystems being it transformed into MeHg. Since 60-70's, several methylation mechanisms are known. In the aquatic environment, MeHg can be formed by two general pathways: chemical methylation (abiotic processes) and microbial metabolism (biotic processes) (Celo et al., 2006). On the other hand,

MeHg can be decomposed abiotically, as for example, by light, or biotically, by various free-living demethylating microorganisms (Segade et al., 2010 and references cited therein). As both processes might occur simultaneously, MeHg presence in the aquatic environments depends on the existing balance of methylation versus demethylation (Fig. 1.1).

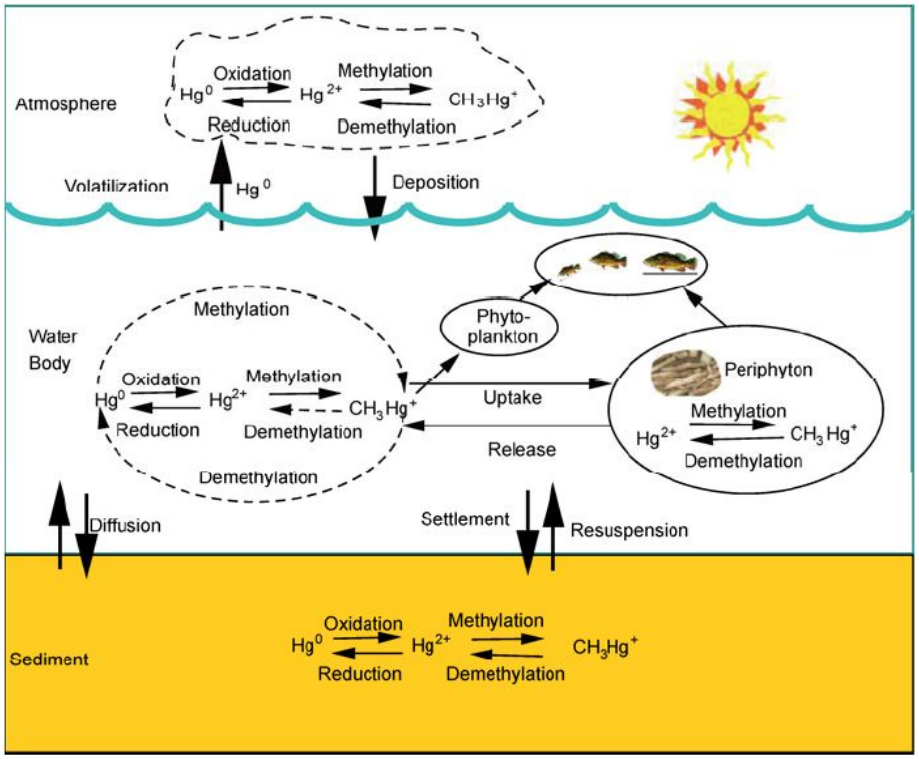


Fig. 1.1. Cycling of mercury in aquatic environment (Li and Cai, 2012).

1.3 Mercury methylation and demethylation in aquatic environments

The knowledge of the efficiency of the different pathways of mercury methylation and demethylation is one of the key steps to predict MeHg concentrations in the different environmental compartments and to estimate the mercury bio-accessibility to the organisms. However, the factors that influence the competing methylation and demethylation reactions are yet insufficiently understood and little to no attempt has been made to determine end products. The relative importance of each reaction and the resulting net effect will probably depend on the environmental conditions and biological factors with spatial and temporal variations (Hintelmann et al., 2000). In this sense, it is important to consider that the net amount of biologically available MeHg is a function of the processes that regulate its formation, degradation and exchanges between compartments. So, methylation and demethylation are two important processes regulating the mercury cycle in natural environments (Monperrus et al., 2007a) and they can be driven by both biotic and abiotic mechanisms.

The biogeochemical cycle of mercury has been extensively studied whereas the mechanism of natural mercury methylation in the environment is not still clear. If MeHg production, for example, is the most significant process that is occurring in the aquatic environment, hazardous effects on living organisms may occur due to

MeHg presence and its related high toxicity. Microbial methylation (biotic processes) is widely accepted as the main conversion mechanism of inorganic mercury into MeHg in natural environment (Barkay et al., 2003; Eckley and Hintelmann, 2006; Monperrus et al., 2007a; 2007b; Raposo et al., 2008). Nevertheless, the relative importance of mercury chemical methylation (abiotic processes) is ambiguous. Some authors emphasize that the abiotic pathway is possible in natural environments but it appears to play a minor role (Ullrich et al., 2001; Gårdfeldt et al., 2003; Eckley and Hintelmann, 2006; Dominique et al., 2007; Monperrus et al., 2007a), especially photochemical methylation (Dominique et al., 2007). On the other hand, other authors suggest that the biotic processes can't account for all the MeHg formed naturally (Celo et al., 2006).

If demethylation of MeHg is occurring in a significant extent, this is advantageous; however, in some situations the substrate of mercury methylation might be formed, inducing in this way the MeHg formation. On the other hand, the demethylation process that occurs in the aquatic environments depends also on abiotic and biotic factors. Generally, the existent relationships are quite complex and variable.

1.3.1 Mercury methylation processes

Chemical methylation – Abiotic processes

In the case of the abiotic pathway, mercury methylation is possible only in the presence of a suitable methyl donor (Ullrich et al., 2001; Celo et al., 2006). Moreover, this process may be photochemically induced. The latter reaction mechanism is likely little relevant, since the methyl radicals produced photochemically will be rapidly scavenged by oxygen (Gårdfeldt et al., 2003). Potential methylating agents for abiotic methylmercury formation in natural environments include small organic molecules, such as methyliodide and dimethylsulfide (Celo et al., 2006), and larger organic components of dissolved organic matter, such as fulvic and humic acids (Ullrich et al., 2001; Celo et al., 2006). Transmethylation reactions involving organometallic complexes like methylcobalamin, methyllead or methyltin compounds have also been considered as possible pathways for chemical mercury methylation. Transmethylation reactions can occur as a result of the transference of carbocationic Me^{+}, carbanionic Me^{-} or radical $Me^{\bullet}$, depending on the chemical properties of the metal component of the methylating agent (Celo et al., 2006). Therefore, a large variety of chemical variables may influence the methylation process (Celo et al., 2006).

Methylcobalt (III) compounds like methylcobalamin are considered potential mercury methylators because their ability for the transference of a methyl group to

free Hg^{2+}. Although some authors propose a reaction mechanism based on the enzymatic transference of methyl radicals from methylcobalamin to Hg^{2+} via sulfate-reducing bacteria (SRB) (Barkay et al., 2003), others suggest that the reaction takes place in the absence of biological activity (Celo et al., 2006; Chen et al., 2007). The reaction products of methylcobalamin and Hg^{2+} are MeHg and dimethylmercury. The first specie to be formed is MeHg, the first methylation rate being two times faster than the second one. Chen et al. (2007) also studied inorganic Hg methylation by methylcobalamin in aquatic systems and identified MeHg as the reaction product. Celo et al. (2006) refer that the most favorable environmental conditions to Hg methylation by methylcobalamin are acidic pH, high ionic strength and low chloride concentration that are more usually present in fresh waters. Furthermore, they found that methylcobalamin is unlikely to methlylate in moderate or highly saline environments because it is apparently unreactive towards chloride complexes of Hg^{2+} (Celo et al., 2006). Nevertheless, there are controversies and other authors have also reported that the inorganic Hg methylation by methylcobalamin is possible even in highly saline solutions, which emphasizes its importance in aquatic environments (Chen et al., 2007).

Organotin compounds, particularly methyltin species, are suitable methyl donors and their role in abiotic Hg methylation has been evidenced in the aquatic environment (Rosenkranz et al., 1997). Furthermore, methyltin compounds have been frequently detected in all the environmental compartments of the aquatic

system. The favorable conditions for the transmethylation reaction among methyltin compounds and Hg^{2+} include alkaline pH and the presence of high amounts of chloride (Celo et al., 2006). Therefore, the greater contribution of this methylation mechanism occurs in seawaters than in freshwaters. Celo et al. (2006) estimate that the Hg methylation rate is 0.5 pg/L/day for typical environmental concentrations of monomethyltin (~ 1,200 ng Sn/L) and Hg^{2+} (~ 1 ng/L) under pH and temperature values appropriate for seawaters (8 and 20 ºC, respectively). Evidence that methyllead compounds may also methylate Hg exists (Rosenkranz et al., 1997), being MeHg produced by transmethylation.

Humic matter contains different kinds of functional groups and, besides the linkage of oxidized mercury to thiol groups, it has most likely an additional complexation to neighboring carboxylic groups. Taking into account that organic acids with methyl groups in the α-position show high methylation efficiency for mercury (Falter, 1999a,b), humic matter is the most promising environmental methylating agent as consequence of its high concentration in waters and sediments and of its association with the solubility and thus mobility of Hg in freshwaters and marine waters. In terms of fulvic acids, all of them are able to methylate inorganic mercury but the lower molecular weight compounds (M.W. 200) are the most active ones.

Biotic processes

Several microorganisms are able to methylate mercury, such as some sulfate-reducing bacteria (SRB) (Kerry et al., 1991; King et al., 2001; Ekstrom and Morel, 2008; Jeremiason et al., 2006), iron-reducing bacteria (Fleming et al., 2006), sulfide and sulfur-oxidizers (Rodríguez Martín-Doimeadios et al., 2004), among others (Rodríguez Martín-Doimeadios et al., 2004). Nevertheless, the first have been identified as the dominant methylators in the aquatic environments. Some studies involving Hg methylators have been done in order to get insight on the pathway of carbon and on the nature of methyl donors used. Several pathways have been proposed but one of the most studied and well understood is the referred to the *Desulfovibrio desulfuricans* (Berman et al., 1990; Choi et al., 1994a). The most likely source of the methyl group seems to be the C-3 of serine. This compound is the principal methyl donor to tetrahydrofolate and is formed during the carbon flow from the pyruvate. The proposed pathway is represented in Figure 1.2 (Berman et al., 1990; Choi et al., 1994a; Segade et al., 2010).

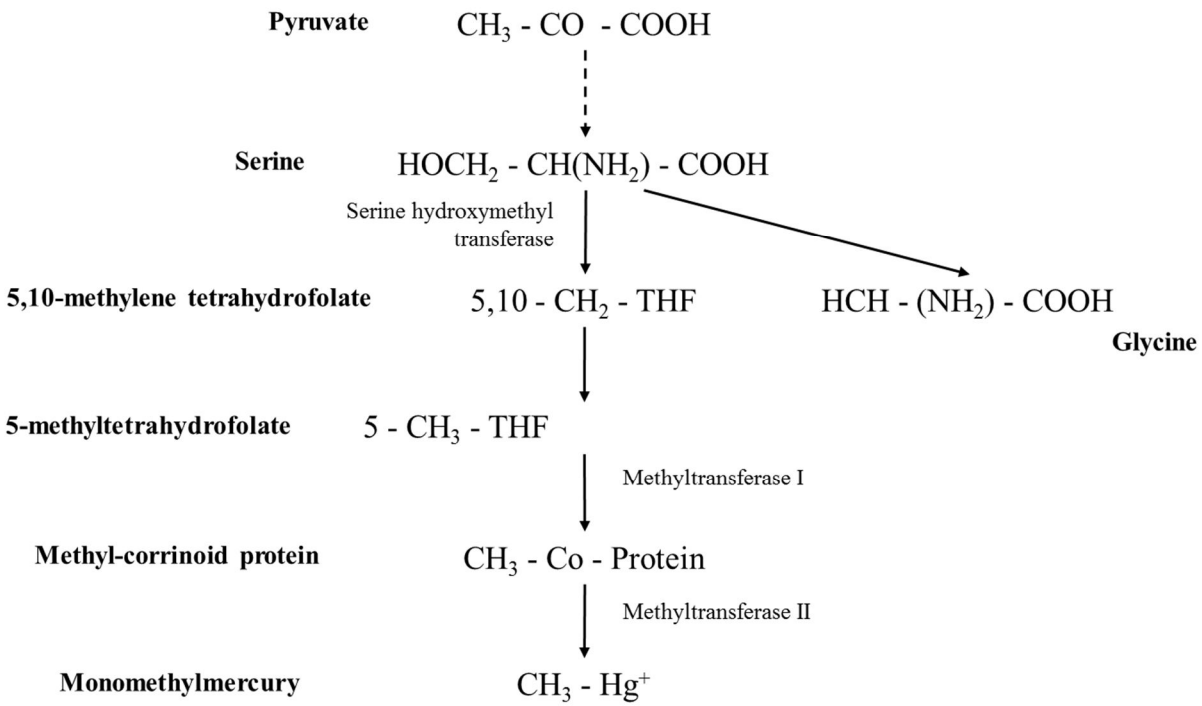


Fig. 1.2. Proposed pathway for methylation of mercury in *Desulfovibrio desulfuricans* (Segade et al., 2010).

In biological systems, beyond methylcorrinoid derivatives (such as, methylcobalamin), there are two possible other microbial methylating agents: S-adenosylmethionine (SAM) and 5-methyltetrahydrofolate (5-MTHF). Nevertheless, Gadd (1993) refers that the major methylating agent involved in Hg methylation is the methylcobalamin. Due to the important role of the enzymes mentioned before, sometimes it might be difficult to differentiate between biotic and abiotic methylation because it has been suggested that the formation of abiotic MeHg may result from dead communities of bacteria that can continue to methylate Hg by releasing enzymes (Eckley and Hintelmann, 2006). Thus, these enzymes seem to have potential to promote extracellular methylation (Eckley and Hintelmann, 2006). Regarding culture conditions, these also play an important role on MeHg synthesis. It has been reported, for example, that *Desulfovibrio desulfuricans* produced more MeHg under fermentative than under sulfate-reducing conditions (Choi and Bartha, 1993).

1.3.2 Mercury demethylation processes

Chemical demethylation – Abiotic processes

The photolytic decomposition of MeHg remains the only abiotic demethylation mechanism that is significant in surface waters exposed to sunlight (Sellers et al., 1996; Gårdfeldt et al., 2001; Chen et al., 2003; Hammerschmidt and Fitzgerald, 2006; Monperrus et al., 2007a). However, the overall impact on the aquatic Hg cycle is still unclear and the end products of the MeHg degradation have not been clearly identified yet. Hammerschmidt and Fitzgerald (2006) demonstrate that the MeHg decomposition in surface waters is an exclusively abiotic and sunlight-induced process. Monperrus et al. (2007b) estimate demethylation rates of MeHg in coastal and marine waters (6.4 to 24.5 % day^{-1}) and suggest that an important part of the demethylation is mostly driven by sunlight because those rates decrease severely under dark conditions. Monperrus et al. (2007b) and Whalin et al. (2007) refer that, in coastal and marine surface waters, although MeHg is mainly photo-chemically degraded, the demethylation yields observed under dark conditions may be attributed to microbial mediated pathways. Furthermore, higher demethylation potentials are predicted in marine surface waters in comparison with the water masses located deeper in the euphotic zone as the MeHg degradation is inhibited under dark conditions. In sediments, the abiotic mechanism is also more conductive

to the environmental MeHg decomposition than the biotic one (Rodríguez Martín-Doimeadios et al., 2004).

Hammerschmidt and Fitzgerald (2006) demonstrated that the rate of the MeHg degradation is positively correlated with the intensity of photo-synthetically active radiation (PAR) at a 0.75 to 6 m depth in the water column. Nevertheless, MeHg can be degraded more rapidly at lower depths due to the additional influence of the ultraviolet (UV) light. In this sense, other authors suggested that the MeHg photo-decomposition is largely limited to the upper 0.5 to 1 m layer of surface waters, which is consistent with the penetration of the UV light in the water column (Krabbenhoft et al., 2002). Moreover, Lehnherr and Vincent (2009) attribute the most important driver of the MeHg photo-decomposition to the UV radiation in freshwaters because wavelengths in the visible spectrum degrade MeHg at a much slower rate than the former. However, they also recognize that the visible light plays an important role in deepening waters as it is attenuated much less rapidly than the UV radiation. Therefore, the modeling of the MeHg photo-decomposition requires the mechanistic knowledge of the role of the UV radiation versus visible light, since wavelengths in both UV and visible regions of the solar spectrum are attenuated at very different rates in the water column of freshwaters.

It is important to take into account that photo-decomposition rates are comparable among several lakes with widely varying water chemistry. It suggests that the kinetics of the MeHg photo-decomposition is not influenced by

environmental factors apart from those affecting the light intensity and MeHg concentration in natural surface waters (Sellers et al., 1996; Hammerschmidt and Fitzgerald, 2006).

Since MeHg cannot absorb sunlight wavelengths at all and thus the direct photo-degradation cannot occur, the only possible mechanism is the indirect photolysis involving the photochemical formation of aqueous free radicals in sunlit natural waters. Chen et al. (2003) investigated the kinetics and mechanism of the methylmercury photo-degradation mediated by hydroxyl radicals ($OH^{\bullet}$). They used the nitrate photolysis from 285 to 800 nm as the OH radical source. The products identified were Hg^{2+}, Hg^{0}, chloroform and formaldehyde, the main aqueous product being divalent Hg. The effects of chloride concentration and MeHg speciation have also been investigated. The presence of chloride can lead to a higher MeHg degradation rate that can be attributed to the chlorine radicals produced during the aqueous oxidation of chloride by OH radicals. The chlorine radicals formed may also attack the C-Hg bond and lead to an enhanced MeHg degradation. Although the pH value does not significantly affect the degradation rate constant for reactions induced by OH radicals, a small decrease in the degradation rate is observed when the pH value increases from 5 to 8.5. It seems to be due to an increase in the relative concentration of methylmercury hydroxide (CH_3HgOH), whose degradation rate is lower than that of methylmercury chloride (CH_3HgCl). The two mechanisms proposed for the MeHg degradation by OH radicals are both the dissociation of CH_3

group ($CH_3HgCl + OH^{\bullet} \rightarrow CH_3 + HgOHCl$) and the dissociation of HgCl ($CH_3HgCl + OH^{\bullet} \rightarrow CH_3OH + HgCl$) to form HgOHCl or other divalent Hg products. So, the Hg-C bond is attacked by the electronically excited OH radicals. Based on the typical concentration of OH radicals in natural waters, the MeHg degradation rate was calculated. It ranges from 0.008 to 3.204 ng L^{-1} d^{-1} assuming a MeHg concentration of 0.9 ng L^{-1} in natural waters, except for seawaters due to their lower OH radical concentration. The MeHg photo-degradation mediated by OH radicals may be one of the most important pathways in sunlit surface waters.

Other possible mechanisms of indirect photo-degradation could involve the singlet oxygen ($^1O_2$) mediated pathway or the organic peroxy radical ($RO_2^{\bullet}$) mediated pathway. However, no laboratory data is available to assess the importance of these reactions for the MeHg decomposition.

As above mentioned, the MeHg photo-decomposition occurs via indirect photolysis and, therefore, it requires the presence of a photosensitizing species such as nitrate or dissolved organic matter (Chen et al., 2003). Several studies have shown that this reaction is enhanced in the presence of organic compounds (Sellers et al., 1996; Gårdfeldt et al., 2001). Lehnherr and Vincent (2009) showed that the contribution of the UV radiation to the MeHg degradation is greater in high dissolved organic matter waters (76 %) than in low ones (54 %) where the visible light acquires a similar role (46 %). Within UV radiation, the region A (320-400 nm) is a more important driver of the MeHg photo-decomposition than the region B

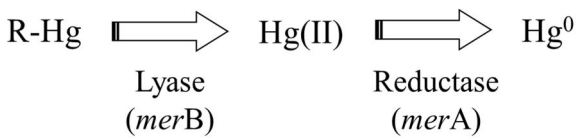
(280-320 nm). On the other hand, the photosensitization of dissolved organic matter by wavelengths in the PAR spectrum appears to be an important factor influencing the MeHg photo-decomposition in not very surface photic zones (Hammerschmidt and Fitzgerald, 2006). However, the demethylation mechanism of MeHg by PAR (400-700 nm) still remains unknown.

The chemical MeHg demethylation mediated by selenoamino acids via a bis(methylmercuric)selenide ($(CH_3Hg)_2Se$) intermediate has been suggested, which is readily degraded to mercury selenide (HgSe) and dimethylmercury ($(CH_3)_2Hg$) (Khan and Wang, 2010). The latter one is then decomposed further to MeHg. This demethylation reaction can occur in vivo. In the aquatic environment, although there has been no report on the concentrations of selenoamino acids in natural waters, their sulfur counterparts have been reported in surface and sediment pore waters. Similarly, the sulfur-aided demethylation pathway gives mercury sulfide ($HgS_{(S)}$) as ultimate reaction product.

