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Research Paper

Microbially-Mediated Methyl Iodide Production in Water Samples from an Estuarine System

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Abstract

The dynamics of aquatic systems are known to facilitate the formation of aggregates of nutrient-rich particulate matter which provide suitable substrate for microbial colonization. Although bacteria-aggregate associations often result in the degradation of Particulate Organic Matter (POM) in aquatic systems, little is known about their contribution to the production of methyl iodide (CH_3I), an environmentally important biogas that has a potential impact on atmospheric chemistry. This study investigated the role of microbial communities in the production of CH_3I and its facilitation by particle aggregation in the Tamar estuary in the South-West of England. Macroaggregates were generated through a laboratory simulated aggregation process. Assessment of bacterial abundance, production and elevated concentration of CH_3I indicated that microbial activity in the experimental setups resulted in the production of CH_3I. A statistical test of significance between aggregated and non-aggregated setups also indicated that the presence of bacterial aggregates significantly enhanced CH_3I production by about 32% of the concentration attributed to microbial activity. This suggests that estuarine systems are potentially ideal environments for microbially-mediated CH_3I production.

Keywords: *Macroaggregates, Microbial Assemblage, Methyl Iodide Production, Estuarine System, Tamar Estuary*

1. Introduction

Particle aggregates are a combination of predominantly Particulate Organic Matter (POM) in the form of marine/estuarine snow (floccules of dead algae) and faecal pellets that are obtained mainly from planktonic organisms and activities (Alldredge & Silver, 1988) and are enriched in nutrients, especially inorganic phosphorus and nitrogen (Shanks & Edmondson, 1989 and Simon et al, 2002). Particle aggregates are ubiquitous in the marine environment (Turley, 1992), lakes (Grossart & Simon, 1993), rivers (Böckelmann et al, 2000) and estuarine environment (Eisma et al, 1980 and Eisma, 1986).

The formation of these macroaggregates in the aquatic environment is facilitated by the coupling of both physical processes, such as surface mixing (Kumar et al, 2001), water circulation through tidal forcing and river discharges and biological processes that involve the self-aggregation of dead algae (Ittekkot, 1996). Formation of macroaggregates also occurs through the excretion of faecal materials by meso-grazers (Ittekkot, 1996), bacterial and algal production of mucus that facilitate adhesion (Biddanda & Pomeroy, 1988) and chemically through ionic attraction between particles. The process of aggregation, therefore, provides a mechanism for transferring organic material from the dissolved state into the particulate state (Burd & Jackson, 2009). Macroaggregation, however, begins with the formation of adhesive mucus matrices from pico- and microaggregates that act as nuclei for adsorption onto other components, such as faecal pellets (Turley, 1992). Particulate materials from the estuarine environment that are constituted in aggregates are chiefly composed of either detritus brought in by rivers or amorphous aggregations of suspended sediments (Zhang et al, 2007).

Dense microbial assemblages of which bacteria form a major part have been found to inhabit nutrient-rich macroaggregates (Artolozaga et al, 2002). In the aquatic environment, bacterial communities become associated with aggregates during formation of the latter. Aggregates provide suitable microenvironment for microbially mediated transformation of POM (Tranvik & Seiburth, 1989) into Dissolved Organic Matter (DOM) with the aid of extracellular enzymes (Cho & Azam, 1988) whilst biogases are formed as by-products. This is a form of remineralization through decomposition (Caron et al, 1982) in the aquatic environment, thus making the microbial community in aquatic environments an important group in the cycling of nutrients (Delong & Karl, 2005).

Following attachment to aggregates, bacteria remain active and can increase their population density (Kaltenböck & Herndl, 1992) and begin the enzymatic breakdown of POM (Delille & Razouls, 1994). During the process of degradation, most of the aggregates could be consumed by the associated bacterial assemblage within 2-3 days (Pomeroy et al, 1984). Therefore, in the estuarine environment, the association of microbial assemblages and aggregates is important in the conversion of POM into DOM through extracellular enzyme activity (Smith et al, 1992).