Biotic processes

Microorganisms in contaminated environments have developed resistance to Hg and play a major role in natural decontamination. Hg resistance occurs widely on Gram negative and Gram positive bacteria, in environmental (Chatziefthimiou et al., 2007; Ramond et al., 2008), clinical (Soge et al., 2008) and industrial isolates. On contrary to Hg methylation that seems to be restricted to a subset of bacteria, Hg demethylation appears to be a process that is more widely spread. Research works on molecular biology shows that MeHg degradation performed by microorganisms generally proceeds through two distinct vias (Hines et al., 2006), oxidative and reductive, being the last one mainly linked to the mercury resistance (*mer*) operon. Both biotic pathways for MeHg degradation are encountered in the environment.

The reductive MeHg degradation might occur through two pathways, one involving the *mer* operon and other that does not; however, the former process is the most studied and considered to be the most common. When microorganisms use the reductive pathway via *mer* operon to perform MeHg degradation, two stages are involved which are catalyzed by two enzymes. The *mer*-mediated MeHg degradation pathway may be represented easily by:



In general terms, the organomercurial lyase breaks the carbon-mercury bond in toxic substrates, such as MeHg and phenylmercury, being released methane or benzene, respectively, and Hg(II), which is subsequently reduced to Hg^0 by the action of the mercuric reductase. Based on the organization of *mer* operon genes, two modes of mercury resistance are encountered in bacteria (Hines et al., 2006): narrow-spectrum resistance and broad-spectrum resistance. In the first, Hg(II) is reduced to the less toxic, inert and volatile elemental form (Hg^0), by the action of the mercuric reductase (*mer*A). On contrary, in broad-spectrum resistance, both organic and Hg(II) will be remediated due to the presence of a *mer*B gene that encodes an enzyme organomercurial lyase, beyond the presence of the mercuric reductase.

Oxidative demethylation is another demethylation pathway of MeHg found in the environment (Oremland et al., 1991; Oremland et al., 1995; Hines et al., 2006) that has been observed under aerobic and anaerobic conditions (Oremland et al., 1991; Marvin-Dipasquale and Oremland, 1998; Hines et al., 2000; Marvin-Dipasquale et al., 2000; Hines et al., 2006). Several kinds of microorganisms have been proposed to be involved in the process; however, the most common are sulfate reducers (Oremland et al., 1991; Marvin-Dipasquale and Oremland, 1998; Marvin-Dipasquale et al., 2000) and methanogens (Oremland et al., 1991; Marvin-Dipasquale and Oremland, 1998; Marvin-Dipasquale et al., 2000).

In oxidative demethylation, MeHg is converted primarily to CO_2 and inorganic Hg, on contrary to the reductive degradation pathway of *mer*-detoxification,

characterized by the nearly exclusive production of methane; however, it has been suggested that different microbial groups are capable of oxidative demethylation but with different stoichiometric end-product CO_2/CH_4 ratios and/or at different rates (Oremland et al., 1995; Marvin-Dipasquale et al., 2000). For methanogenic bacteria, for example, is expected the production of both CO_2 and CH_4 during oxidative demethylation, since these are the products of the C_1 metabolism by methanogens (Oremland et al., 1995). Moreover, some of the CO_2 formed by the demethylators can be fixed into acetate pools by acetogenic bacteria (Oremland et al., 1991).

The following reactions for the oxidative demethylation pathways used by sulfate reducers (Eq. (1-1)) and methanogens (Eq. (1-2)) have been proposed (Marvin-Dipasquale and Oremland, 1998), respectively:

$$SO_4^{2-} + CH_3Hg^+ + 3H^+ \rightarrow H_2S + CO_2 + Hg^{2+} + 2H_2O \qquad (1\text{-}1)$$

$$4CH_3Hg^+ + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4Hg^{2+} + 4H_2 \qquad (1\text{-}2)$$

Considering Eq. (1-2), the oxidative metabolism of methylmercury during methanogenis will yield methane and carbon dioxide at a ratio of 3:1 (Oremland et al., 1995), while CO_2 will be the only product formed under conditions of sulfate reduction or of respiration of other anaerobic electron acceptors (Oremland et al., 1995). Nevertheless, the knowledge about oxidative demethylation is limited and it

is not certain in what form and by what mechanism MeHg is taken up (Drott et al., 2008).

1.4 Objectives

Mercury (Hg) and its compounds are a class of highly toxic and pervasive pollutants. During the biogeochemical cycling of Hg, methylmercury (MeHg), a potent neurotoxin, can be produced and subsequently bio-accumulated along the food chain in aquatic ecosystems. MeHg is among the most widespread contaminants that pose severe health risks to humans and wildlife.

In most aquatic ecosystems, in situ production (methylation of inorganic Hg to MeHg), rather than input from runoff water or atmospheric deposition, is the major source of MeHg. In addition to methylation of inorganic Hg, the reverse process, demethylation of MeHg, simultaneously occurs in the environment. Both processes are important for the cycling of MeHg, determining the levels of MeHg in aquatic environments. In this sense, it is important to consider that the net amount of biologically available MeHg is a function of the processes that regulate its formation, degradation and exchanges between compartments.

Methylation and demethylation can be driven by both biotic and abiotic mechanisms. The biogeochemical cycle of Hg has been extensively studied whereas the mechanism of natural Hg methylation and demethylation in the environment is not still clear, especially photochemical processes. Thus, the main objective of this study was to investigate the possible photochemical process and mechanism of Hg

methylation and demethylation in aquatic environment. Following are the specific research objectives:

(1) To investigate the influence of several environmental factors including light intensity, natural photosensitizers (i.e., nitrate and ferric ions), and other water constituents such as dissolved organic matters and bicarbonate ion on photo-decomposition of MeHg
(2) To investigate the production of dissolved gaseous mercury (DGM) from MeHg photo-degradation at various salinity in order to understand the mechanism of MeHg demethylation process in water flow from freshwater to seawater
(3) To assess the possibility of different methyl donors such as acetate, malonate, dimethylsulfoxide, and litter-derived DOM for photochemical methylation of Hg(II) under various conditions

This dissertation is divided into five chapters (Fig. 1.3). Chapter 1 mainly describes a background information and literature review. Chapter 2 focuses on the effect of natural water constituents on the photo-decomposition of MeHg and the role of hydroxyl radical, suggesting possible abiotic mechanisms for demethylation. Chapter 3 focuses on the abiotic photo-degradation of MeHg at different salinity, determining the kinetics of the photo-demethylation of MeHg in water flow from

freshwater to seawater. Chapter 4 presents the methylation of Hg(II) by various dissolved organic matters, showing the possible photochemical methylation process and mechanism of Hg in aquatic environments. Lastly, Chapter 5 presents the conclusions and significance of this study.

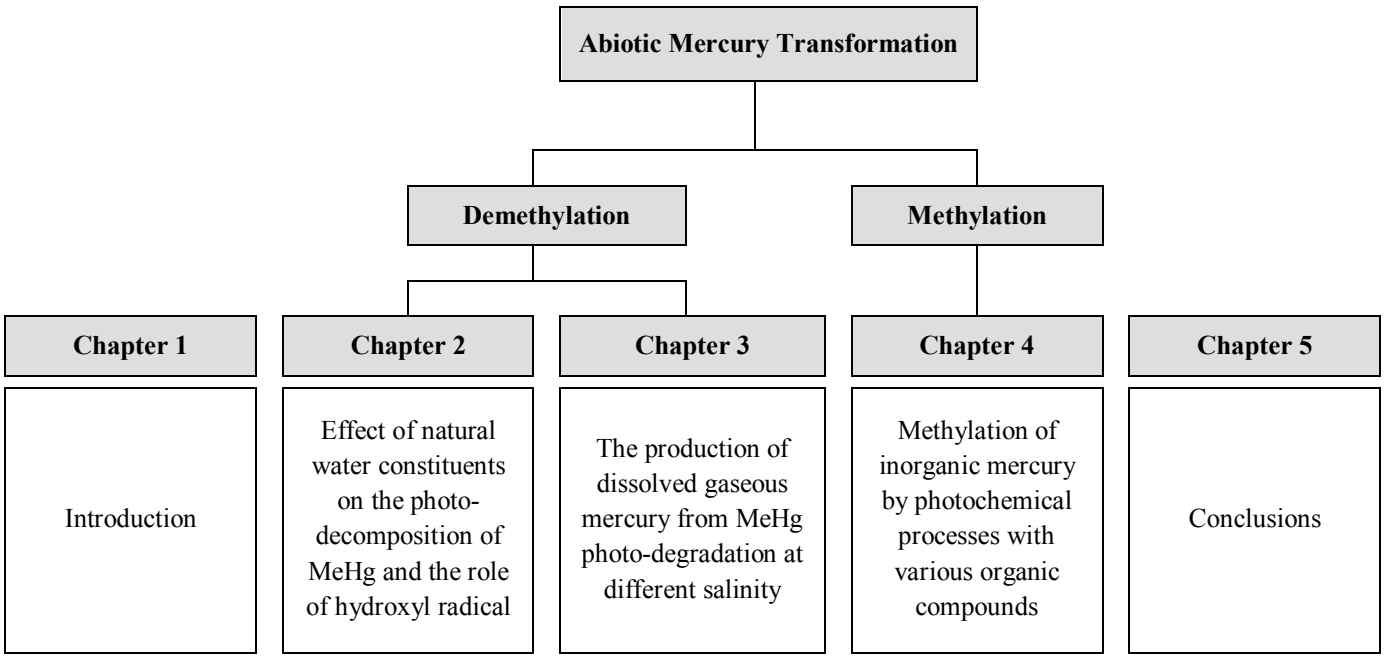


Fig. 1.3. Schematic diagram of the overall composition in the dissertation.

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Chapter 2. Effect of Natural Water Constituents on the Photo-decomposition of Methylmercury and the Role of Hydroxyl Radical

2.1 Introduction

Methylmercury (MeHg) is a highly toxic form of mercury (Hg) that is bioaccumulated in aquatic food chains (Wiener et al., 2003). Accumulation in fish is of primary concern because fish consumption is the main route for human exposure to Hg (Renzoni et al., 1998; Björnberg et al., 2005). The concentration of MeHg in natural waters is influenced by biotic (i.e., biological processes by bacterial activity) and abiotic factors (i.e., photochemical processes by solar radiation or photosensitive materials) (Sellers et al., 1996; Schaefer et al., 2004; Hammerschmidt and Fitzgerald, 2006; Monperrus et al., 2007; Lehnherr and St. Louis, 2009).

Although MeHg decomposition can be microbially mediated in water (Schaefer et al., 2004; Monperrus et al., 2007) and sediment (Marvin-Dipasquale et al., 1998; 2000), photo-induced abiotic decomposition of MeHg in the water column is known as an another important pathway to reduce MeHg levels in the aquatic food chain (Sellers et al., 1996; Hammerschmidt and Fitzgerald, 2006; Lehnherr and St. Louis,

2009). In fact, Sellers et al. (1996) suggested that abiotic photo-decomposition of MeHg is a more important process in lake waters than biological demethylation. Hammerschmidt and Fitzgerald (2006) demonstrated that MeHg decomposition was abiotically mediated by solar radiation in surface water of Toolik Lake, Alaska. They estimated that yearly photo-decomposition of MeHg in the lake accounted for approximately 80% of MeHg annually redistributed from in-situ sediment.

Previous work has shown that aqueous MeHg can be decomposed directly by photolysis with sunlight (> 280 nm) and UV radiation (< 400 nm) (Inoko, 1981; Suda et al., 1993), and indirectly by reactions with reactive oxygen species (ROS) (e.g., hydroxyl (OH) radicals) and singlet oxygen ($^1O_2$) (Zepp et al., 1987; Suda et al., 1991; Suda and Takahashi, 1992; Suda et al., 1993; Chen et al., 2003; Hammerschmidt and Fitzgerald, 2010; Zhang and Hsu-Kim, 2010). ROS such as the OH radical play an important role in the photo-transformation of organic pollutants due their high oxidizing potential (Chowdhury et al., 2011).

Natural constituents present in surface waters such as nitrate (NO_3^-), ferric iron (Fe^{3+}), and bicarbonate (HCO_3^-) ions can participate in the photo-decomposition of MeHg. NO_3^- ions are known to produce OH radicals in water when excited by solar UV light (Brezonik and Fulkerson-Brekken, 1998; Vione et al., 2006; Zepp, 1987). The Fe^{3+} ion has also been shown to be a photoreactive species in terms of OH radical generation with the highest quantum yield (Feng and Nansheng, 2000; Zhang et al., 2006). Fe^{3+} ion mediated photochemical decomposition of MeHg was reported

for natural waters by Hammerschmidt and Fitzgerald (2010). Carbonate (CO_3^-) and bicarbonate (HCO_3^-) ions, that are responsible for alkalinity, are the most common inorganic salts present in natural surface water. These do not absorb solar UV radiation, however, HCO_3^- is known to act as OH radical scavenger (Brezonik and Fulkerson-Brekken, 1998; Ma and Graham, 2000; Chin et al., 2004; Chowdhury et al., 2011).

Another important natural constituent in photo-initiated processes in surface water is a dissolved organic matter (DOM) (Brenzonik and Fulkerson-Brekken, 1998; Vione et al., 2006). DOM is ubiquitous in the aquatic environment, formed by abiotic and microbial transformations of plants and animal materials. Humic acid is the fraction of DOM that is not soluble at higher pH, with an average molecular weight between 2000 and 3000 Da. Fulvic acid is the fraction of DOM that is soluble at all pH values, with average molecular weight of less than 1000 Da. DOM has a high portion of oxygen-containing functional groups, such as OH and COOH groups. These functional groups absorb solar radiation between 300 and 500 nm to reach an excited state, hence generating free radicals that cause photo-oxidation of organic contaminants (Vaughan and Blough, 1998; Chin et al., 2004; Chowdhury et al., 2011; Jacobs et al., 2011).

DOM can also strongly interact with Hg, thus affecting fate, transformation, and bioavailability of Hg in water systems (Haitzer et al., 2002; Ravichandran, 2004). These compounds play a key role in the photochemical reduction of ionic

mercury (Hg(II)) to elemental mercury (Hg(0)), and in subsequent reoxidation of Hg(0) to (Hg(II)), thus affecting volatilization loss and bioavailability of Hg to organisms (Ravichandran, 2004). DOM also affects the production and bioavailability of MeHg, the most bio-accumulative Hg species in fish (Ravichandran, 2004). Specifically the fulvic acid fraction can absorb UV light to reach an excited state, resulting in generation of ROS such as OH and $^1O_2$ radicals and triplet DOM (^{3}DOM), but primarily OH radicals (Miller and Chin, 2002; Chowdhury et al., 2011; Jacob et al., 2011). In contrast, Brezonik and Fulkerson-Brekken (1998) reported the quenching effect of DOM expressed as the dissolved organic carbon (DOC) on aquatic contaminants. Lam et al. (2003) reported that colored DOM (mainly humic acid) acted as a radiation filter and played an important role in scavenging radicals.

Although photo-decomposition of MeHg is an important process in water, the detailed mechanisms of MeHg abiotic demethylation are not clear because natural surface waters have different chemistry. This work hypothesized if the OH radical is an important factor controlling the photo-decomposition of MeHg in water then photosensitive materials such as DOM, NO_3^-, and Fe^{3+} ion would significantly affect photo-decomposition of MeHg. To test this hypothesis, MeHg photo-decomposition was investigated using a well-characterized laboratory solution irradiated using UVA light. The role of these photosensitive materials and the OH radical on the photo-decomposition of MeHg were examined.

2.2 Materials and Methods

2.2.1 Reagents and sample preparation

To examine the effect of parameters on MeHg photo-decomposition, various environmental factors were evaluated such as light intensity, pH, DOM (i.e., humic acid and fulvic acid), and the presence of NO_3^-, Fe^{3+}, and HCO_3^- ions.

Fe^{3+} stock solution was reagent grade $Fe_2(SO_4)_3$ (Sigma-Aldrich, St. Louis, MO). NO_3^- stock solution was prepared with reagent grade KNO_3 (Kanto Chemical Co., Tokyo, Japan). HCO_3^- stock solution was prepared by dissolving $NaHCO_3$ in water (reagent grade; Sigma-Aldrich).

Standard Suwanee River fulvic (SRFA) and humic acid (SRHA) (International Humic Substances Society, St. Paul, MN) were used to examine the roles of DOM on the photo-decomposition of MeHg. Stock solutions for fulvic and humic acids were prepared with deionized water and then filtered through a 0.45-μm nitrocellulose filter (Adventec, Tokyo, Japan).

Solutions were prepared with laboratory grade (18 MΩ) deionized water from Millipore (MILLIQ®; Billerica, MA). The standard solution of MeHg (as CH_3HgCl; Alfa Aesar, Ward Hill, MA; 1000 μg mL^{-1}) was diluted to make a MeHg stock solution (1 ng mL^{-1}), and the initial concentration of MeHg was fixed to 20 ng L^{-1} by

diluting stock solution, and kept constant throughout all experiments. For the Fe^{3+} ion experiment, Fe^{3+} ions in solution were spiked immediately after MeHg addition to minimize the reaction between Fe^{3+} and MeHg during preparation. Other than Fe^{3+} ions, the addition of chemicals such as DOM, NO_3^-, and HCO_3^- was before MeHg spiking.

2.2.2 Photo-reactor and experimental design

The schematic design of the photo-reactor is shown in Fig. 2.1. Quartz cells were located inside the box-type photo-reactor and irradiated for 90 min. The reactor was equipped with five UVA (λ=365 nm) lamps. UVA intensity was controlled by the number of lamps, and all experiments (except light intensity tests) were conducted under 0.3 mW cm^{-2} of UVA irradiation (λ=365 nm). MeHg photo-decomposition experiments with changing UV intensity were performed with five different UVA intensities of 0.3, 0.7, 1.2, 2.0, and 3.0 mW cm^{-2}, which can simulate natural sunlight conditions.

The solar spectrum has wavelengths greater than 290 nm; wavelengths above 400 nm do not have enough energy to promote a photoreaction with most chemicals (Stumm and Morgan, 1996; Chen et al., 2003). UVA intensity in natural sunlight ranges from 0.24 to 1.32 mW cm^{-2} during the summer in Korea (Park et al., 2008). Thus, a UVA lamp (λ=320 to 400 nm, maximally 365 nm, 20 W; Sanko Denki,

Tokyo, Japan) was selected to mimic actual UVA radiation of sunlight. The UVB (λ=280 to 320 nm) component of the solar spectrum may also contribute to MeHg photo-decomposition (Lehnherr and St. Louis, 2009). This UVB range was not tested in this study because these wavelengths are not as easily transmitted to the Earth's surface.

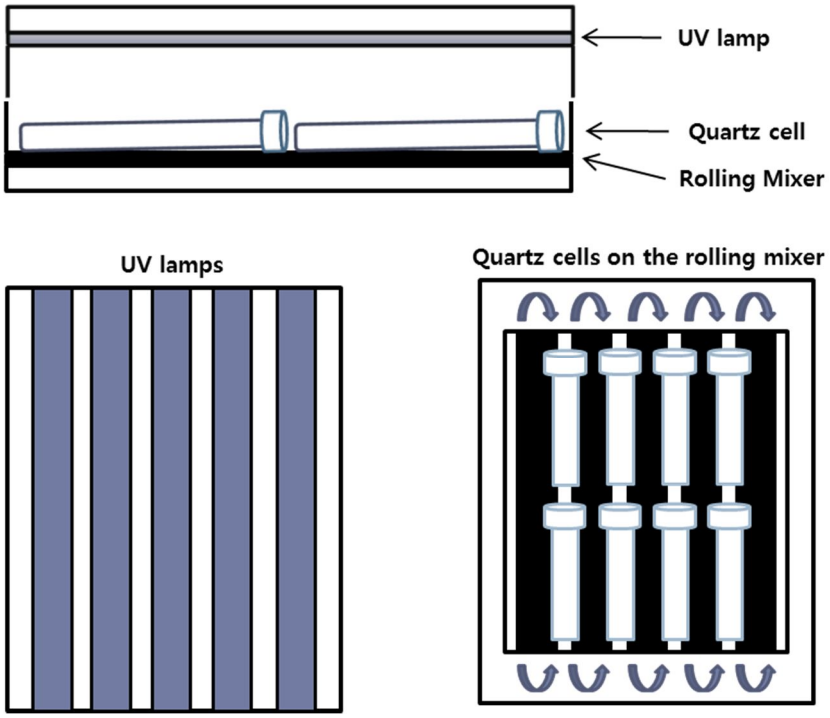

Fig. 2.1. Schematic diagram of experimental design for photo-decomposition of MeHg (the reactor size: 66 (L) × 50 (W) × 40 (H) cm).

2.2.3. Analytical methods

To determine MeHg concentrations, the analytical methods of Hammerschmidt and Fitzgerald (2006) and *EPA Method* 1630 (US EPA, 2001) were used. Preliminary experiments were conducted to examine the effect of distillation on the each solution, and no significant difference was observed in MeHg concentration for the samples analyzed both directly and after distillation (n = 16, paired t-test, p = 0.27). Accordingly, the distillation step was excluded from analytical processes of this study.

Samples were transferred to a 125-mL purging vessel, adjusted to pH 4.9 with 2 M acetate buffer, and ethylated by the addition of sodium tetraethyl borate ($NaBEt_4$) in the closed vessel. After 15 min, ethylated Hg complexes were separated from solution by purging with nitrogen gas for 15 min and trapped on Tenax. Trapped Hg complexes were carried through a gas chromatography column for separation, converted to Hg^0 via a pyrolytic column and detected using cold vapor atomic fluorescence spectrometry (CVAFS) (model III; Brooks Rand, Seattle, WA).

Analyses of MeHg in samples were performed after daily calibration with a MeHg stock solution (1 ng mL^{-1}). Recoveries of the known amount of MeHg from test waters containing DOM, added before analysis, ranged from 97% to 113% (average 105%, n = 28). The detection limit (standard deviation × 3.143) for 50-mL water samples was 0.006 ng L^{-1}. Light intensity of UVA radiation in the photo-

reactor was checked regularly before the start of every experiment using a VLX-3W radiometer (Cole-Parmer, Vernon Hills, IL) with UVA sensor at 365 nm. The concentration of DOM was analyzed by TOC-V_{CPH} (Shimadzu, Kyoto, Japan). The pH was adjusted using 1N H_2SO_4 and NaOH solutions. The solution pH of test waters was measured using 415 CP pH/ISE/conductivity meter (Istek Co., Daejeon, Korea). The absorbance spectrum of MeHg and DOM in water was measured using an Infinite® M200 (TECAN, Mãnnedorf, Switzerland).

Results of repeated experiments were expressed as the mean value ± standard deviation. This study carried out linear regression analysis using the SAS system for window version 9.1.3 (SAS Institute, Cary, NC).

2.3. Results and Discussion

2.3.1 Effect of UV light intensity

The variation in UV light intensity is an important parameter to consider when evaluating UV light-driven processes because the photon generation rate increases with light intensity. The MeHg photo-decomposition experiments with changing UV light intensities showed that MeHg concentrations declined exponentially with time (Fig. 2.2). All experiments produced linear plots of $ln(MeHg_t/MeHg_0)$ versus time, indicating that the photo-decomposition of MeHg followed the pseudo-first-order kinetics. The rate constant was calculated using Eq. (2-1):

$$ln(MeHg)_t = ln(MeHg)_0 - R_{deMeHg} \times t \qquad (2\text{-}1)$$

where $(MeHg)_t$ and $(MeHg)_0$ are MeHg concentrations at time t and 0, respectively, R_{deMeHg} is the first-order rate constant as the MeHg photo-decomposition rate (min^{-1}).

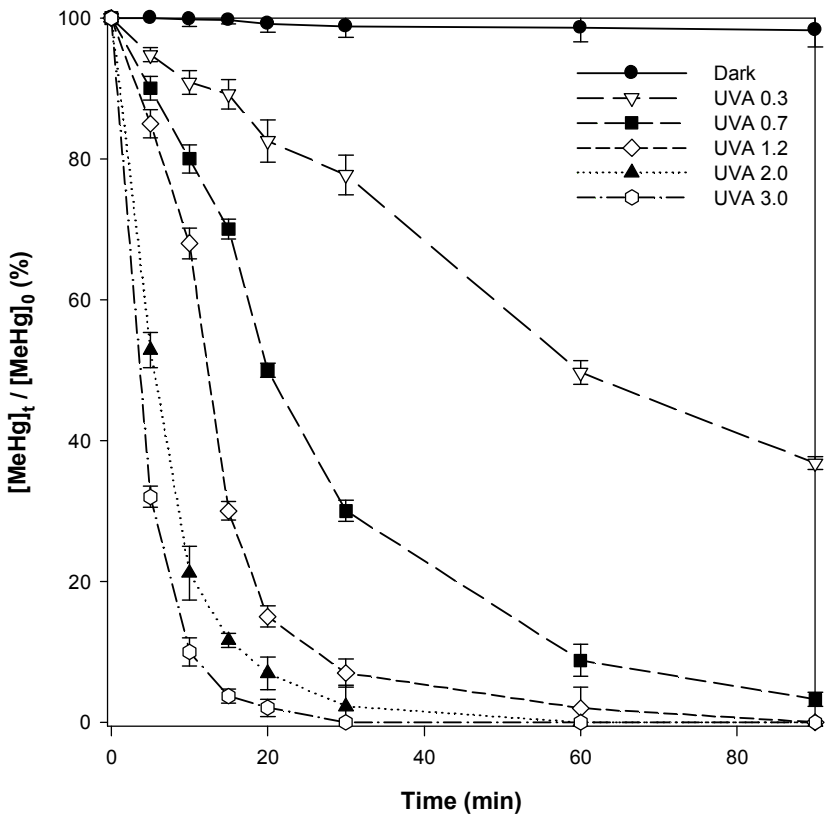

Fig. 2.2. Effect of UVA intensity on the photo-decomposition of MeHg (20 ng L^{-1}) (Error bars represent the standard deviation of triplicate experiments. Unit of UVA intensity in the legend is mW cm^{-2}.).

Increasing the UV intensity resulted in an increase of the MeHg photo-decomposition rate (R_{deMeHg}) (Table 2.1). This shows that direct decomposition of MeHg by UVA light occurred mainly, being consistent with previous work (Hammerschmidt and Fitzgerald, 2006; Lehnherr and St. Louis, 2009). According to Hammerschmidt and Fitzgerald (2006), MeHg is degraded more rapidly at the surface and this may be attributed to an additional influence of ultraviolet light (UV, 280 to 400 nm). Lehanherr and St. Louis (2009) also found that UV mediated MeHg photo-demethylation accounts for over three-quarters of the overall areal flux, with UVA radiation the single-most important contributor (58%).

A linear relationship between the MeHg photo-decomposition rates (R_{deMeHg}, min^{-1}) and UVA light intensities (I_{UVA}) was obtained, as shown in Eq. (2-2):

$$R_{deMeHg} = 0.0668 \times I_{UVA} \qquad (2\text{-}2)$$

Table 2.1. Photo-decomposition rate constants (R_{deMeHg}) and half-lives ($t_{1/2}$) as a function of UVA intensities

UVA intensity	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	r^2
0.3 mW cm^{-2}	0.011 ± 0.005	63	0.99
0.7 mW cm^{-2}	0.038 ± 0.010	18	0.99
1.2 mW cm^{-2}	0.072 ± 0.020	10	0.94
2.0 mW cm^{-2}	0.132 ± 0.008	5	0.99
3.0 mW cm^{-2}	0.208 ± 0.002	3	0.98

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

r^2 is coefficient of determination.

2.3.2 Effect of pH

Solution pH can influence the photo-degradation of organic compounds in natural aquatic environments because the protonated and deprotonated species have different absorbance for UV light wavelengths. According to Ku and Chang (1997), the photolytic rate of diazinon decreased under acidic solutions. Gal et al. (1992) also demonstrated that the photochemical degradation rate of parathion in aqueous solutions increased as the pH increased. Castrantas and Gibiliso (1990) suggested that the photochemical removal of phenol from alkaline solutions should be more efficient than that from neutral and acidic ones.

In this study, to evaluate the effect of pH on MeHg photo-decomposition, the experiments were performed at pH values ranging from 3.0 to 10.0 and the MeHg photo-decomposition rates (R_{deMeHg}) were compared. The experiments were conducted at a UVA intensity of 0.3 mW cm^{-2} and MeHg initial concentration of 20 ng L^{-1}. Higher rates of MeHg degradation occurred at higher pH values. The decomposition rate constant (R_{deMeHg}) in the alkaline pH range was significantly higher than that in the acidic pH range (Fig. 2.3). This result is explained by the fact that OH radicals are effectively produced at higher pH levels due to the effective production of hydroxide (OH^-) ion. The increased concentration of hydroxide ion at an alkaline pH enhances the generation of OH radicals through photo-oxidation, as

indicated in Eq. (2-3), which was previously reported (Legrini et al., 1993; Nriagu, 1994).

$$H_2O + OH^- + light \rightarrow OH^{\bullet} + H^+ + e^- \qquad (2\text{-}3)$$

In contrast, the change in the decomposition rate of MeHg in the acidic pH ranges (3 to 6) was small compared to the rate changes in basic pH (Fig. 2.3).

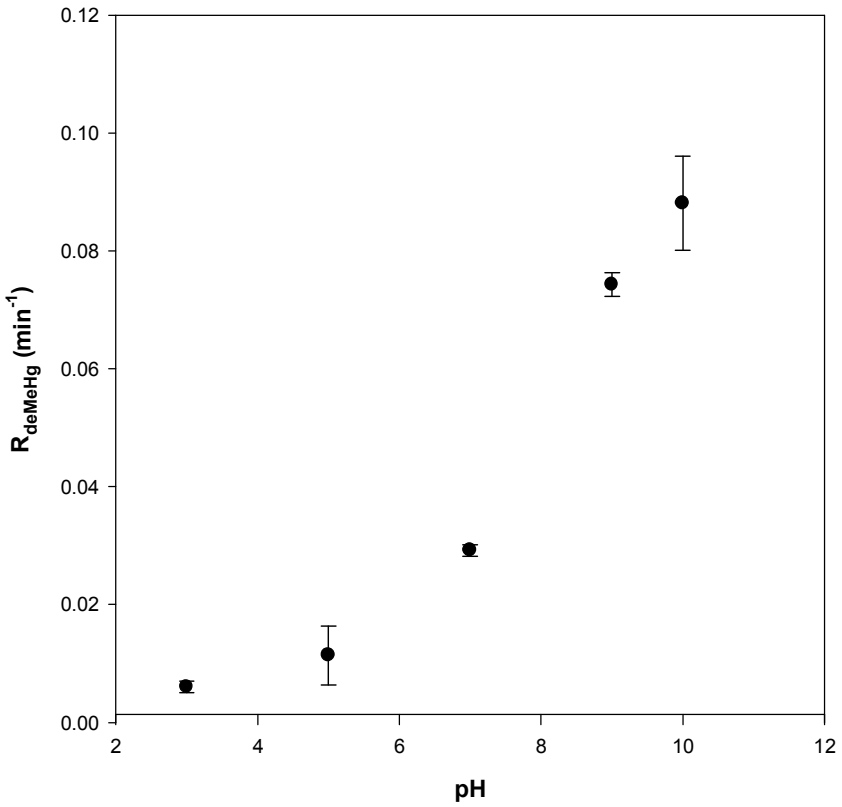

Fig. 2.3. Effect of pH on the photo-decomposition rate of MeHg (UV light intensity at 365 nm = 0.3 mW cm^{-2}, $[MeHg]_{initial}$ = 20 ng L^{-1}, Error bars represent the standard deviation of triplicate experiments).

2.3.3. Effect of Fe^{3+} ions

Dissolved iron species especially Fe^{3+} ions are often present in natural surface water at concentrations ranging from 1.2~16.8 μM depending on the geographical location (Lofts et al., 2008). Fe^{3+} ions exist different species in aqueous solution, such as Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, and dimer $Fe_2(OH)_2^{4+}$, can absorb solar irradiation ($\lambda > 290$ nm) at pH < 5, and yield OH radials and Fe^{2+} ions according to Eq. (2-4) (Mailhot et al., 1999; Feng and Nansheng, 2000):

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \qquad (2\text{-}4)$$

Among Fe^{3+}-aquo complexes, $Fe(OH)^{2+}$ is a prevalent species at pH between 2.5 and 5. This is the predominant photoreactive species in terms of OH radical generation with the highest quantum yield according to Eq. (2-5) (Feng and Nansheng, 2000; Zhang et al., 2006):

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \qquad (2\text{-}5)$$

Hence, in order to examine the effect of Fe^{3+} ion on the photo-decomposition of MeHg, the experiments were performed using Fe^{3+} ion concentrations that ranged from 0 to 50 μM. The photo-decomposition rate of MeHg increased by 2 times with

increasing Fe^{3+} concentrations from 0 to 2.5 μM, and the degradation rate increased by 7.5 times when Fe^{3+} concentrations were increased from 2.5 to 50 μM (Table 2.2). The pH in the solution decreased from 5.8 to 3.6 by increasing Fe^{3+} ion concentration from 2.5 to 50 μM, however, the change in the photo-decomposition rate of MeHg associated with this pH decrease was negligible (see Fig. 2.3) compared to the rate change by increasing Fe^{3+} ion itself (Table 2.2). This result implies that the increase in the photo-decomposition rate in the presence of Fe^{3+} ion is mainly due to the effective production of OH radicals, as shown in Eq. (2-5).

Similar results were obtained for the photo-degradation of MeHg in the presence of Fe^{3+} by the research of Hammerschmit and Fitzgerald (2010). They suggested that photochemical reduction of Fe^{3+} has an important role in affecting MeHg decomposition in natural surface water, and this may be caused by production of OH radical from the photo-Fenton reaction. Fisher et al. (2006) also observed that domoic acid in solution quickly photo-degraded while exposing to simulated sunlight in the presence of Fe^{3+}. Vermilyea and Voelker (2009) also suggested that the photo-Fenton reaction can contribute significantly to the oxidation of water-soluble organic contaminant.

Table 2.2. Effect of Fe^{3+} ion concentration (μM) on the photo-decomposition rate (R_{deMeHg}) of MeHg (UVA intensity = 0.3 mW cm^{-2})

Fe^{3+} concentration (μM)	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	r^2
0	0.011 ± 0.005	63	0.99
1	0.015 ± 0.003	460	1.00
2.5	0.022 ± 0.004	31	0.99
50	0.165 ± 0.003	4	0.95

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

r^2 is coefficient of determination.

2.3.4. Effect of NO_3^- ions

NO_3^- ions are generally present in natural surface water at various concentrations depending on the agricultural and geographic location (typical range is 10^{-5} to10^{-3} M) (Shankar et al., 2007). Experiments were performed using NO_3^- ion concentrations ranging from 0 to 0.65 mM, similar to natural surface water conditions (Chowdhury et al., 2011).

The photo-decomposition rate of MeHg in the presence NO_3^- ions followed the pseudo-first-order kinetics (Fig. 2.4). The decomposition rate (R_{deMeHg}) of MeHg increased proportionately with increasing NO_3^- concentrations (Table 2.3). The presence of NO_3^- ions did not significantly affect the solution pH. NO_3^- ions are known to produce OH radical when excited by UV light (Stumm and Morgan, 1996; Nelieu et al., 2004; Shanker et al., 2007). The mechanism of OH radical generation from NO_3^- photolysis is shown Eqs. (2-6) and (2-7). According to Eq. (2-6) and (2-7), OH radical production is proportional to NO_3^- ion concentration. Since the reaction of MeHg with OH radical is equimolar, the degradation rate of MeHg should be proportional to NO_3^- ions, as shown in Eq. (2-8).

$$NO_3^- + h\nu \rightarrow NO_3^{-\bullet} \rightarrow NO_2^- + O^{-\bullet} \quad (2\text{-}6)$$

$$O^{-\bullet} + H_2O \rightarrow OH^{\bullet} + OH^- \quad (2\text{-}7)$$

$$MeHg + OH^{\bullet} \rightarrow Demethylation \quad (2\text{-}8)$$

However increasing NO_3^- concentration by 4 times (from 0.16 to 0.65 mM) resulted in an increase of the photo-degradation rate constant by 1.66 times (from 0.013 to 0.022 min^{-1}) (Table 2.3). This result can be explained by the inhibition of OH radical by the produced nitrite (NO_2^-) ion during photolysis of NO_3^- ion. Mack and Bolton (1999) suggested that NO_2^- ion can be produced as a byproduct from NO_3^- photolysis, and this intermediate ion might be acted as an OH radical scavenger, as shown in Eq. (2-9):

$$NO_2^- + OH^{\bullet} \rightarrow NO^{\bullet} + OH^- \qquad (2\text{-}9)$$

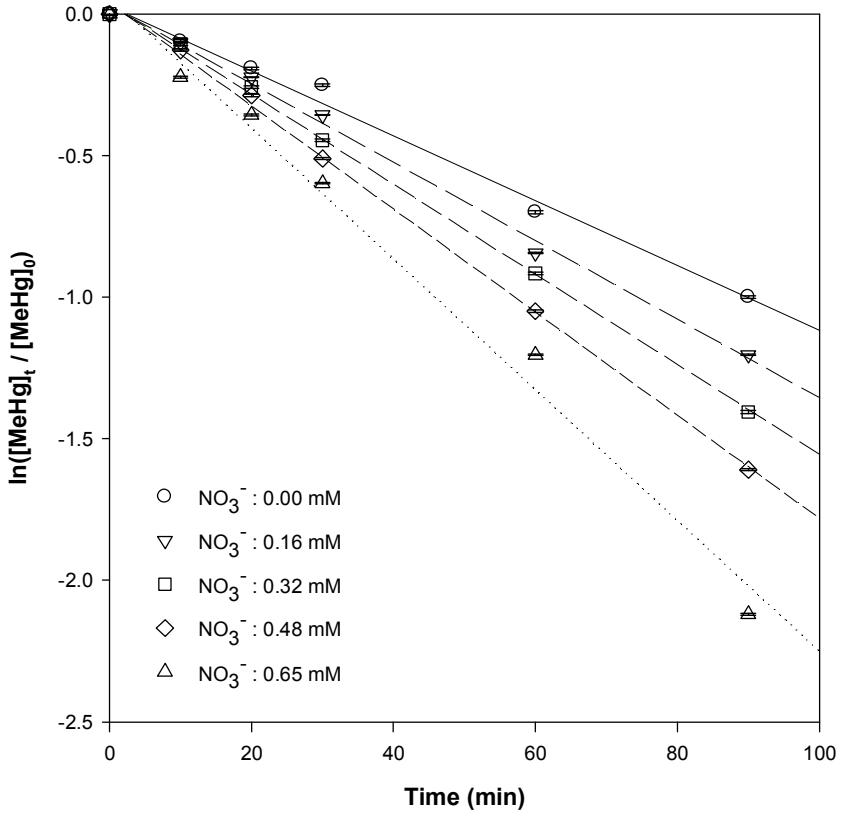

Fig. 2.4 Effect of NO_3^- concentration on the photo-decomposition rate of MeHg (UVA intensity = 0.3 mW cm^{-2}, $[MeHg]_{initial}$ = 20 ng L^{-1}, Error bars represent the standard deviation of triplicate experiments).

Table 2.3. Effect of NO_3^- ion concentration on the photo-decomposition rate (R_{deMeHg}) of MeHg (UVA intensity = 0.3 mW cm^{-2})

NO_3^- concentration (mM)	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	r^2
0	0.011 ± 0.005	63	0.99
0.16	0.013 ± 0.002	52	0.99
0.32	0.015 ± 0.005	45	1.00
0.48	0.018 ± 0.004	39	1.00
0.65	0.022 ± 0.003	31	0.99

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

r^2 is coefficient of determination

2.3.5. Effect of HCO_3^- ions

Since the main form of carbonate in solution at neutral pH is HCO_3^-, experiments were performed by adding HCO_3^- ion ranging from 0 to 50 mM in the presence of 50 μM of Fe^{3+} ion to provide the condition of producing OH radicals when excited by UV light, as shown in Eq. (2-5) (Mailhot et al., 1999; Feng and Nansheng, 2000; Zhang et al., 2006).

The presence of HCO_3^- ions significantly reduced the rate of MeHg decomposition in the presence of 50 μM of Fe^{3+} ion (Table 2.4). Higher concentrations of HCO_3^- ions caused a greater reduction in the rate of MeHg decomposition, implying that HCO_3^- ion clearly acted as an OH radical scavenger according to Eq. (2-10) (Ma and Graham, 2000):

$$HCO_3^- + OH^{\bullet} \rightarrow CO_3^{\bullet} + H_2O \qquad (2\text{-}10)$$

Similar results were obtained by Chowdhury et al., (2011). They observed that the degradation rate of 17 β-estradiol in the presence of NO_3^- ion, which can produce OH radical when excited by UV light, decreased markedly with increasing HCO_3^- concentration. Ma and Graham (2000) also showed the effect of OH radical scavenger in the presence of HCO_3^- ion. They observed that the presence of HCO_3^-

ion inhibited the formation of OH radicals when ozone (O_3) was used, resulting in a lower degradation rate for atrazine pesticide.