Although there are extensive discussions in the literature concerning the role of microbial assemblages in the conversion of POM into DOM in the aquatic environment (e.g. Karner & Herndl, 1992 and Smith et al, 1992), very few reports are found which directly link microbially-mediated degradation of aggregates to the production of ecologically important biogases such as methyl halides (CH_3X), especially in estuarine systems. For example, there have been reports of significant CH_3I production by a photosynthetic bacterium from the marine environment in a laboratory culture (Smythe-Wright et al, 2006 and Brownell et al, 2010).

CH_3I (the halocarbon to be investigated in this study) is considered one of the main iodine carriers from the aquatic environment to the atmosphere (Heumann et al, 1987 and Moore & Tokarczyk, 1993). The production and consumption of CH_3I in the environment, therefore, serve an important and integral part of the iodine cycle. CH_3I has also been reported to have the potential to affect the lower stratospheric ozone (Solomon et al, 1994). CH_3I has a global annual ocean to atmosphere flux rate of between 0.9-2.5 x 10^9 mol y^{-1} (i.e.128-355 Gg y^{-1}) (Moore & Groszko, 1999). This makes it one of the major and most dominant of all iodide compounds responsible for the delivery of iodine into the atmosphere from aquatic environments (Heumann et al, 1987 and Moore & Tokarczyk, 1993) as well as the main source to land (Nightingale, 2003).

The present study, therefore. Investigates: 1) the role of microbial community in the production of CH_3I (an environmentally important methyl halide) in estuarine environment through their association with macroaggregates and 2) how the process of aggregation influences the rate of microbially-mediated CH_3I production in the estuarine environment.

2. Materials and Methods

2.1. Description of the Study Site

The Tamar estuary is located in South-West England (50° 26' 35.30"N, -4° 12' 25.42"W) (Figure 1). It is known to exhibit a strong Turbidity Maximum Zone (TMZ) (Grabemann et al, 1997) in its upper reaches during summer (Uncles & Stephens, 1993) and in the middle reaches during winter due to increased river discharge (Tattersall et al, 2003). This estuary also experiences highly dynamic changes physically, chemically and biologically during the transition from the freshwater to the marine environment (Uncles & Lewis, 2001). The Tamar estuary, therefore, represents a suitable estuarine system for the study of environmentally important processes.

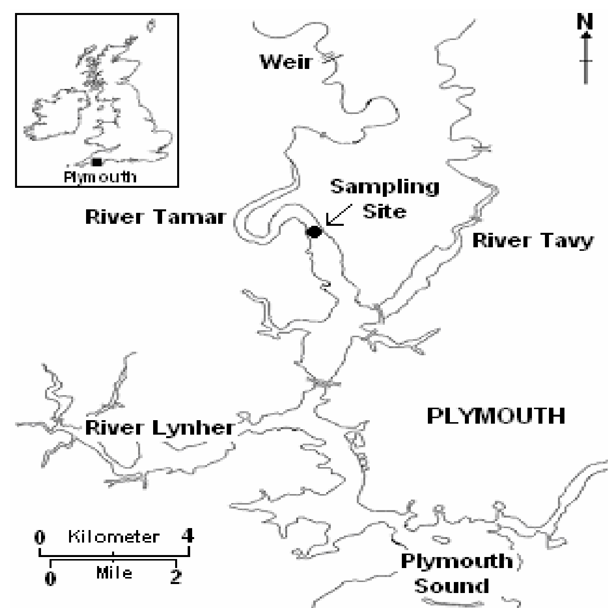

Figure 1. Map of the Tamar Estuary Showing the Sampling Site

2.2. Water Sampling and Treatment

Surface water samples were taken from the TMZ of the Tamar estuary for two laboratory-simulated experiments

using a stainless steel bucket. The samples were stored in a 10-litre glass reagent bottle (Pyrex) with a glass stopper (avoiding any headspace) at the prevailing water temperature during time of sampling and transported to the laboratory.