Although the solution pH increased from initially 3.6 to 6.5 due to an increase in alkalinity by the increase in HCO_3^- ion from 0 to 50 mM, the effect of increase in pH on the photo-decomposition rate was negligible compared the rate decrease by the increase in HCO_3^- ion (see Fig. 2.3, Table 2.4). This result implies that the addition of HCO_3^- ion slowed down the photo-degradation rate of MeHg, mainly by the OH radical scavenging effect.

Table 2.4. Effect of HCO_3^- ion concentration on the photodegradation of MeHg in the presence of 50 μM Fe^{3+} ion (UVA intensity = 0.3 mW cm^{-2})

HCO_3^- concentration (mM)	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	% Reduction	r^2
0	0.165 ± 0.003	4	-	0.95
0.5	0.117 ± 0.004	6	29.34	1.00
5	0.083 ± 0.005	8	29.31	1.00
10	0.053 ± 0.002	13	36.36	0.98
50	0.003 ± 0.010	239	94.48	0.60

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

r^2 is coefficient of determination.

2.3.6 Effect of DOM

The experiment of MeHg photo-decomposition in the presence of DOM at a UVA intensity of 0.3 mW cm^{-2} was performed using several concentrations of fulvic acid (SRFA) and humic acid (SRHA) at concentrations between 0 to 40 mg C L^{-1}, while the MeHg concentration was maintained at 20 ng L^{-1}. The change in the photo-decomposition rate of MeHg in the presence of fulvic and humic acids is shown in Table 2.5. Since fulvic and humic acid are relatively weak acids, the pH of the solution also did not change significantly during MeHg photo-decomposition experiment, resulting in a negligible effect on photo-decomposition rate.

In the presence of fulvic acid, the photo-decomposition rate of MeHg increased with an increasing fulvic acid concentration. Several studies have shown that DOM such as fulvic acid can generate ROS such as OH radicals (Vaughan and Blough, 1998; Jacobs et al., 2011). Vaughan and Blough (1998) found a linear relationship between the rate of OH production and fulvic acid concentration during UV irradiation at 310 and 320 nm and suggested that fulvic acid was the source of the OH. Jacobs et al. (2011) also reported that the presence of fulvic acid significantly increased the degradation rate of organic compounds by producing OH radicals and other ROS. This result implies that OH radicals produced in the presence of fulvic acid result in photo-decomposition of MeHg.

However, in the presence of humic acid, the photo-decomposition rate of MeHg

significantly decreased with increasing humic acid concentration. This inhibition could be the result from the combining effects of photon-attenuation, and the competition for absorbing light, and OH radical scavenging. First, the transmittance of UV light decreased from 89% to 71% when humic acid concentration increased from 10 to 40 mg L^{-1} (Table 2.5). Similarly, Li et al. (2010) observed relatively lower percent reduction in MeHg in the Everglades could be attributed to its higher DOC concentration (20 mg L^{-1}). They suggested that the decreasing sunlight penetration by increasing DOC concentration would decrease the degradation of MeHg. The effect of increasing humic acid concentration on the subsequent decrease in MeHg photo-decomposition is related to photon attenuation, which is the reduction in light penetration,

Another reason for the decrease in the MeHg photo-decomposition rate can be attributed to the competition between MeHg and humic acid for absorbing UV light (Haag and Hoigné, 1985; Lam et al., 2003; Vione et al., 2006). As shown in Fig. 2.5, humic acid can absorb UV light more effectively at wavelengths less than 400 nm. The effect of increasing humic acid concentration on the subsequent decrease in MeHg photo-decomposition is directly related to the competition between MeHg and humic acid to absorb UV light.

Table 2.5. Effect of fulvic and humic acid concentrations (mg C L^{-1}) on the photo-decomposition rate (R_{deMeHg}) of MeHg (UVA intensity = 0.3 mW cm^{-2})

Concentration (mg C L^{-1})	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	T (%)[b]	r^2
Fulvic acid				
0	0.011 ± 0.005	63	100	0.99
10	0.013 ± 0.002	54	95	0.99
20	0.016 ± 0.004	44	89	0.97
40	0.023 ± 0.003	30	80	0.96
Humic acid				
0	0.011 ± 0.005	63	100	0.99
10	0.005 ± 0.003	151	89	0.80
20	0.002 ± 0.002	301	80	0.82
40	0.001 ± 0.001	533	71	0.90

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

[b] T means the transmittance of light at 365 nm.

r^2 is coefficient of determination.

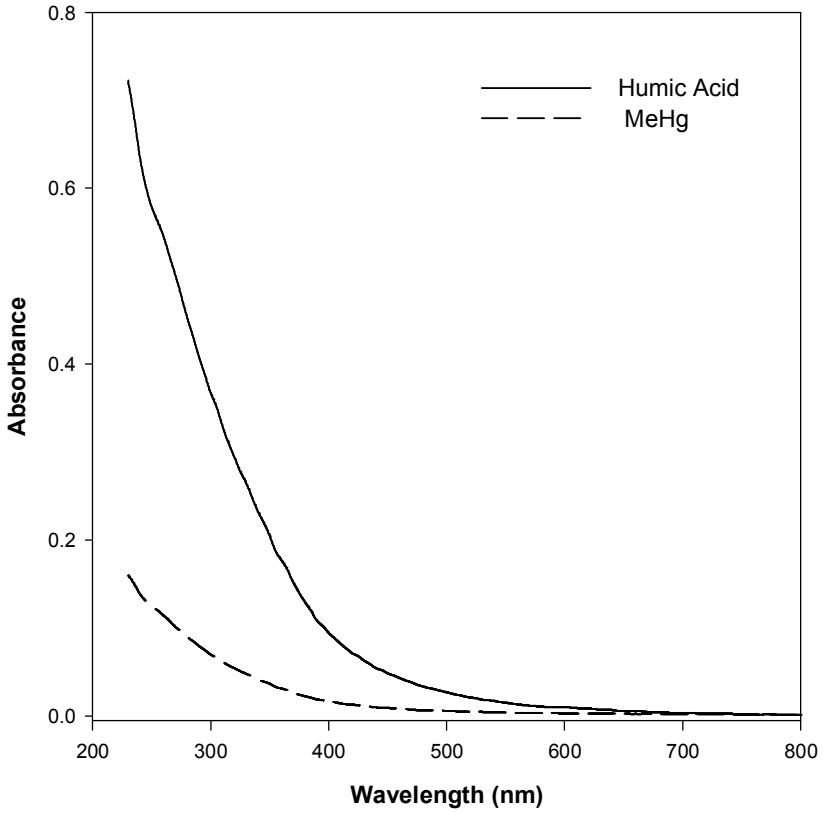

Fig. 2.5. Comparison of absorbance spectrum of humic acid and MeHg ([MeHg] = 100 ng L^{-1}, [humic acid] = 20 mg C L^{-1}).

Finally, in order to examine the effect of humic acid as an OH radical scavenger on MeHg decomposition rate, experiments were conducted at a UVA intensity of 0.3 mW cm^{-2} with an initial MeHg concentration of 20 ng L^{-1} in the presence 10 mg L^{-1} NO_3^- ion known to produce OH radical effectively when excited by UVA light. The presence of even only 5 mg L^{-1} humic acid significantly reduced the rate of MeHg degradation (Table 2.6). Previous research suggested that natural scavengers such as DOM can rapidly consume OH radicals generated by photo-oxidation (Brezonik and Fulkerson-Brekken, 1998; Mehrvar et al., 2001; Vione et al., 2006). This result indicates that the presence of humic acid decreased the photo-decomposition rate of MeHg mainly by OH radical scavenging effect.

Thus, the effect of DOM on the photo-decomposition rate of MeHg is dependent on DOM chemistry. Additional complexities associated with the influence of DOM on MeHg degradation may arise due to the relative proportion of DOM fractions.

Table 2.6. Effect of humic acid on the photo-decomposition rate (R_{deMeHg}) of MeHg in the presence of NO_3^- ion (UVA intensity = 0.3 mW cm^{-2}, $[NO_3^-]$ = 10 mg L^{-1})

	R_{deMeHg} (min^{-1}) ± SD[a]	$t_{1/2}$ (min)	r^2
without humic acid	0.013 ± 0.002	52	0.99
with humic acid (5 mg L^{-1})	0.004 ± 0.001	193	0.98

[a] The SD is the standard deviation of average rate constant from triplicate experiments.

r^2 is coefficient of determination.

2.4 Conclusions

The effects of pH, NO_3^-, Fe^{3+}, HCO_3^-, fulvic acid, and humic acid on photo-decomposition of MeHg in water under UVA irradiation were evaluated. The photo-decomposition of MeHg followed pseudo-first-order reaction kinetics. Approximately 63% of MeHg was degraded by direct photolysis. The half-life of MeHg varied from 3 to 63 min depending on the UVA intensity. The photo-decomposition rate increased in the presence of NO_3^-, Fe^{3+}, and fulvic acid due to photosensitization effect, whereas humic acid and HCO_3^- ions reduced the decomposition rate mainly due to OH radical scavenging effect. These results support that abiotic decomposition of MeHg in surface water is mainly induced by direct and indirect photoreaction from UV irradiation, and OH radical is a critical parameter in the photo-decomposition of MeHg. Moreover, when estimating loss from the photo-decomposition of MeHg, natural water constituents are important, and MeHg concentration in natural water can be affected by seasonal changes in solar UV radiation, variation in DOM by runoff, and the flux of solar radiation.

The results of this study imply that the changes in natural water constituents such as nitrate, ferric, and bicarbonate ions can influence the photo-decomposition rate of MeHg in the aquatic system and ultimately control the potential bioavailability of MeHg in surface waters.

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Chapter 3. The Production of Dissolved Gaseous Mercury from MeHg Photo-degradation at Different Salinity

3.1 Introduction

The pollution of water with mercury (Hg) and its compounds has received much attention due to the high toxicity of these species and their tendency to bio-accumulate, even at very low concentrations. Methylmercury (MeHg) is the form of Hg that generates most concern because it is readily biomagnified in aquatic food chains and can reach levels harmful to both humans and wildlife (Clarkson et al., 2006; Fitzgerald and Lamborg, 2007). MeHg generally accounts for > 90% of the Hg found in most fish (Bloom, 1992), and its bio-magnification to harmful levels has resulted in fish consumption advisories throughout the US, Canada, and Europe (e.g., EPA, 2006).

The levels of MeHg in natural water bodies are influenced by both biological and photochemical processes. Although MeHg decomposition can be microbially mediated in water, many researchers have identified photo-degradation as another important removal mechanism for MeHg degradation in freshwater lakes, thus resulting in the reduced bioavailability of MeHg for accumulation through aquatic food webs (Sellers et al, 1996; Hammerschmidt and Fitzgerald, 2006; Lehnherr and

ST. Louis, 2009; Li et al., 2010).

Recent studies have reported that environmental factors, such as the intensity of solar radiation, water constituents (e.g., dissolved organic carbon (DOC) and specific ions), are significant variables affecting abiotic MeHg degradation (Chen et al., 2003; Hammerschmidt and Fitzgerald, 2010; Zhang and Hsu-Kim, 2010; Black, 2012). Sellers et al. (1996) found that the MeHg concentration decreased in sunlight, but not in dark conditions. They also found that MeHg photo-degradation followed first-order kinetics with respect to the MeHg concentration and light intensity. They further found that MeHg photo-degradation rates decreased exponentially with depth below a lake surface due to the decrease in sunlight intensity.

Naturally occurring constituents of surface waters such as nitrate (NO_3^-), ferric iron (Fe^{3+}), and bicarbonate (HCO_3^-) ions can also participate in the photo-decomposition of MeHg (Zepp, 1987; Brezonik et al., 1998; Vione et al., 2006). Nitrate and ferric ions are known to produce OH radicals in water when excited by solar UV radiation. However, the bicarbonate ion does not absorb solar UV radiation, and is known to act as a scavenger of the OH radical (Brezonik et al., 1998; Ma and Graham, 2000; Chin et l., 2004). Dissolved organic matter (DOM) is ubiquitous in the aquatic environment and can also interact with Hg, affecting its speciation, solubility, mobility, and toxicity (Haag and Hoigné, 1985; Vaughan and Blough, 1998; Lam et al., 2003; Vione et al., 2006; Jacobs et al., 2011; Kim and Zoh, 2013). However, the effects of these parameters on the photo-degradation of MeHg in the

seawater environment are not fully understood, and relatively little research has been conducted on the fate and stability of MeHg in seawater. In fact, seawater could be potentially more important than other water sources as both a sink and source of MeHg because of its large global volume.

Dissolved gaseous mercury (DGM, Hg^0) is a major volatile species of Hg that is released from natural surface waters. DGM production in natural surface water is mediated by solar radiation, and can be affected by other abiotic factors (Zhang and Lindberg, 2001). Although DGM can be released by the decomposition of MeHg, no studies have been published that have investigated the degradation of MeHg to DGM. Only studies investigating DGM production by the reduction of divalent inorganic mercury (Hg^{2+}) are available (Zhang and Lindberg, 2001; O'Driscoll et al., 2003; Ravichandran, 2004; Zhang et al., 2006).

This study investigated the effect of environment factors (i.e., light intensity and wavelength and initial MeHg concentration) and primary water constituents (i.e., DOM, nitrate and bicarbonate ions) on the photo-degradation of MeHg at different salinity. This work measured the production of DGM during MeHg photo-degradation and also investigated the photo-degradation kinetics of MeHg under various conditions to determine the underlying mechanism of MeHg demethylation in water flow from freshwater to seawater.

3.2 Materials and Methods

3.2.1 Sampling and materials

Seawater samples were collected from the Yellow Sea, located at latitude 37° N and longitude 126° E. After filtering through a 0.45-μm nitrocellulose filter (Adventec, Tokyo, Japan), the samples were stored in the dark at 4°C until required for experiments. For the different salinity experiment, the seawater samples were diluted with deionized water by 1:2 serial dilutions. Nitrate (NO_3^-) stock solution was prepared with reagent-grade KNO_3. Standard Suwanee River humic acid (SRHA) was used to investigate the role of DOM in the photo-decomposition of MeHg. Stock solutions of HA were prepared with deionized water and then filtered through a 0.45-μm nitrocellulose filter. A standard solution of MeHg (1000 μg mL^{-1}) was diluted to make a MeHg stock solution (1 ng mL^{-1}). The initial concentration of MeHg was fixed at 20 ng L^{-1} by diluting the stock solution; this was kept constant throughout all experiments.

3.2.2 Photo-reactor and experimental design

Figure 3.1 is a schematic diagram of the photo-reactor (Fig. 3.1). The PTEF

bottles were placed inside a box-type photo-reactor and irradiated for 180 min. The reactor was equipped with five UV lamps. UV intensity was controlled by the number of lamps used. The solutions were shaken after the addition of MeHg (20 ng L^{-1}) and natural water constituents, then immediately transferred into PTEF bottles (250 mL each) to leave no headspace inside. During the reaction, one bottle was removed every 30 min to determine MeHg and DGM concentrations. To provide dark conditions, PTEF bottles were covered with aluminum foil. The temperature of the reactor was maintained at 24 to 26 °C for the entire incubation.

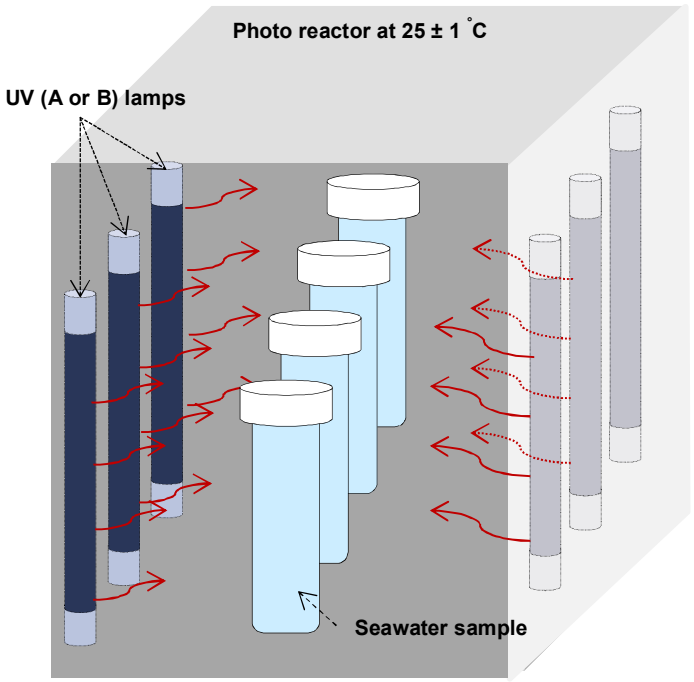

Fig. 3.1. A schematic diagram of the experimental design used to investigate the photochemical decomposition of MeHg.

3.2.3 Analytical methods

For the determination of MeHg, the samples were analyzed immediately before the digestion process commenced. A series of preliminary experiments indicated that no significant differences were observed in the MeHg concentration of samples analyzed both before and after distillation ($n = 20$, paired t-test, $p < 0.05$). Therefore, the samples used for MeHg analysis were taken from the reactor at different reaction times, and were filtered through a nitrocellulose Millipore membrane (2.5 cm, 0.2 μm). Filtered samples were transferred to a 125-mL purging vessel, adjusted to pH 4.9 with 2 M acetate buffer, then ethylated by the addition of sodium tetraethyl borate (NaBEt4) in the closed vessel. After 15 min, ethylated Hg complexes were separated from solution by purging with nitrogen gas for 15 min and then trapped on Tenax. Trapped Hg complexes were carried through a gas chromatography column for separation, converted to Hg^0 via a pyrolytic column and detected using cold vapor atomic fluorescence spectrometry (CVAFS).

DGM was measured using a Hg vapor analyzer (2537A: Tekran Instruments, Canada) connected to an automated purging system. This is a slightly modified version of the method described in Lindberg et al. (2000). The DGM was displaced by bubbling with zero-air gas and passed through the reaction bottle into the Hg vapor analyzer after humidity was removed from the solution by a soda-lime trap to protect the instrument. The DGM that entered the Hg vapor analyzer was adsorbed

on a gold trap cartridge, and then thermally desorbed from the cartridge by a resistive heater. The response of the CVAFS detector was then integrated to provide a quantitative measure of the amount of DGM desorbed from the gold trap.

The intensity of the UVA (λ=365 nm) and UVB (λ=312 nm) regions in the photo-reactor were measured regularly before the start of each experiment using a radiometer (VLX-3W: Cole-Parmer, Vernon Hills, IL). The concentration of DOC was determined using a total organic carbon analyzer (TOC-VCPH: Shimadzu, Kyoto, Japan). The pH of water was measured using 415 CP pH/ISE/conductivity meter.

Results of replicated experiments were expressed as the mean values ± standard deviation. Linear regression analysis was conducted using IPM SPSS statistic 21.0 version (IBM Corp., New York, USA).

3.3 Results and Discussion

3.3.1 Effect of UV light wavelength and intensity on MeHg degradation

Initially a blank test was conducted. In dark conditions, MeHg removal from a seawater sample was less than 5% within a 2-h period (data not shown). In contrast, exposure to UV radiation significantly enhanced MeHg photo-degradation (Fig. 3.2). MeHg photo-degradation in seawater was dependent on the source of the light (UVA or UVB), and intensity. Increasing the UV intensity resulted in an increase in the MeHg photo-degradation rate (Fig. 3.2). MeHg photo-degradation in seawater followed pseudo-first-order kinetics. A plot of ln (C_t/C_0) versus reaction time (min) with changing UV radiation intensity produced a straight line with a correlation coefficient (r^2) greater than 0.98. This indicates that the direct photo-decomposition of MeHg occurred following exposure to UV radiation, which is consistent with other recent reports (Hammerschmidt and Fitzgerald, 2006; Lehnherr and ST. Louis, 2009; Li et al., 2010; Kim and Zoh, 2013).

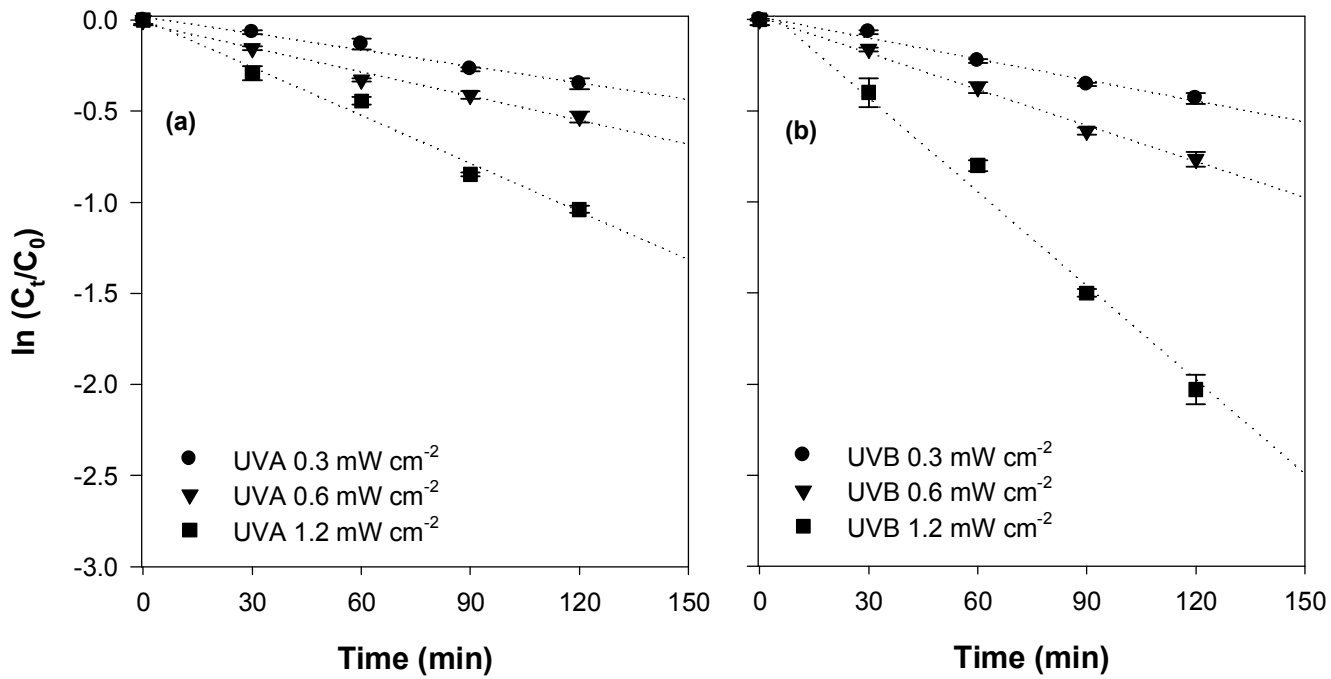

Fig. 3.2. Photo-degradation kinetics of MeHg at pH = 8.3, salinity = 30 ppt (parts per thousand), and $[MeHg]_{initial}$ = 20.0 ng L^{-1}, under (a) UVA and (b) UVB (Error bars represent the standard deviations of experiments performed in triplicate).

There was a positive linear relationship between the MeHg photo-degradation rate and UV radiation intensity at each UV wavelength (Fig. 3.3). UVB was more effective than UVA for MeHg photo-decomposition. This may be because UVB radiation has a shorter wavelength and higher energy. The increase in the photo-degradation rate constant with increasing UV radiation intensity implies that the photo-degradation of MeHg in seawater is likely to decrease with water depth as a consequence of light attenuation.

Figure 3.4 shows the variation in MeHg photo-degradation at different initial MeHg concentrations (from 10 to 40 ng L^{-1}). Under UVA irradiation, the rate constant was 0.258, 0.162, and 0.096 hr^{-1} at initial MeHg concentrations of 10, 20, and 40 ng L^{-1}, respectively. The rate constants also ranged from 0.276 to 0.114 hr^{-1} under UVB irradiation across a range of initial MeHg concentrations. Increasing initial MeHg concentration led to a decrease in the rate constant. The lower photo-degradation rates at higher MeHg concentrations were probably due to the competition for the photon flux between the increased number of MeHg molecules, and/or the MeHg photo-degradation intermediates.

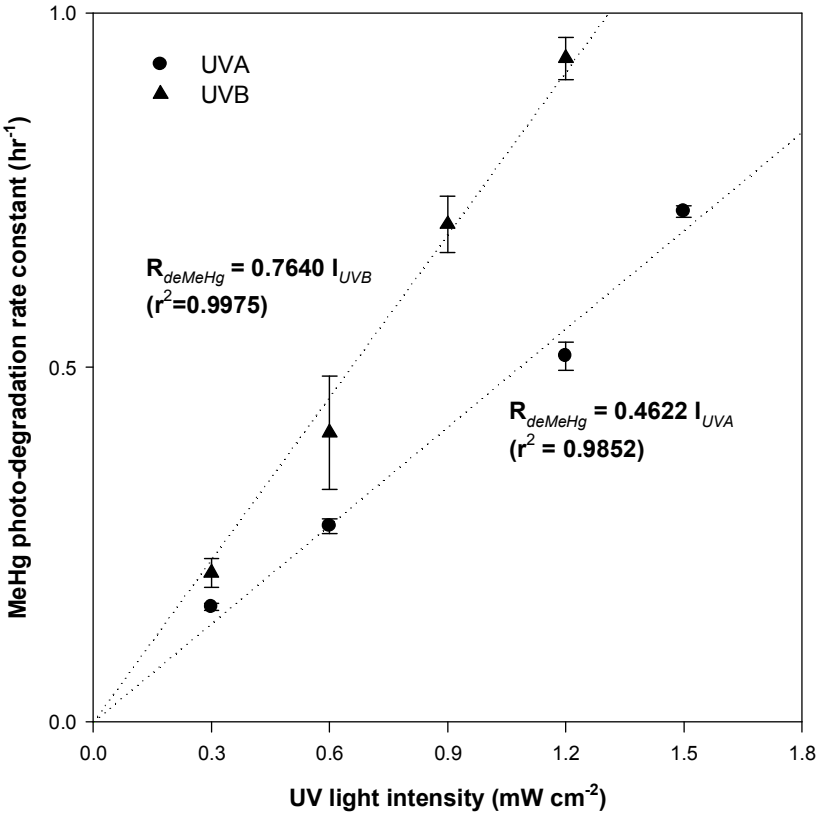

Fig. 3.3. Photo-degradation rate constants as a function of different UV intensities (Error bars represent the standard deviations of experiments performed in triplicate).

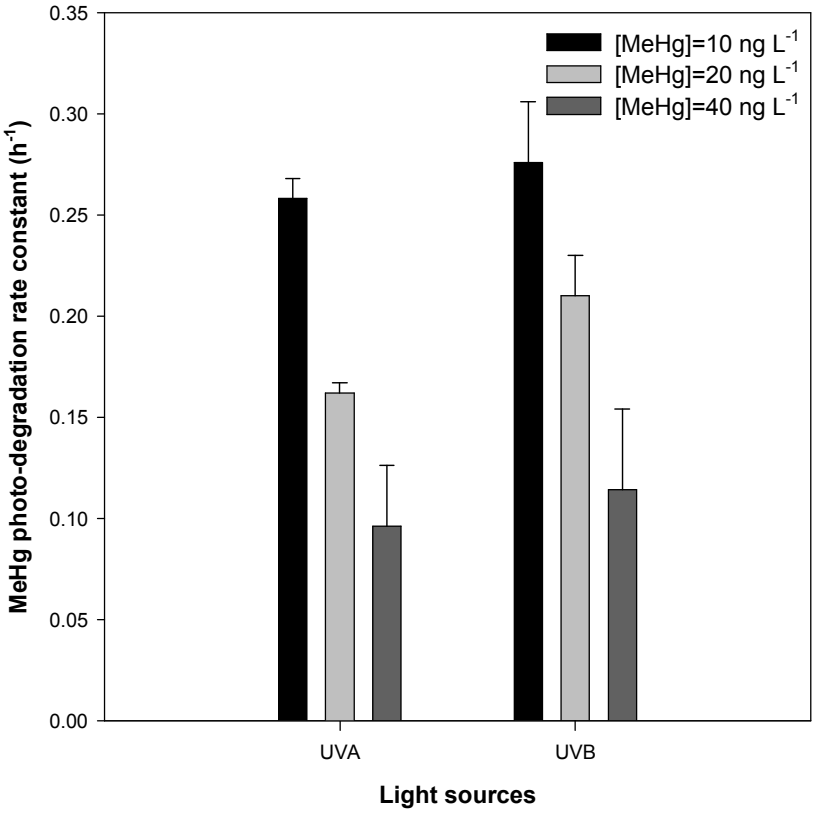

Fig. 3.4. The effect of the MeHg concentration on the rate of photo-degradation under UVA and UVB at pH = 8.3 and salinity = 30 ppt (Error bars represent the standard deviations of experiments performed in triplicate).

3.3.2 Effect of salinity on MeHg degradation

The average ocean salinity is 35 ppt. This number varies between about 32 and 37 ppt. Rainfall, evaporation, river runoff, and ice formation cause the variations. For example, the Black Sea is so diluted by river runoff, its average salinity is only 16 ppt.

The first-order rate constants of MeHg photo-degradation were calculated at different salinities (Table 3.1). The photo-degradation rate decreased from 26.4% to 37.2% as salinity increased from 7.5 to 30 ppt, respectively. The decrease in photo-degradation rates with increasing salinity agreed with recent reports (Whalin et al., 2007; Zhang and Hsu-Kim, 2010; Black et al., 2012; Sun et al., 2013), showing that photo-degradation rates in water samples decreased with increasing chloride ion concentration.

Sun et al. (2013) showed that the presence of high concentrations of chloride prevented MeHg chloride (CH_3HgCl) complexes from being readily converted into other Hg species. Their study revealed that, as the chloride ion concentration in water samples decreased, MeHg speciated gradually into CH_3HgOH. The strength of the Cl-Hg bond in CH_3HgCl is much greater than the strength of the Hg-OH bond in CH_3HgOH because of the strong complexation capacity of the chloride ion. Thus, the rate of MeHg photo-degradation is considerably lower at high salinity than at low salinity.

Zhang and Hsu-Kim (2010) also reported that the chloride ion concentration has a significant effect on MeHg degradation. They suggested that CH_3HgCl complexes, which are the main species of MeHg in coastal marine waters, are less susceptible to photolytic decomposition at higher salinity. This also agrees with the results of a study by Black et al. (2012). These findings imply that salinity is an important parameter that affects various MeHg species and modulates the various photo-degradation mechanisms and pathways.

Table 3.1. The effect of salinity on the rate of MeHg photo-degradation under UVA at $[MeHg]_{initial}$ = 20.0 ng L^{-1}.

Salinity (ppt)	Rate constant (h^{-1}) ± SD[a]	r^2
0.0	0.660 ± 0.005	0.9874
7.5	0.486 ± 0.020	0.9955
15.0	0.360 ± 0.080	0.9836
22.5	0.258 ± 0.040	0.9235
30.0	0.162 ± 0.030	0.9470

[a] SD is the standard deviation of the average rate constant from experiments performed in triplicate.

r^2 is the coefficient of determination

3.3.3 DGM production during MeHg photo-degradation

The photo-degradation of MeHg in water is a key stage in the biogeochemical cycling of Hg in the environment. Many studies have investigated the photo-degradation of MeHg in water systems, but few have considered the importance of the DGM flux resulting from MeHg photo-degradation and the fate of inorganic Hg species from MeHg photo-degradation. Therefore, this study measured DGM, which can be released from MeHg photo-degradation in seawater under UV irradiation. DGM can be produced from the photolysis of MeHg (Sun et al., 2013), as shown in Eqs. (3-1) ~ (3-4);

$$CH_3HgCl + h\nu \rightarrow CH_3^{\bullet} + HgCl^{\bullet} \quad (3\text{-}1)$$

$$2HgCl^{\bullet} + h\nu \rightarrow Hg_2^{2+} + 2Cl^{-} \quad (3\text{-}2)$$

$$Hg_2^{2+} + h\nu \rightarrow 2Hg^{+} \quad (3\text{-}3)$$

$$2Hg^{+} + h\nu \rightarrow Hg^{2+} + Hg^{0}\ (DGM) \quad (3\text{-}4)$$

These equations imply that DGM can be generated from MeHg photo-degradation, and it is therefore an important Hg species to study to better understand the mechanism of MeHg photo-degradation in seawater.

Figure 3.5 shows DGM production from MeHg photo-degradation under UVA and UVB irradiation in seawater samples. As shown in Fig. 3.5, DGM was produced

from the photolysis of MeHg. The total DGM production under UVB irradiation was higher than under UVA irradiation, which might be due to UVB radiation having a shorter wavelength and higher energy than UVA. Some recent studies have reported that reduced inorganic Hg and DGM emissions at the air/water interface were significantly and positively associated with solar radiation (Byrne et al., 2009; Choi and Holen, 2009; Zhang et al., 2012).

The trend of DGM production according to reaction time varied (Fig. 3.5). DGM production increased due to MeHg photo-degradation from the initial reaction time to 60 or 90 min, while DGM production decreased after 90 min. This phenomenon may be due to the re-oxidation of DGM by chlorine or the OH radical (Amyot et al., 1994; Lalonde et al., 2001; 2004), as shown in Eqs. (3-5) ~ (3-6);

$$Hg^0 + HOCl \rightarrow Hg^{2+} + Cl^- + OH^- \qquad (3\text{-}5)$$

$$Hg^0 + 2OH^{\bullet} \rightarrow Hg_2^{+} + OH^- \qquad (3\text{-}6)$$

Therefore, this study suggest that DGM is produced by the photo-reduction of MeHg and can also be rapidly oxidized in seawater. In seawater (pH 7.4 - 8.1), less than 50% of the chlorine is available as fast acting hypochlorous acid (HOCl). Previous studies have reported that the DGM oxidation rate increases in the presence of chloride ions (Amyot et al., 1997; Lalonde et al., 2001).

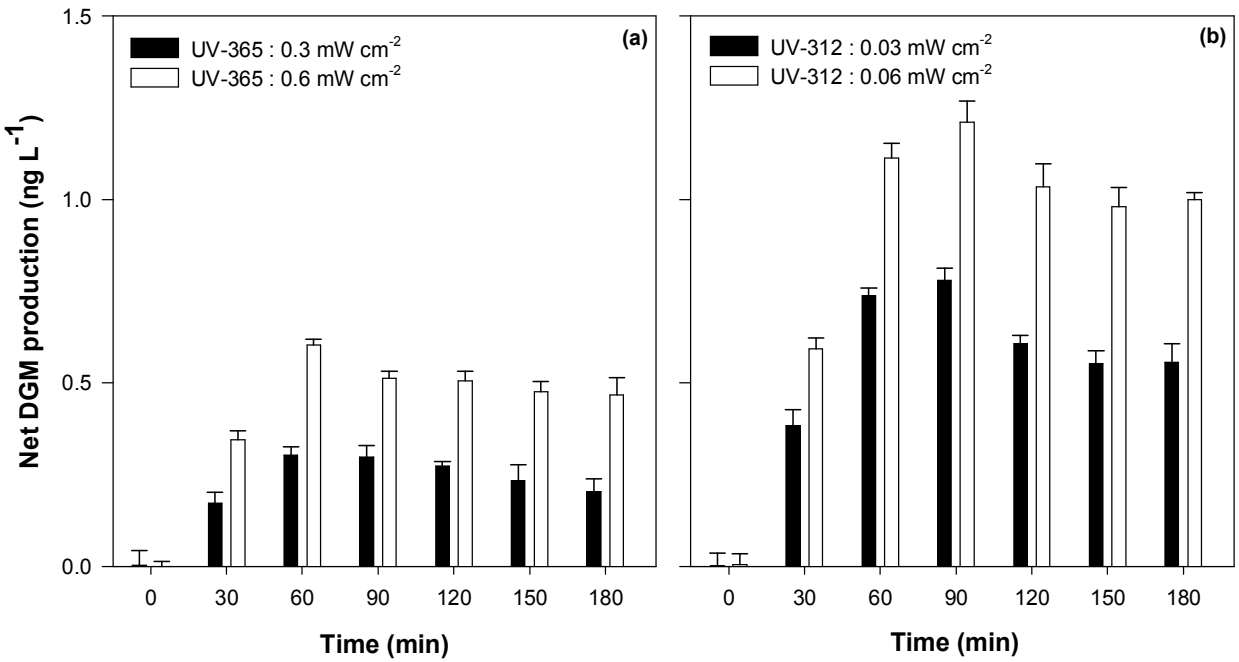

Fig. 3.5. Dissolved gaseous mercury production from MeHg photo-degradation under UVA and UVB at pH = 8.3, salinity = 30 ppt, and $[MeHg]_{initial}$ = 20.0 ng L^{-1} (Error bars represent the standard deviations of experiments performed in triplicate).

3.3.4 Effect of salinity on DGM production in the presence of nitrate and bicarbonate ions

This study also investigated the effect of salinity on DGM production through the photo-degradation of MeHg. The results are shown in Table 3.2. DGM production decreased with increasing salinity, likely due to the salinity inhibiting the photoreaction of MeHg through a strong affinity between Hg and chloride.

In addition, this study assessed the effect of salinity in the presence of nitrate and bicarbonate ions on DGM production. Although seawater contains more dissolved ions than any freshwater system, the nitrate ion is present at lower levels in the ocean (from 0.01 to 0.08 mM) than other natural water sources, whereas the bicarbonate ion concentration in the ocean is only 2.8-fold higher than in surface water. However, nitrate ion is known to produce the OH radical when excited by UV light (Stumm and Morgan, 1996; Nelieu et al., 2004; Shanker et al., 2007; Kim and Zoh, 2013), and plays an important role in MeHg transformation (Shanker et al., 2007; Kim and Zoh, 2013) (Eqs. (3-7) ~ (3-9)). In contrast, the bicarbonate ion is known to inhibit the photo-decomposition of MeHg through radical scavenging (Ma and Graham, 2000; Kim and Zoh, 2013) (Eq. (3-10)).

$$NO_3^- + h\nu \rightarrow NO_3^{-\bullet} \rightarrow NO_2^- + O^{-\bullet} \qquad (3\text{-}7)$$

$$O^{-\bullet} + H_2O \rightarrow OH^{\bullet} + OH^- \qquad (3\text{-}8)$$

$$MeHg + OH^{\bullet} \rightarrow Demethylation \quad (3\text{-}9)$$

$$HCO_3^{-} + OH^{\bullet} \rightarrow CO_3^{\bullet} + H_2O \quad (3\text{-}10)$$

The effects of nitrate and bicarbonate ions on the MeHg photo-degradation rate are shown in Tables 3.3 and 3.4, respectively. Under UVA irradiation, the MeHg photo-degradation rate increased significantly in the presence of nitrate, whereas the MeHg photo-degradation rate decreased dramatically in the presence of bicarbonate (Table 3.3). This suggests that nitrate can promote the photoreaction of MeHg by producing the OH radical when excited by UV light. Likewise, DGM production increased in the presence of nitrate, but decreased in the presence of bicarbonate (Table 3.4). However, the photo-degradation of MeHg and DGM production decreased with increasing salinity in the absence and presence of both nitrate and bicarbonate. Therefore, this study suggest that Hg may be more significantly affected by salinity (Tables 3.3 and 3.4).

Table 3.2. The effect of salinity on DGM production from MeHg photo-degradation under UVA ($[MeHg]_{initial}$ = 20.0 ng L^{-1}, reaction time is 180 min)

Salinity (ppt)	Gross DGM (ng L^{-1}) ± SD[a]	r^2
0.0	2.4430 ± 0.02	0.9856
7.5	2.2087 ± 0.04	0.9476
15.0	2.0943 ± 0.02	0.9728
22.5	1.9841 ± 0.05	0.9609
30.0	1.4881 ± 0.03	0.9855

[a] SD is the standard deviation of the average rate constant from experiments performed in triplicate.

r^2 is the coefficient of determination.

Table 3.3. The effect of salinity on the rate of MeHg photo-degradation in the presence of nitrate or bicarbonate ions under UVA ($[MeHg]_{initial}$ = 20.0 ng L^{-1}, reaction time is 180 min, $[NO_3^-]$ = 0.08 mM, and $[HCO_3^-]$ = 20 mM).

Salinity (ppt)	Rate constant (h^{-1}) ± SD[a]		
	Without NO_3^-/HCO_3^-	With NO_3^-	With HCO_3^-
0.0	0.660 ± 0.01	0.732 ± 0.01	0.354 ± 0.03
15.0	0.360 ± 0.08	0.480 ± 0.02	0.207 ± 0.04
30.0	0.162 ± 0.03	0.289 ± 0.01	0.140 ± 0.01

[a] SD is the standard deviation of the average rate constant from experiments performed in triplicate.

Table 3.4. The effect of salinity on DGM production in the presence of nitrate or bicarbonate ion under UVA ($[MeHg]_{initial}$ = 20.0 ng L^{-1}, reaction time is 180 min, $[NO_3^-]$ = 0.08 mM, and $[HCO_3^-]$ = 20 mM).

Salinity (ppt)	Gross DGM (ng L^{-1}) ± SD[a]		
	Without NO_3^-/HCO_3^-	With NO_3^-	With HCO_3^-
0.0	2.443 ± 0.02	2.622 ± 0.03	2.104 ± 0.03
15.0	2.094 ± 0.03	2.414 ± 0.05	1.741 ± 0.01
30.0	1.546 ± 0.01	1.785 ± 0.04	1.326 ± 0.04

[a] SD is the standard deviation of the average rate constant from experiments performed in triplicate.