2.3. Experimental Design for the Laboratory Simulated Aggregation

Two experiments were conducted in the laboratory to facilitate the formation of macroaggregates. The approach taken in both experiments was adapted from Shanks & Edmondson (1989).

2.3.1. Experiment 1

The experiment was carried out in a culture cabinet under controlled physical conditions (i.e. at the prevailing *in situ* temperature of 17 ^{o}C and no illumination over a 14 days period) to assess the role of aggregation in microbially-mediated CH_3I production. Four sets of samples labelled A, B, C and D were used for the experiment (A and B for simulated aggregation, C and D as controls - for non-aggregation). Each set contained 8 subsets of samples (with 3 replicates for every subset) analysed on 8 different days within the 14 days period (See Table 1 for details).

Table 1. Four Simulated Aggregation and Non-Aggregation Sample Sets (A, B, C and D) for Experiment 1, Each With 8 Subsets (3 Replicates for Every Subset)

Days of Analysis	Aggregated		Non-Aggregated	
	Set One (Live)	Set Two (Dead)	Set Three (Live)	Set Four (Dead)
0	A1	B1	C1	D1
2	A2	B2	C2	D2
4	A3	B3	C3	D3
6	A4	B4	C4	D4
8	A5	B5	C5	D5
10	A6	B6	C6	D6
12	A7	B7	C7	D7
14	A8	B8	C8	D8

Live – Samples with no Antibiotic Treatment
Dead – Samples Treated with Antibiotic Solution

All replicates of sample sets B and D were treated with 1 ml of antibiotic solution (antimycotic cocktail), comprising of penicillin G (10000 units ml^{-1}), streptomycin sulphate (10 mg ml^{-1}), and amphotericin B (25 µg ml^{-1}) to prevent microbial growth and activity in these control samples. Glass reagent bottles (30 ml capacity) were filled with the water samples and sealed (avoiding any headspace in the process) for all replicates. Simulated aggregation was carried out for sample sets A and B by placing all replicates on low profile electric rollers (Stovall) set at a speed of 30 revolutions per minute (rpm). Replicates for sample sets C and D were placed on a bench to prevent the formation of macroaggregates. Water samples of replicates from all subsets were used to analyse for CH_3I concentration, bacterial production and enumeration over the 14 days period.

2.3.2. Experiment 2

The second experiment was carried out over 8 days using similar procedure as in Experiment 1. This experiment was carried out to ascertain the possible effect of light on the rate of CH_3I production in an estuarine system. However, sample sets A, B, C and D for this experiment were all aggregated at the prevailing *in situ* temperature of 11 ^{o}C. Simulated aggregation of sample sets A and B were carried out under continuous fluorescent illumination whilst sets C and D were kept under constant darkness in the culture cabinet. The following denotations were used in the experiments to describe the various conditions applied; aggregated samples (Rolled), unaggregated samples (Unrolled), non-antibiotically treated samples (Live), antibiotically treated samples (Dead), illuminated samples (Light) and non-illuminated samples (Dark).

2.4. CH_3I Concentration Analysis

Replicate water samples were analysed for dissolved CH_3I concentration using gas chromatographic (GC) method described by Krysell & Nightingale (1994). This method involves the stripping of dissolved gases which are then trapped through cryogenic focusing before injected into a GC unit for separation and detection (electron capture detector was used in this analysis).

2.5. Bacterial Production and Enumeration

In order to establish a possible link between the increase in bacterial activity and CH_3I production in the experimental media, there was the need to estimate the specific bacterial activity (a ratio of leucine incorporation to cell numbers). To achieve this, bacterial protein synthesis and abundance were also investigated.