3.3.5 Effect of DOM

DOM has various functions and plays important roles in chemical, biological, and even physical oceanography. In general, the major source of open ocean DOM is planktonic primary producers, whereas the large amounts of DOM in coastal environments are mostly terrestrial humic and fulvic acids from rivers and runoff (Ogawa and Tanoue, 2003; Nelson et al., 2004). Previous studies have reported DOC concentrations in surface waters in the range 0.6~1.2 mg C L^{-1} in large areas of the ocean (Ogawa and Tanoue, 2003). In coastal waters, more than 60% of the total dissolved Hg is associated with organic matter or suspended particles (Paraquetti et al., 2004). DOC appears to control the bioavailability of Hg due to its high Hg-binding capacity (Benoit et al., 2001; Zhang and Hsu-Kim, 2010).

As the chromophoric portion of DOM, the role of humic acid in MeHg photo-degradation presents a paradox. Humic acid can promote MeHg photo-degradation by acting as a photosensitizer through the generation of the $OH^{\bullet}$ and $O_2^{\bullet-}$ radicals (Miller and Chin, 2002; Chowdhury et al., 2011; Jacobs et al., 2011). Alternatively, humic acid can compete with MeHg for photons or reactive oxygen species (ROS), leading to the suppression of MeHg photo-decomposition (Brezonik and Fulkerson-Brekken, 1998; Lam et al., 2003; Vione et al., 2006; Kim and Zoh, 2013). Thus, the overall role of humic acid in MeHg photo-degradation depends on the balance between these two opposing effects.

This study examined the effect of DOC concentrations on the rate of MeHg photo-degradation and DGM production. The initial DOC concentration was 2 mg C L^{-1} in the seawater samples used, and humic acid was added to examine the effect of DOM. As shown in Table 3.5, MeHg photo-degradation was suppressed by increasing the DOC concentration under UV light exposure. This inhibition occurred due to the combined effect of photon-attenuation and the competition for photons. MeHg photo-degradation was suppressed by DOM added under UVA irradiation, which indicated that suppression due to photo-absorption or ROS quenching by DOM was more significant than photosensitization effects. Higher DOC concentrations inhibited MeHg photo-degradation, which may be due to DOM competing with MeHg to absorb photons or interact with ROS.

Recently, Li et al. (2010) and Kim and Zoh (2013) investigated the role of DOM in MeHg photo-degradation in freshwater under UVA irradiation. They found that the effect of increasing DOC concentration on the subsequent decrease in MeHg photo-decomposition is related to the competition between MeHg and DOM to absorb UV radiation, which is the same result obtained in this study.

Table 3.5. The effect of DOC concentration on the rate of MeHg photo-degradation and DGM production under UVA ($[MeHg]_{initial}$ = 20.0 ng L^{-1}, pH = 8.3, salinity = 30 ppt, and reaction time = 180 min).

DOC (mg L^{-1})	Rate constant (h^{-1}) ± SD[a]	Gross DGM (ng L^{-1}) ± SD[a]
2	0.1620 ± 0.03	1.8190 ± 0.02
6	0.1200 ± 0.03	1.3530 ± 0.03
10	0.0767 ± 0.01	1.1830 ± 0.01
20	0.0300 ± 0.01	1.1840 ± 0.02

[a] SD is the standard deviation of the average rate constant from experiments performed in triplicate.

3.4 Conclusions

The photo-demethylation experiments reported here demonstrated that while numerous chemical and physical factors can influence the rate of MeHg photo-degradation, few exerted any substantial control of the transformation. The most important factors for predicting MeHg photo-degradation rates in natural seawater were found to be UV wavelength, intensity, salinity, nitrate ion, and DOC concentration. MeHg photo-degradation rate constants varied with the UV wavelength and intensity of radiation, demonstrating that MeHg photo-degradation in seawater is dependent on the radiation source and its intensity. MeHg photo-degradation rates and the DGM flux resulting from photo-degradation decreased with an increase in the chloride ion concentration, suggesting that the presence of chloride could prevent MeHg photo-degradation. The rate of MeHg photo-degradation and DGM production recorded in this study under various conditions indicated that UV radiation is responsible for MeHg photo-degradation together with chloride, and that salinity has a significant influence on MeHg photo-degradation and DGM photo-production in water. Therefore, this study imply that MeHg in freshwater could be more rapidly demethylated than that in seawater and MeHg flowing into the lake or river would be almost removed by photo-demethylation. However, MeHg flowing to seawater would be hardly removed, which could have more chance for bioaccumulation in seawater.

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Chapter 4. Photochemical Methylation of Inorganic Mercury by Various Organic Compounds

4.1 Introduction

Mercury (Hg) compounds have long been of great public concern because of their adverse effect on wildlife and humans. It is well known that the toxicity of Hg compounds depends on their species, which include inorganic, methyl, ethyl and phenyl Hg. Amongst these compounds, methylmercury (MeHg) is the most toxic form in the environment. One famous case of severe MeHg poisoning occurred in Minamata, Japan, which was caused by the consumption of seafood contaminated with MeHg compounds discharged from a chemical plant in the 1950s and 1960s.

The methylation of inorganic Hg in waters and sediments constitutes a key step in the cycling of Hg in aquatic systems and takes place in both remote and impacted environments (Fitzgerald and Mason, 1997). It is important to note that since both methylation and demethylation processes occur, environmental MeHg concentrations reflect net methylation rather than actual rates of MeHg synthesis. It appears that the combined effect of MeHg production and degradation leads to a state of equilibrium with a near constant level of MeHg in sediments that rarely exceeds 1 to 1.5% of total Hg concentration, whereas the proportion of MeHg in fish

and other aquatic biota may be much higher. On the basis of mass balance studies, estimated rates for MeHg production in temperate freshwater lakes currently range from 0.5 to 5 g MeHg per km^2 per year (Ullrich et al., 2001).

Methylation occurs predominantly in sediments and to a lesser extent in the water column (Olson and Cooper, 1974; Xun et al., 1995), but it should be borne in mind that water column methylation is potentially more important, because the volume of water is typically much larger than the volume of surficial sediments.

Methylation of elements such as Hg, As and Sn is an important transformation and transportation pathway of elements in the environment, and has been widely studied (Chen et al., 2007). Natural conversion from inorganic Hg to MeHg was first demonstrated by Jensen and Jernelov (1969). There exist two methylation pathways of inorganic Hg: the biotic process and the abiotic process in the aquatic environment (Celo et al., 2006). Although many scientists have provided evidence for abiotic methylation of Hg in the environment (Akagi and Takabatake, 1973; Weber, 1993; Siciliano et al., 2005; Hammerschmidt et al., 2007), it is widely accepted that biotic methylation accounts for most or all methylation in the environment, especially by sulfate reducing bacteria (SRB) (Benoit et al., 2003). However, the relative importance of abiotic methylation of inorganic Hg(II) should not be neglected (Celo et al., 2006).

Chemical methylation of inorganic Hg(II) can occur only if suitable methyl donors exist in the environment. Nagase (1982) demonstrated that inorganic Hg(II)

can be methylated by fulvic and humic acids, which are large organic components of dissolved organic matter. It is also possible that inorganic Hg can be methylated by acetic acid, propionic acid and ethanol in aquatic environments under the irradiation of sunlight or ultraviolet light (Gårdfeldt et al., 2003; Siciliano et al., 2005; Celo et al., 2006). Transmethyl reaction between inorganic Hg(II) and other methylmetals, such as the methyl transfer from methyltin to inorganic Hg(II), have also been shown to be possible in aquatic environments (Cerrati et al., 1992). Moreover, methyl transfer from methylcobalamin to inorganic Hg(II) was suggested as the most probable mechanism (Yin et al., 2012; Jiménez-Moreno et al., 2013). Recently, Hammerschmidt et al. (2007) suggested that MeHg is possibly formed in wet deposition through the chemical methylation of labile Hg^{2+} by a methylating agent (especially acetate).

A comprehensive study on the possible methylating donors and related mechanism for Hg methylation is needed. Low molecular-weight organic compounds (LMWOCs) including ketones, aldehydes, and low-molecular-weight organic acids, are important metabolites of micro-organisms (Musa-Veloso et al., 2006). LMWOCs (Atkinson and Arey, 2003) are widely present in surface water (Hudson et al., 2007), soil (Jones et al., 2003), and the atmosphere (Poschl et al., 2001) and are potential methyl donors for Hg methylation.

In this paper, the methylation of inorganic Hg(II) by high- and low-molecular-weight organic compounds (HMWOC and LMWOM, respectively) in aquatic

systems was investigated. LMWOC and HMOWC which were used `as methylation donors in this study were acetate, malonate, and dimethylsulfoxide (DMSO), and litter-derived dissolved organic matter (DOM), respectively. This study considered influencing factors such as pH, light source, and initial concentrations of the methyl donor. This work can help improve our understanding of the possible photochemical methylation process and mechanism of Hg in natural environment.

4.2 Materials and Methods

4.2.1 Materials

All reagents were obtained commercially and used without further purification unless otherwise stated. Low-molecular-weight organic acids were analytical grade (Sigma Aldrich, Korea). Methylmercury chloride (CH_3HgCl) and mercury chloride (HgCl) were obtained from Alfa Aesa (USA).

Litter samples were collected from forest and raw litter samples were ground using a blender, producing homogeneous samples. Litter extracts were made by dilution in de-ionized water with 1:100 (w:w) for litters. After 24 hour extraction in the incubator (T: 25±1 °C), litter solutions were filtered with 0.45 μm pore size filter.

To investigate the effect of salinity on methylation, seawater samples were collected from the Yellow Sea, located at latitude 37° N and longitude 126° E. After filtering through a 0.2-μm nitrocellulose filter (Adventec, Tokyo, Japan), the samples were stored in the dark at 4°C until required for experiments. In addition, seawater samples unfiltered were used for the biotic methylation experiment.

4.2.2 Photochemical experiments

The solar spectrum at the surface of the earth has wavelengths greater than 290

nm and wavelengths above 400 nm do not have enough energy to promote the photoreaction with most chemicals (Bonzongo and Donkor, 2003). Accordingly, UVA (λ=365 nm) and UVB (λ=312 nm) were chosen as light sources for the experiments, considering that they would play a leading role in MeHg productions.

For the methylation reaction of Hg^{2+} and LMWOMs or DOM derived from litter, working solution was distributed into sterilized Teflon bottles, spiked with Hg(II) and different concentrations of methyl donor, and incubated under irradiation at the temperature of 25 °C for 3 days. Also, photochemical MeHg production in seawater was investigated using dimethylsulfoxide (DMSO) as methyl donor.

Each experiment consisted of three replicates (three Teflon bottles per each experimental condition) to examine the variation of MeHg concentration according to exposure time. One bottle at a time was taken out for MeHg analysis.

4.2.3 Molecular weight fractionation of DOM experiment

Dialysis was conducted following the method described by Chen et al (2010). Briefly, the specific membranes were used to isolate DOM-fractions at molecular weight of 3.5 and 10 kDa, respectively. The membrane bag containing 20 mL of the extract was soaked in deionized water over night and rinsed with distilled water. Then, the sample solution was poured into the membrane bag. Dialysis was conducted in a beaker containing 1 L of deionized water in darkness at 4 °C. The

solutions were constantly stirred with a magnetic stirrer for 5 days. Dialysis began with the membrane of 3.5 kDa. When the dialysis was over, an aliquot of solution outside the membrane with the fraction of MW < 3.5 kDa was collected. The sample inside the bag (3.5 kDa < MW) was carefully transferred into the membrane bag of 10 kDa for further dialysis. When the dialysis was over, the membrane bag containing the fraction of 10 kDa < MW and the outside solution with the fraction of 3.5 < MW < 10 kDa were separated. TOC contents of both fractions were determined using a total organic carbon analyzer. Each of dialysis of the samples was repeated in triplicate.

4.2.4 Analysis of mercury and other environmental parameters

THg concentrations in water samples were analyzed using the Tekran 2600 Hg analyzer (Tekran Inc., Canada), based on EPA method 1631, by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry (CVAFS) (US EPA, 2002; 2006). To convert all mercury species to $HgCl_2$, and to prevent volatilization, samples were mixed with 12 N HCl or bromine monochloride (BrCl) solutions. Prior to analysis, all Hg in a sample aliquot was oxidized to Hg^{2+} with BrCl. After oxidation, the sample was sequentially reduced with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) to destroy the free halogens, and then reduced with stannous chloride ($SnCl_2$) to convert Hg^{2+} to the volatile Hg^0. Hg^0 was separated from the solution

using nitrogen, and collected using a gold trap. The Hg was then thermally desorbed from the gold trap into an inert gas stream carrying the released Hg^0 to a second gold (analytical) trap. The desorbed Hg^0 from the analytical trap entered the gas stream, carrying Hg into the cell of a CVAFS for detection. Quality assurance and quality control (QA/QC) during THg analysis was performed each measurement day by analyzing known amounts of $HgNO_3$, achieving a high level of precision ($r^2 >$ 0.9995). The relative percent difference (RPD; 100×difference/mean) in the analysis of duplicate samples was typically less than 15% (average 9.9%).

For MeHg analysis, this study used automated MeHg analyzer based on *EPA Method 1630* (MERX-4400, Brooks Rand Inc., USA). Analyses of MeHg in samples were performed after daily calibration with a MeHg stock solution (1 ng mL^{-1}). The detection limit (standard deviation × 3.143) was 0.0025 ng L^{-1}. The RPD during analysis of duplicate samples was typically less than 15% (average 5.7%) and spike recoveries were within ± 20%.

Light intensity of UVA or UVB radiation in the photo-reactor was checked regularly before the start of every experiment using a VLX-3W radiometer (Cole-Parmer, Vernon Hills, IL) with UVA sensor at 365 nm and UVB sensor at 312 nm. The concentration of DOM was analyzed by TOC-V_{CPH} (Shimadzu, Kyoto, Japan). The pH was adjusted using 1N H_2SO_4 and NaOH solutions. The solution pH of test waters was measured using 415 CP pH/ISE/conductivity meter (Istek Co., Daejeon, Korea).

Results of repeated experiments were expressed as the mean value ± standard deviation. IBM SPSS statistic 21.0 version (IBM Corp., New York, USA) was used to determine the coefficients by linear regression analysis.

4.3 Results and Discussion

4.3.1 Effect of UV irradiation and incubation time

The MeHg production by acetate in relation to the incubation time was investigated for 3 days under UV irradiation. A large excess of acetate over inorganic Hg(II) was designed in the kinetic experiments so that the concentration of acetate remained essentially constant in the methylation system. The results are shown in Fig. 4.1; the MeHg production increased with the incubation time. Under UV irradiation in the presence of acetate, the yield of MeHg increased significantly, indicating that photo-irradiation lead to the production of MeHg. Effect of UVB irradiation on the methylation of Hg by acetate was relatively higher than that of UVA (Fig. 4.1). This may be because UVB radiation has a shorter wavelength and higher energy.

The reaction mechanism for inorganic Hg(II) and acetate can be described as follows (Yang et al., 2008):

$$CH_3COO^- + h\nu \rightarrow CH_3^{\bullet} + {}^{\bullet}COO \quad (4\text{-}1)$$

$$CH_3^{\bullet} + Hg^{2+} \rightarrow CH_3Hg^+ \quad (4\text{-}2)$$

As shown in Eq. (4-1) and (4-2), acetate can be photo-dissociated forming a

methyl radical, and then it can react with inorganic Hg(II) to yield of MeHg.

The methylation reaction can be considered as pseudo-first-order for Hg(II), because of the linear relationship between $\ln[(C_0 - C_t)/C_0]$ and the incubation time, where C_0 is the initial concentration of inorganic Hg(II) and C_t is the concentration of CH_3Hg in the methylation system at time t. The results of the first order kinetic fit are shown in Fig. 4.2. The high correlation coefficient (above $r^2 = 0.95$) corroborates the linear relationship between amount of MeHg produced and the incubation time. Thus, it could be deduced that this methylation reaction was first-order for inorganic Hg(II). The first-order reaction rates were 3.5908e-7 and 2.0915e-7 hr^{-1} in the solution under UVB and UVA irradiation, respectively.

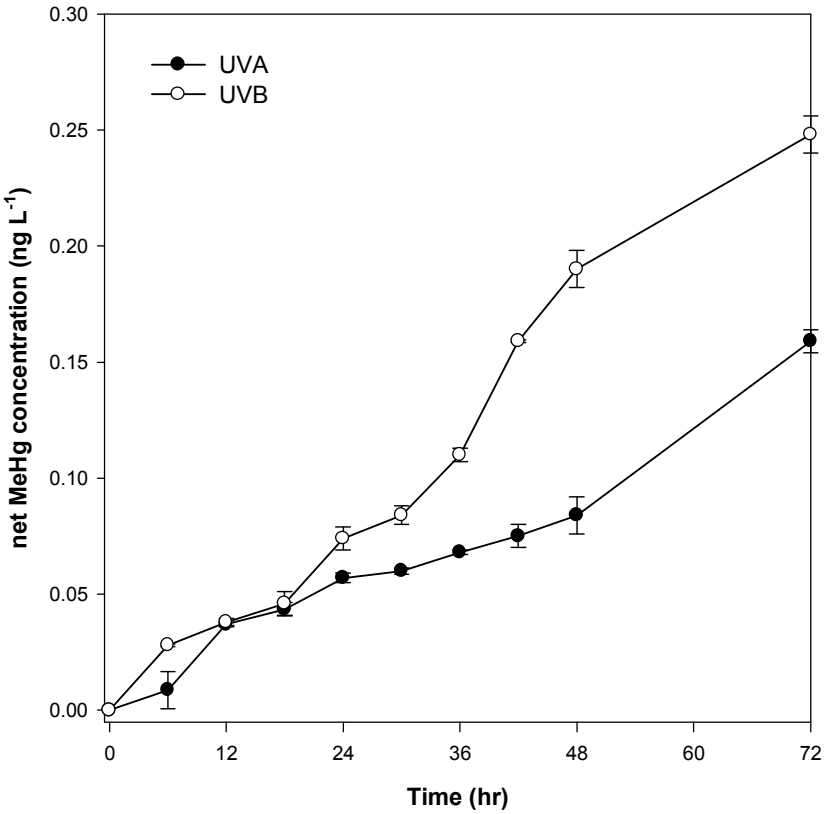

Fig. 4.1. Effect of UV irradiation on the methylation of Hg in the presence of acetate (Light intensity of UVA (λ=365 nm) and UVB (λ=312 nm) is 0.8 and 0.6 mW cm^{-2}, respectively. $[Hg^{2+}]_{initial}$ is 100 ng L^{-1} and $[acetate]_{initial}$ is 40 mg L^{-1}. Error bars represent the standard deviation of triplicate experiments).

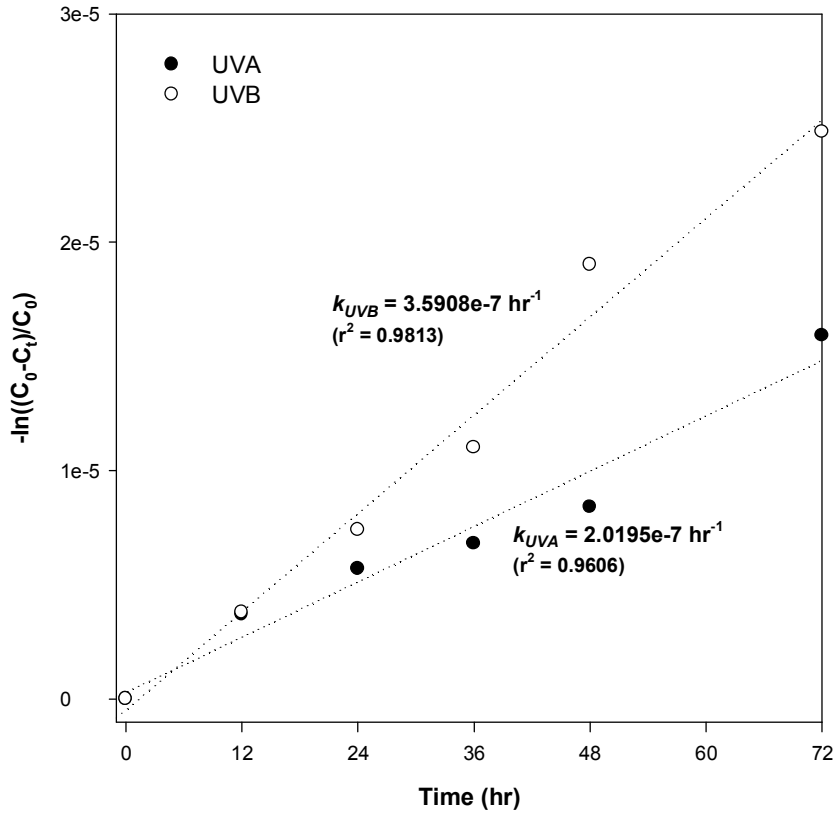

Fig. 4.2. First-order rate plots at different UV irradiation (Light intensity of UVA (λ=365 nm) and UVB (λ=312 nm) is 0.8 and 0.6 mW cm^{-2}, respectively. $[Hg^{2+}]_{initial}$ is 100 ng L^{-1} and $[acetate]_{initial}$ is 40 mg L^{-1}).

4.3.2 Effect of different LMWOMs

Some potential methylation donors between acetate and malonate were compared for their capabilities to methylate Hg(II), as shown in Fig. 4.3. The result indicated that MeHg yield of acetate had much higher MeHg yield than those of malonate. These data did not support the hypothesis of his study that molecules containing carbonyl groups exhibited relatively higher methylation capabilities. It is possible because malonate may have functioned as ethyl donors despite it don't contain an ethyl group (Si and Ariya, 2008).

The effect of concentration of methyl donors (i.e., acetate and malonate) on the methylation reaction was also investigated, as shown in Fig. 4.3. Methylation efficiency of Hg^{2+} is enhanced with an increased concentration of methyl donor at low concentrations ranged from 10 to 40 mg L^{-1}. This result indicated that methyl donors are responsible for the formation of Hg^{2+}-organic molecule complexes, facilitating photo-methylation of Hg^{2+}. In contrast, at high concentration, the results were different between methyl donors. The yield of MeHg peaked at 40 mg L^{-1} of acetate and then decreased with increasing acetate, while that for malonate kept increasing within the range of investigated concentrations from 10 mg L^{-1} to 80 mg L^{-1}. For acetate as methyl donor, an increased concentration of the methyl donor suppresses Hg^{2+} methylation, which may be attributed to the reduction of penetration depth of the UV irradiation into the bulk solution.

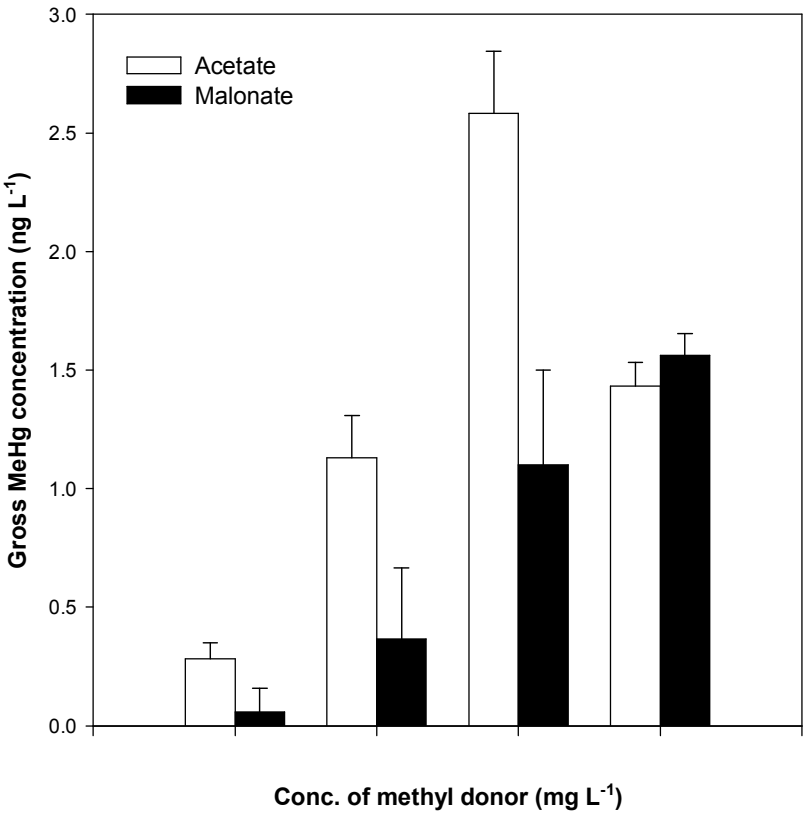

Fig. 4.3. Effect of concentration of methyl donors under UV irradiation on the methylation of Hg (Light intensity of UVA (λ=365 nm) is 0.8 mW cm^{-2}. $[Hg^{2+}]_{initial}$ is 100 ng L^{-1}. Error bars represent the standard deviation of triplicate experiments).

4.3.3 Effect of pH

The effect of pH on the MeHg production was investigated. The methylating ability of acetate was determined under different conditions of pH. The experiments were conducted at a UVA intensity of 0.8 mW cm^{-2} and acetate concentration was 40 mg L^{-1}. Inorganic Hg concentrations was 100 ng L^{-1}, and they were incubated at 25°C for 3 days in the UV irradiation.

As shown in Fig. 4.4, MeHg production was enhanced with the time in all pH ranges. However, the methylating ability of acetate was highest at pH 5 and decreased with the solution pH increased, being consistent with previous work (Celo et al., 2006). It was due to the availability of inorganic Hg (II) in the solution. The equilibrium exists between Hg^{2+} and $Hg(OH)_2$ as a function of pH in the aqueous solution. Free Hg^{2+} is considered to have a higher methylation reactivity than that of $Hg(OH)_2$ (Gårdfeldt et al., 2003; Chen et al., 2007; Yin et al., 2012). The increased pH of the reaction solution decreases the availability of Hg^{2+}, resulting in the decrease in the yield of MeHg production.

Methylation reaction also followed the pseudo-first-order kinetics for Hg(II) (Fig. 4.4). In Fig. 4.4, y axis represents the $\ln(([THg]_{initial}-[MeHg]_{produced})/[THg]_{initial})$. From these plots, this study calculated the first-order reaction rates constants, being 1.7133e-5, 1.2335e-5, and 0.8217e-5 hr^{-1} under pH 5, pH 7, and pH 10 in the solution, respectively. All methylation reaction under various pH conditions had the

high correlation coefficient with incubation time ($r^2 = 0.97$). The pH of the reaction solution of acetate influences not only the availability of Hg(II), but also the photolysis of LMWOMs and the interaction between Hg(II) and LMWOMs.

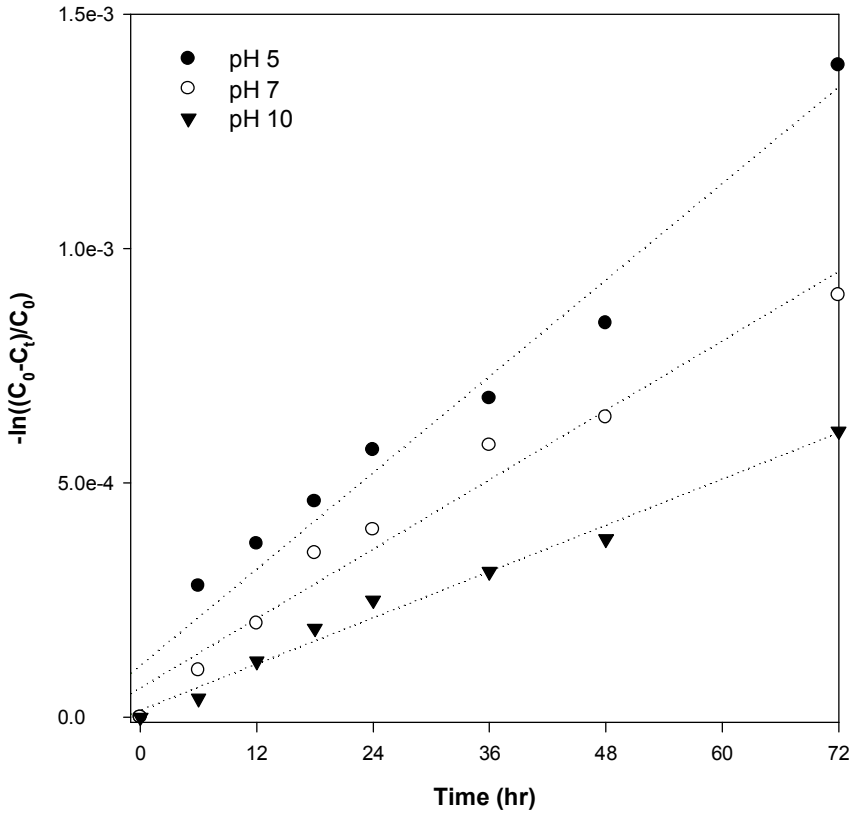

Fig. 4.4. Effect of pH on the methylation of Hg in the present of acetate (Light intensity of UVA (λ=365 nm) is 0.8 mW cm^{-2}. $[Hg^{2+}]_{initial}$ is 100 ng L^{-1} and $[acetate]_{initial}$ is 40 mg L^{-1}).

4.3.4 Effect of DOM derived from litter

Litter represents an important pathway for Hg entry into aquatic ecosystems for two reasons. First, Hg concentrations are elevated in litter because the foliar burden of Hg increases during the growing season (Grigal, 2002). Consequently, litterfall Hg flux is higher on an areal basis than either wet deposition or throughfall in temperate and boreal forests (Grigal, 2002). Second, Hg transported to aquatic ecosystems via litterfall is accompanied by a large supply of labile organic matter. Therefore, litter entering lakes, wetlands, or streams under low flow conditions may possibly elevate in situ net Hg methylation rates because of high organic carbon and inorganic Hg availability from litter (Balogh et al., 2002). Thus, in this study, the production of MeHg in the presence of litter-derived DOM (LDOM) with and without UVA irradiation (λ=365 nm, 0.8 mW cm^{-2}) was examined. LDOM solutions with Hg(II) were incubated at 25°C for 3 days; the MeHg produced was measured. LDOM were 20 mg C L^{-1} and Hg(II) concentration was 100 ng L^{-1}. The result for the MeHg production via LDOM was shown in Table 4.1.

As shown in Table 4.1, under UVA irradiation, the initial MeHg concentration for pine needles (0.2080 ng/L) was around 7 times greater than for chestnut leaves (0.0304 ng/L). At the end of the experiment, net concentration of MeHg was 0.1501 and 0.0394 ng/L for pine needles and chestnut leaves, respectively (Table 4.1). The increased MeHg concentration was observed only for chestnut leaves, but for pine

needles. The percent THg that was MeHg (%MeHg) in the chestnut leaves treatment increased over 3 times during total incubation time, while %MeHg in the pine needles treatment slightly decreased (Table 4.1). Therefore, under UVA irradiation, predominant net reaction was the demethylation of original MeHg in the pine needles treatment, but in the chestnut leaves treatment the formation of new MeHg (methylation) predominated for total incubation time.

In contrast, under dark condition, chestnut leaves exhibited an increase of above 540% of initial MeHg concentration, compared to increase of only 18% of initial concentration for pine needles (Table 4.1). The %MeHg in the chestnut leaves treatment increased by 11-fold (increase from 0.03 to 0.35) while that in the birch leaves treatment increased only 2-fold (increase from 0.19 to 0.40) (Table 4.1). These results may be because the more easily decomposable chestnut leaves relative to pine needles result in the higher production of MeHg.

Therefore, this experiment results support that there was production of new MeHg as a result of the decomposition of flooded plant tissues. However, the results of this study are not consistent with other studies that examined MeHg increases in coniferous needles compared to deciduous leaves and grasses (Heyes et al., 1998; Hall et al., 2004). According to Heyes et al. (1998), black spruce needles sampled from litterbags placed in an experimentally flooded wetland at the Experimental Lakes Area in northwestern Ontario exhibited an increase of 800% of original MeHg mass, compared to increases of 630% of original mass in *Sphagnum fuscum* moss

and 50% of original mass in sedge grass (*Carex rostrata*) stalks.

This study propose a possible explanation as the difference in rates of demethylation among treatments as to why there was greater production of MeHg associated with flooded chestnut leaves compared to flooded pine needles. For example, if the organic carbon derived pine needle stimulated rates of Hg(II) methylation, but also enhanced rates of demethylation, it would be expected to see less net methylation in the pine needles treatment compared to those in the chestnut leaves treatment. Thus, this study suggest that rates of both methylation and demethylation would be increased via LDOM derived from pine needles, resulting in lower net methylation via LDOM derived from pine needles compared to those via LDOM derived from chestnut leaves which possibly only Hg(II) methylation would be stimulated.

Table 4.1. Concentration of MeHg and THg the present of LDOM with and without UVA irradiation

		Pine tree			Chestnut tree		
		THg (ng/L)	MeHg (ng/L)	%MeHg	THg (ng/L)	MeHg (ng/L)	%MeHg
With UVA	C_0[1]	108.62	0.2080	0.1915	99.68	0.0304	0.0305
	C_t[2]	80.00	0.1501	0.1876	41.78	0.0394	0.0943
Without UVA	C_0[1]	108.62	0.2080	0.1915	99.68	0.0304	0.0305
	C_t[2]	62.00	0.2457	0.3963	56.18	0.1944	0.3460

[1] C_0 presents the initial concentrations of mercury before incubation.

[2] C_t presents the final concentrations of mercury after 3 days incubation.

4.3.5 Effect of LDOM-fractions on methylation

The molecular size distribution of DOM may be a useful indicator of DOM quality in water as well as a potential predictor of DOM on the fate (Choi et al., 1998) and bioavailability of trace chemicals (Amon and Benner, 1996). However, there is still a lack of knowledge regarding how MeHg binding changes with molecular size of DOM (Hintelmann et al., 1995). The structure of DOM is directly related to its ability to bind Hg. Different DOM constituents may affect Hg fate and transport in a different way. Therefore, in this study litter-derived DOM was separated by size-fractionation into three molecular size group: MW < 3.5 kDa, 3.5 < MW < 10 kDa, and 10 kDa < MW. Effects of DOM-fractions on Hg methylation were analyzed using approaches of batch experiments.

First, the DOC values of individual fractions measured after dialysis and the size distributions of LDOM from chestnut leaves were shown in Table 4.2. From the initial mass and mass recovered of LDOM, 7.15% was lost in the consecutive two-step dialysis procedure, which was acceptable in light of the experimental and analytical procedures. The LDOM with MW < 3.5 kDa was most enriched, accounting for 43.83% of their total DOC content. The second large was the fraction with 10 kDa < MW, accounting for 42.08% in LDOM. DOM-fraction with 3.5 < MW < 10 kDa constituted the smallest group. These results indicated that the low molecular weight fraction (MW < 3.5 kDa) was predominant, but the

macromolecule fractions were also important (Chen et al., 2010). In addition, we 131analyzed the fluorescence spectrum of individual fractions and showed in Fig. 4.5. The LDOM with MW < 3.5 kDa exhibited the highest fluorescence intensity at wavelengths from 270 to 350 nm, which implies protein-like compounds. However, the LDOM with 10 kDa < MW exhibited the highest fluorescence intensity at wavelengths more than 450 nm except ranging 270~350 nm, which implies humic-like substances.

Table 4.3 shows the effect of DOM-fractions on methylation of inorganic Hg. This study observed that methylation efficiency of Hg^{2+} was the highest in the low molecular weight fractions (MW < 3.5 kDa). These results imply that low molecular weight of LDOM (MW < 3.5 kDa) is structurally favorable to increased MeHg binding. MeHg bioaccumulation is generally known to be dependent on DOM concentration and binding (Amirbahman et al., 2002). However it is unclear which components of DOM have the highest binding strength in different freshwater environments (Ravichandran, 2004). MeHg is known to have a high affinity for reduced sulfur functional groups in DOM (Hintelmann et al., 1997; Karlsson and Skyllberg, 2003) and will bind with these preferentially over the abundant carboxylic groups and other oxygen containing groups found in DOM (Ravichandran 2004).

This research highlights the importance of low molecular weight DOM in MeHg fate. The results imply that Hg preferentially binds to small aromatic DOM

fractions in the litter extracts. Small free amino acids degradation products and fragments of aromatic humic substances may have increased binding preference for Hg due to sulfur containing functional groups. Future work should combine size fractionation with an analysis of thiol content in the DOM of different systems to further elucidate this phenomenon. The relationship between MeHg and DOM size is dynamic and may not apply in all ecosystems. The origin and structural character of the DOM will be influenced by physical and chemical parameters such as pH, DOM concentration, solar attenuation, the distribution of plant and animal matters, and the hydrological cycle variation in an aquatic catchment. Future research should examine this relationship between catchment characteristics and DOM structure in more detail in order to clarify the implications for MeHg bioavailability and transport in aquatic environment.

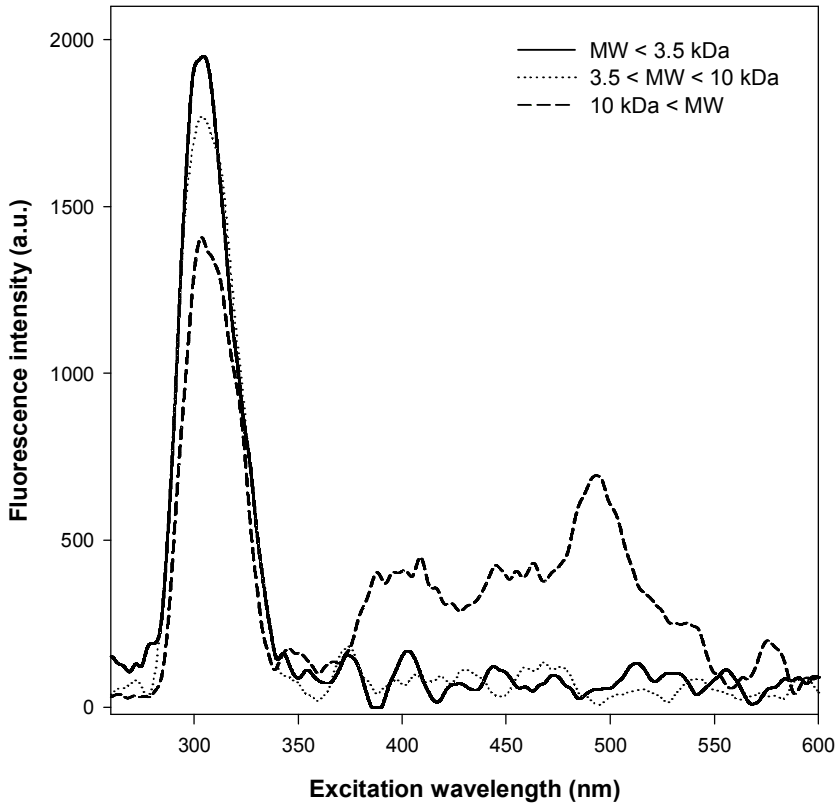

Fig. 4.5. Comparison of fluorescence spectrum of LDOM size-fractionation into three molecular size group.

Table 4.2. Total organic carbon concentration of the resulting fractions after dialysis

LDOM[1]	DOC (mg/L)	Percentage (%)	Initial mass (mg DOC)	Mass recovered (mg DOC)
MW < 3.5 kDa	170.7	43.83	41.95	38.95
3.5 < MW < 10 kDa	54.9	14.09		
10 kDa < MW	163.9	42.08		

[1] LDOM presents the extracts of chestnut leaves.

Table 4.3. The effect of DOM-fractions on methylation of Hg(II) with UVA irradiation

LDOM[1]	THg (ng/L)[2]	MeHg (ng/L)[2]	%MeHg
MW < 3.5 kDa	79.35	0.0361	0.0455
3.5 < MW < 10 kDa	83.14	0.0174	0.0209
10 kDa < MW	65.06	0.0606	0.0931

[1] LDOM presents the extracts of chestnut leaves and were 20 mg C L^{-1}.

[2] These values present the final concentrations of mercury after 3 days incubation.

4.3.6 Reactions between Hg(II) and dimethylsulfoxide in seawater

Dimethylsulfoxide (DMSO) is produced in seawater by both photo-oxidation and bacterial transformation of dimethylsulfide (DMS) which is one of the most important volatile sulfur compounds in the global sulfur cycle (Kiene and Gerard, 1994; Herscu-Kluska et al., 2008). According to Kiene and Gerard (1994), DMSO has been founded at concentrations up to 220 nM in seawater and at lower concentrations ranging from 1 to 70 nM in lakes, rivers and rainwater. Thus, this study investigated MeHg formation of Hg(II) using DMSO as methyl donor in seawater. Seawater samples were collected from the Yellow Sea, located at latitude 37° N and longitude 126° E. After filtering through a 0.45-μm nitrocellulose filter (Adventec, Tokyo, Japan), the samples were used for experiments.