Bacterial protein synthesis experiment was carried out by incubating water samples from the replicate reagent bottles (3 replicates per subset) with radiolabelled leucine. The method used in this analysis was used to estimate the rate of incorporation of radiolabelled leucine into bacterial protein as described by Smith & Azam (1992). In this method, 5 µl of L-[4, 5-^{3}H] leucine (specific activity of around 5 TBq mmol^{-1} stored at 4 ^{o}C), was added to 1.7 mL of the water sample to achieve a final sample concentration of 20 nmol L^{-1} and incubated for 1 hour. After incubation, bacterial activity was terminated by the addition of 89 µl of 100% trichloroacetic acid (TCA). Bacterial protein was then extracted and the incorporated ^{3}H-leucine counted with a scintillation counter (Wallac Winspectral 1414).

Enumeration of bacterial abundance in water samples was conducted following the technique of analytical flow cytometry (AFC) described by Shapiro (1995). Prior to counting, samples were ultra-sonicated to disintegrate aggregates present and SYBR Green I added. The method of SYBR Green I application is also explained by Marie et al (1997).

3. Results and Discussion

3.1. CH_3I Concentration in Experimental Media

The results from the two experiments carried out in this study clearly show that there were changes in CH_3I concentration in the experimental media which peaked at Day 8 and 6 for Experiment 1 and 2 respectively (Figure 2 a-b). The Live-Rolled samples of Experiment 1 recorded the highest mean concentration of 6.26 ± 0.17 pmol L^{-1} whilst Live-Light samples of Experiment 2 had the highest of 5.26 ± 0.26 pmol L^{-1}.

Between the two aggregated sets of treatments in Experiment 1 (Live-Rolled and Dead-Rolled samples), the highest cumulative concentration difference of 1.64 pmol L^{-1} of CH_3I was recorded on day 8 (Figure 3a). This result was identical to that of Experiment 2 (Figure 3b) when the highest cumulative concentration difference of 1.65 pmol L^{-1} was recorded between Live-Light and Dead-Light samples on Day 6 of the experiment. With Experiment 1 as reference, the average elevation of CH_3I concentration for the entire study period was estimated at 0.42 ± 0.09 pmol L^{-1} d^{-1}. However, another highly elevated CH_3I concentration difference of 1.16 pmol L^{-1} was observed between Live-Unrolled and Dead-Unrolled samples during day 12 of the experiment (Figure 3a).

A conducted ANOVA revealed that there was statistically significant difference between all treatments of Experiment 1 (with p-values of < 0.0001). A further comparison of the change in CH3I concentration between Live-Rolled and Dead-Rolled, Live-Rolled and Live-Unrolled, Live-Unrolled and Dead-Unrolled over the entire study period also indicated a statistically significant difference ($p < 0.05$) between all respective data sets. There was, however, no statistical difference between Dead-Rolled and Dead-Unrolled over the same study period ($p = 0.50$).

From Experiment 2, changes in CH_3I concentration over the study period exhibited a significant difference between Live-Light and Dead-Light as well as between Live-Dark and Dead-Dark (both with p-values of < 0.05). The estimated rates of CH_3I increase in the experimental media were 0.38 ± 0.23 pmol L^{-1}d^{-1} and 0.37 ± 0.16 pmol L^{-1}d^{-1} for non-illuminated aggregated samples (Live-Dark) and illuminated samples (Live-Light) respectively. From these estimates, the elevation of CH_3I concentration in the presence of light is statistically indistinguishable (p = 0.724) from concentration in the dark. The Dead-Light and Dead-Dark data sets also had no significant difference between them ($p = 0.927$).

3.2. Bacterial Production and Abundance

Based on the protein synthesis incubation, a maximal ^{3}H-leucine incorporation rate (peak protein synthesis) of 670 ± 90 pmol L^{-1}h^{-1} and 420 ± 50 pmol L^{-1}h^{-1} were recorded for Live-Rolled and Live-Unrolled samples respectively during Experiment 1. From these results, an estimated difference of 250 pmol L^{-1} h^{-1} (student's t-test; $p < 0.01$), representing about 37.3% of the total productivity, was observed between the two sample sets on Day 8 of the experiment when peak CH_3I elevation was observed. This may represent the rate at which aggregate-attached bacteria contributed to the overall bacterial activity in the experimental media.