This work observed that reaction between Hg(II) and DMSO in the dark condition yielded no detectable MeHg. However, DMSO can produce methyl and peroxomethyl radicals in the presence of oxygen under UV radiation according to the following reactions (Herscu-Kluska et al., 2008):

$$(CH_3)_2SO + H_2O + h\nu \rightarrow (CH_3)S(O)OH + CH_3^{\bullet} \quad (4\text{-}3)$$

$$O_2 + CH_3^{\bullet} \rightarrow OOCH_3^{\bullet} \quad (4\text{-}4)$$

And then methyl radicals produced can react with inorganic Hg(II) to format the

MeHg, as previously described in Eq. (4-2).

Fig. 4.6 shows the obtained MeHg concentration under UV irradiation. During 3 days incubation, the MeHg productions through the interactions between Hg(II) and DMSO increased with incubation time regardless salinity concentration. However, the increase in salinity concentration significantly slowed the methylation of Hg(II) by DMSO ($p<0.01$). These results seem to be directly related to the different Hg(II) speciation presented in either a lot of chloride (where Hg-chloride complexes are predominant) or a little chloride conditions (where Hg-DMSO complexes are predominant) and it can be linked to the mechanism of the methylation reaction. According to Craig and Moreton (1985), the chemical alkylation of Hg involves not only the cleavage of a CH_3-S 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 centered on Hg rather than on carbon.

From the results of this study, abiotic methylation reaction appears to be promoted via Hg-DMSO complexes, and limited when the reactant is a chloro complex due to its inhibitory effect probably because of higher stability of the Hg-Cl bond (Compeau and Bartha, 1983).

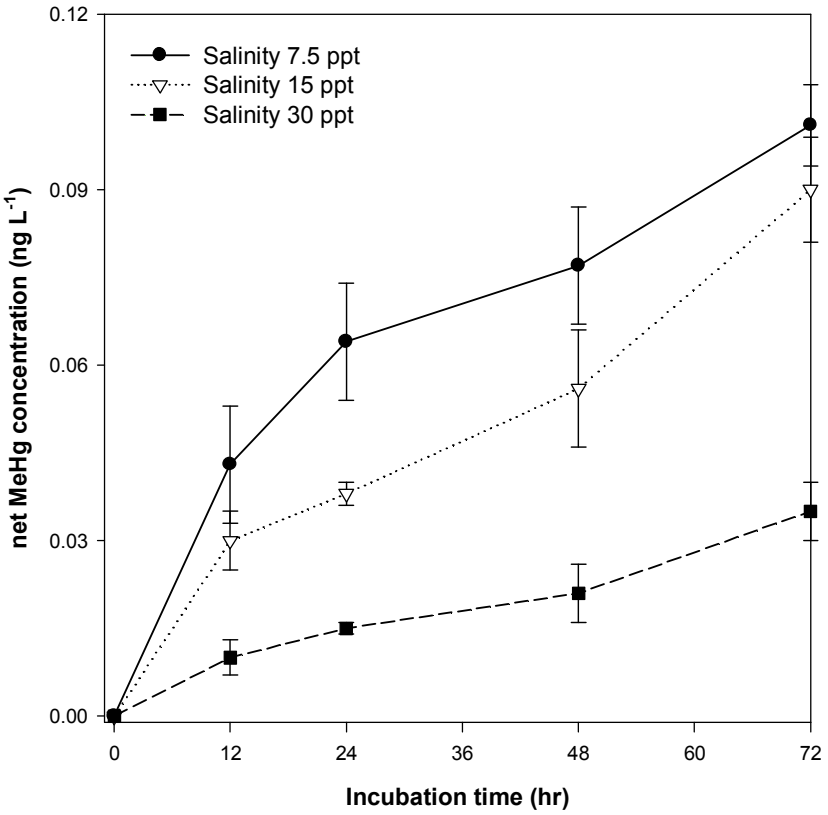

Fig. 4.6. MeHg formation of Hg(II) via DMSO in different salinity (Light intensity of UVA (λ=365 nm) is 0.8 mW cm^{-2}. $[Hg^{2+}]_{initial}$ is 100 ng L^{-1} and $[DMSO]_{initial}$ is 10 nM. Error bars represent the standard deviation of triplicate experiments).

4.4 Conclusion

In this study, the possibility of methylation of inorganic Hg(II) to MeHg in the presence of high- (i.e., litter-derived dissolved organic matter) and low-molecular-weight organic matters (i.e., acetate and dimethylsulfoxide) with and without UV irradiation in aquatic system.

First, under UV irradiation, acetate was photo-dissociated forming a methyl radical, and then it was reacted with Hg(II) to yield of MeHg. And the methylation reaction via acetate was followed the pseudo-first-order for Hg(II), and the methylation ability of acetate decreased with the solution pH increased. Second, in the Hg(II) methylation using litter-derived DOM (LDOM) as methyl donor, LDOM leads to the production of new MeHg under dark condition. This study observed that the more easily decomposable chestnut leaves relative to pine needles resulted in the higher production of MeHg. Third, through the size fractionation experiment, the importance of low molecular weight DOM in MeHg fate is obtained. The results imply that MeHg preferentially binds to small aromatic DOM fractions in the litter extracts. Small free amino acids degradation products and fragments of aromatic humic substances may have increased binding preference for MeHg due to sulfur containing functional groups. Forth, this work investigated MeHg formation of Hg(II) using dimethylsulfoxide (DMSO) as methyl donor in seawater. The results suggest that abiotic methylation reaction appears to be promoted via Hg-DMSO

complexes, and limited when the reactant is a chloro complex due to its inhibitory effect probably because of higher stability of the Hg-Cl bond.

Consequently, these results will contribute to a better understanding pf the photo-photo-generation of MeHg in natural environments. Moreover, many chemical reactions are reported as a result of photochemical methylation, but this work shows a non-dependence upon light as it occurs in the dark; especially when filter sterilized LDOM react with Hg(II) in the microbial free condition, which suggests the possibility that the reaction occurs in the natural aquatic environment. However, the UV irradiation used throughout the photochemical methylation experiments is different from natural sunlight. Further studies are still needed to validate the possibility of photochemical methylation of Hg(II) under natural sunlight.

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Chapter 5. Conclusions

5.1 Conclusions

Methylmercury (MeHg) is among the most widespread contaminants that pose severe health risks to humans and wildlife. It is important to consider that the net amount of biologically available MeHg is a function of the processes that regulate its formation, degradation and exchanges between compartments. Methylation of inorganic Hg to MeHg and demethylation of MeHg are the two most important processes in the cycling of MeHg, determining the levels of MeHg in aquatic ecosystems. Thus, this thesis suggested that the possible photochemical processes and mechanism of Hg demethylation and methylation in water with UV irradiation.

First, in the effect of natural water constituents on the photo-decomposition of MeHg and the role of hydroxyl radical, the photo-decomposition rate increased in the presence of NO_3^-, Fe^{3+}, and fulvic acid due to photosensitization effect, whereas humic acid and HCO_3^- ions reduced the decomposition rate mainly due to OH radical scavenging effect. These results support that abiotic decomposition of MeHg in surface water is mainly induced by direct and indirect photoreaction from UV irradiation, and OH radical is a critical parameter in the photo-decomposition of MeHg. Thus, the changes in natural water constituents such as NO_3^-, Fe^{3+}, and

HCO_3^- can influence the photo-decomposition rate of MeHg in the aquatic system and ultimately control the potential bioavailability of MeHg in surface waters.

Secondly, the abiotic photo-degradation of MeHg in seawater was examined. This study was found that MeHg photo-degradation rates and the DGM flux resulting from photo-degradation decreased with an increase in the chloride ion concentration, suggesting that the presence of chloride could prevent MeHg photo-degradation. The rate of MeHg photo-degradation and DGM production recorded in this study under various conditions indicated that UV radiation is responsible for MeHg photo-degradation together with chloride, and that salinity has a significant influence on MeHg photo-degradation and DGM photo-production in seawater.

Thirdly, in order to show the possible photochemical methylation process and mechanism of Hg, this study conducted the methylation of inorganic Hg experiment using DOM derived from litter as methylation donor. This study was found that Hg methylation is an abiotic chemical reaction; when filter sterilized LDOM react with inorganic Hg in the microbial free condition, they produced MeHg in the different quantities with DOM originated. Many chemical reactions are reported as a result of photochemical methylation, but this work shows a non-dependence upon light as it occurs in the dark which suggests the possibility that the reaction occurs in the natural aquatic environment.

5.2 Implications

Photo-induced decomposition of MeHg and photochemical methylation of mercury are a significant part of MeHg cycling, determining the levels of MeHg in aquatic ecosystems. Photo-demethylation in surface water is suggested to be the major sink of MeHg in aquatic environments. Especially, MeHg in freshwater could be more rapidly demethylated than that in seawater and MeHg flowing into the lake or river would be almost removed by photo-demethylation. However, MeHg flowing to seawater would be hardly removed, which could have more chance for bioaccumulation in seawater. Unfortunately, the relative importance of methylation versus demethylation in aquatic environments has yet to be clear, which is limitation in this dissertation. Much more study is required to estimate the net MeHg production rate, which is important for identifying the major source of MeHg in aquatic ecosystems.

Consequently, based on the results and conclusions, this dissertation proposed that four potential pathways could be responsible for MeHg photo-demethylation (Fig. 5.1). Pathway 1 is a direct photo-demethylation of MeHg. Although resent studies showed that MeHg present in DI water cannot be demethylated under sunlight (Hammerschmidt and Fitzgerald, 2010; Zhang and Hsu-Kim, 2010), the results of *Study 1* in this dissertation showed MeHg present in DI water can be declined exponentially under UV irradiation. Pathway 2 is hydroxyl radical ($^{\bullet}OH$)

induced degradation of MeHg, which dominated the photo-demethylation of MeHg in water. By adding scavengers of OH radical, this dissertation found that OH radical could be responsible for the degradation of MeHg in both DI water and sea water (*Study 1* and *Study 2*, respectively). Dissolved gaseous mercury (Hg^0) resulted in the product of MeHg photo-demethylation. Pathway 3 is direct transfer of electrons from photosensitized DOM (i.e., fulvic acid) to MeHg. In the presence of fulvic acid (FA), the photo-decomposition rate of MeHg increased with an increasing FA concentration (*Study 1*). In addition, a recent study using DOM isolated from natural waters found that MeHg photo-demethylation rate was not significantly decreased after adding scavengers of $^{\bullet}OH$ and $^1O_2$ (Black et al., 2012). On the other hands, Pathway 4 is inhibition effect on MeHg photo-demethylation. In the presence of bicarbonate (HCO_3^-) and humic acid (HA), the photo-degradation rate of MeHg decreased with an increasing these compounds concentration (*Study 1* and *Study 2*). Therefore, the variation of MeHg photo-demethylation pathway in different aquatic systems may be caused by their differences in chemical characteristics (e.g., DOM).

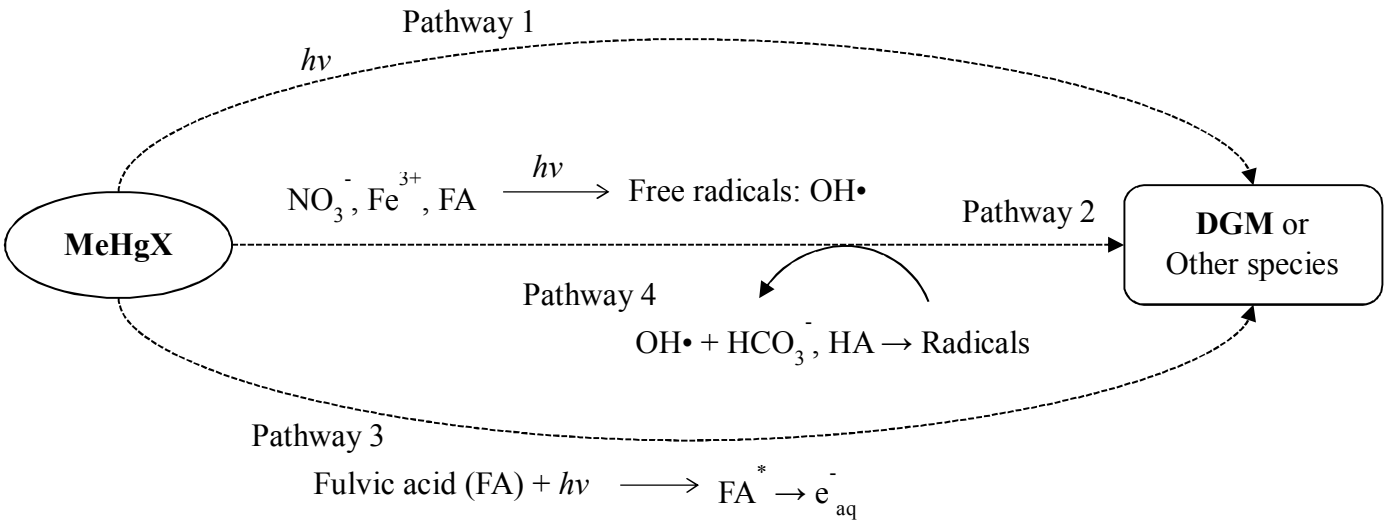

Fig. 5.1. Possible pathways of MeHg photo-demethylation to enhance (dashed arrows) and to inhibit (solid arrow with full arrow head) in aquatic environments.

국문초록

수체 내 수은의 이화학적 메틸화 및 디메틸화 반응의 기작 연구

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인간을 비롯한 야생동물에 심각한 건강상 위해를 가할 수 있는 메틸수은은 널리 분포하는 오염물질 중 하나이다. 수체 내 메틸수은의 농도는 무기수은에 의한 메틸화 반응과 메틸수은의 디메틸화 반응에 의해 좌우되며, 이 두 가지 반응은 메틸수은의 순환과정에 있어서 가장 중요한 부분이다. 이에 이 두 가지 반응에 대한 충분한 이해는 다양한 환경 매체에서의 메틸수은 농도를 예측하고 수은의 생물체 내 이용가능성을 평가하는데 매우 중요한 과정이 된다. 그러나 메틸화 및 디메틸화 반응에 영향을 미치는 요소에 대해서는 아직까지 명확하지 않으며, 이화학적 반응에 관한 자료는 더욱 미흡한 실정이다. 더욱이 수은의 메틸화 및 디메틸화 반응 중 상대적으로 더 중요한 반응 및 그 반응의 결과에 의한

영향은 수 환경 내 조건에 따라 다르게 나타날 것이다. 그럼에도 국내에서의 수체 내 메틸수은의 거동과 관련된 연구는 전무한 실정이다. 따라서 본 연구에서는 수체 내 수은의 이화학적 메틸화 및 디메틸화 반응 메커니즘을 알아보았다. 연구의 주된 목표는 (1) 여러 가지 환경인자 및 수중에 존재하는 이온물질들이 메틸수은의 광분해 반응에 미치는 영향조사, (2) 해수 내 메틸수은의 광분해 특성 및 용존가스상 수은의 배출특성 조사, 그리고 (3) 고분자 및 저분자량 유기물질에 의한 수은의 광화학적 메틸화 반응의 가능성 평가이다.

첫 번째 연구에서는 자연수에 존재하는 이온물질들(NO_3^-, Fe^{3+}, HCO_3^-)과 휴믹산(humic acid; HA) 및 풀빅산(fulvic acid; FA)과 같은 용존유기물질(DOM)이 공존하는 조건에서 인공 광(UV)을 조사함으로써, 메틸수은의 광분해 반응을 조사하였다. 메틸수은의 광분해는 유사 일차 반응(pseudo-first-order kinetics)을 따랐으며, UVA의 광 세기가 0.3 mW cm^{-2}에서 3.0 mW cm^{-2}로 증가함에 따라 광분해 속도 역시 빨라짐을 알 수 있었다. 그리고 질산 이온(NO_3^-), 철 이온(Fe^{3+}), 그리고 풀빅산(FA)이 존재하는 조건에서, 메틸수은의 광분해 속도가 상당히 증가하였는데, 이는 위 물질들이 OH 라디칼(hydroxyl radical)과 같은 광 민감성 반응물질을 발생시킴으로써 메틸수은의 분해 효과를 향상시킨 것으로 판단된다. 그러나 휴믹산(HA)과 중탄산 이온(HCO_3^-)이 존재하는 조건에서는 메틸수은의 광분해 속도가 감소하였는데, 이는 이 둘 물질이 광 반응에 의해 생성되는 라디칼과 반응하여 라디칼을 제거함으로써 메틸수은의

광분해를 저해한 결과라 사료된다. 따라서 본 연구결과는 수중 메틸수은의 광분해 반응에서 OH 라디칼은 매우 중요한 역할을 하며, 자연수 내에 존재하는 이온물질들은 라디칼 생성 및 제거 반응에 관여함으로써 수체 내 수은의 광화학적 디메틸화 반응에 영향을 미칠 수 있음을 시사하였다.

두 번째 연구에서는 해수 조건에서의 메틸수은의 디메틸화 반응 메커니즘을 조사하고자 다양한 환경인자(빛의 파장 및 세기, 염도, 그리고 메틸수은의 농도) 및 수중에 존재하는 이온물질들이 미치는 영향을, 메틸수은의 광화학적 분해에 따른 용존가스상 수은(DGM)의 발생양상을 관찰함으로써, 평가해 보았다. 해수 내 메틸수은의 광분해 속도는 조사되는 광 세기와 양의 상관관계가 있음을 보였으며, 이는 수중 메틸수은의 광분해에 광 투과율이 상당한 영향을 미친다는 것을 뜻한다. 그러나 고농도의 용존 유기 탄소(DOC) 및 염도(salinity)는 메틸수은의 디메틸화 반응을 저해하는 것으로 나타났다. 해수 내 메틸수은의 광분해에 의해 생성되는 용존가스상 수은(DGM)의 양을 측정해 본 결과, 메틸수은의 광분해가 진행되는 동안 용존가스상 수은(DGM)의 생성량이 증가됨을 볼 수 있었다. 그러나 염도(salinity)가 증가함에 따라 용존가스상 수은(DGM)의 생성량은 감소하였는데, 이는 염소 이온에 의한 메틸수은의 광분해 저해 영향에 따른 결과라 사료된다.

세 번째 연구는, 위 두 가지 연구와는 반대로, 수체 내 수은의 이화학적 메틸화 메커니즘을 알아보고자 하였다. 이를 위해 고분자 및

저분자량 유기물질(HMWOC와 LMWOC)을 이용하여, 외부 유입이나 내부 생성에 의해 수중에 존재하는 용존유기물질이 수은의 메틸화 반응에 미치는 영향을 평가하였다. 본 연구에서 사용한 메틸 공여체(methyl donor)는 저분자량 유기물질(LMWOC)로 아세테이트(acetate), 말로네이트(malonate), 그리고 디메틸설폭사이드(DMSO)이며, 고분자량 유기물질(HMWOC)로 낙엽 추출액(LDOM)이다. 아세테이트(acetate)에 의한 메틸화 반응은 유사 일차 속도식(pseudo-first-order kinetics)을 따랐으며, 수중 pH가 증가함에 따라 메틸화 속도는 감소하였다. 낙엽 추출액(LDOM)을 이용한 수은의 메틸화 반응은 빛의 조사 유/무와 상관없이 메틸수은이 생성되었다. 특히, 미생물적 요인이 전혀 배제된 조건 및 빛이 존재하지 않는 어두운 환경에서도 이화학적 반응을 통한 무기수은의 메틸화 반응이 일어난다는 본 연구 결과는 실질적인 자연환경에서도 이화학적 메틸화 반응을 통한 메틸수은의 생성 및 거동의 가능성에 대해 시사하는 바가 크다. 또한, 해수 내 수은의 DMSO에 의한 메틸화 반응의 조사 결과, DMSO에 의한 무생물적 메틸화 반응이 일어남을 알 수 있었으나, 해수 내 존재하는 염소이온과 수은의 높은 친화도에 의한 메틸화 억제 반응 또한 관찰되었다. 따라서 본 세 번째 연구 결과는, 비록 현재까지는 무생물적 이화학 반응을 통한 메틸화 반응은 대부분 빛에 의존하여 발생한다고 알려져 있지만, 빛이 없는 환경에서의 무생물적 메틸화 반응 역시 간과할 수 없는 중요한 반응임을 강조하였다.

결론적으로 본 논문에서는 수체 내 수은의 광화학적 메틸화 및 디메틸화 반응 메커니즘에 영향을 미치는 자연 환경의 조건들을 알 수 있었다. 즉, 기후변화의 영향으로 자연수 내에 도달하는 자외선 양이 증가하여 수 중 광화학적 디메틸화 반응을 촉진함으로써, 수 중에 존재하는 수은의 양은 줄어들 것이며 그 결과 수중 먹이사슬을 통한 메틸수은의 생물 농축의 가능성 역시 저감될 것으로 기대된다.

주요어: 메틸수은, 거동, 광분해, 용존유기물질, OH라디칼, 메틸화, 디메틸화

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