Enumeration of bacterial abundance from Experiment 1 showed maximum mean cell counts of 1.14 ± 0.14 x 10^9 cells L^{-1} and 0.34 ± 0.03 x 10^9 cells L^{-1} for Live-Rolled and Live-Unrolled respectively. Between aggregated and non-aggregated treatments, an estimated difference of 0.79 x 10^9 cells L^{-1} was recorded. This result coincided with the period of highest bacterial activity (with respect to leucine incorporation) and highest CH_3I concentration on Day 8 of the experiment. This suggests that the recorded increase in the overall bacterial activities at those periods may be as a result of an increase in bacterial abundance. The

population of aggregate-attached bacteria was estimated to constitute approximately 37% of the total recorded bacterial abundance from all aggregated samples. Over the 14-day period of the experiment, the mean bacterial abundance for the Live-Rolled samples (a representative of the estuarine environment) was estimated at 0.66 ± 0.32 x 10^9 cells L^{-1}.

insignificant correlations for both aggregated (p = 0.777) and non-aggregated (p = 0.884) samples. The relationship between bacterial abundance and CH_3I concentration however became explicit when bacterial specific activities based on protein synthesis were used in the correlation analyses. From these analyses there were statistically significant linear relationships between bacterial specific

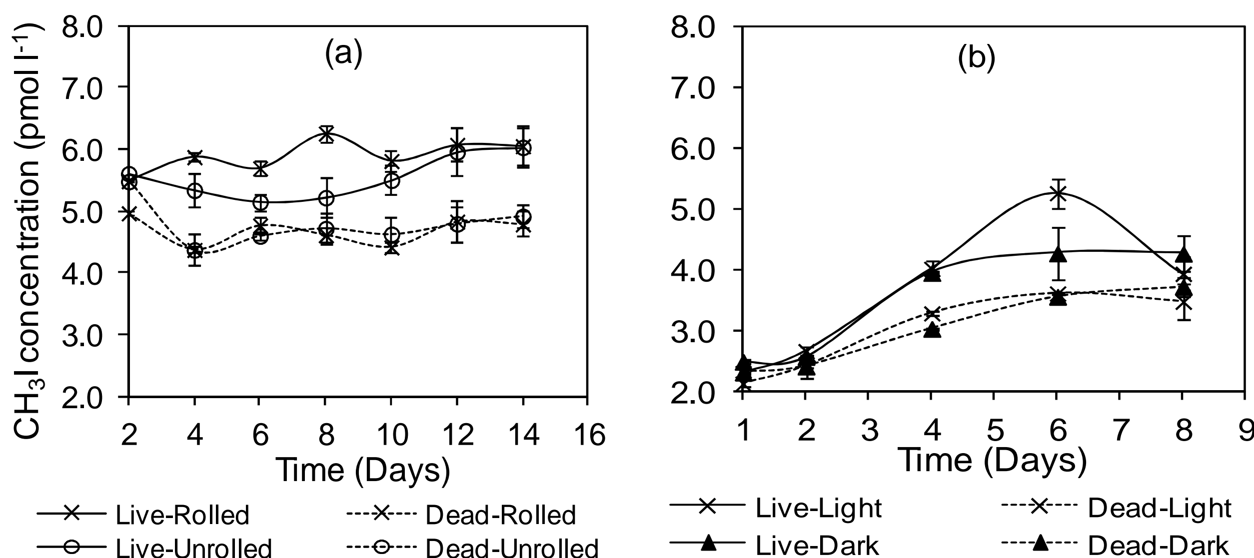

Figure 2 (a & b). Changes in the Mean CH_3I Concentration in (a) Experiment 1: Aggregated and Non-Aggregated Samples and (b) Experiment 2: Illuminated and Non-Illuminated Samples

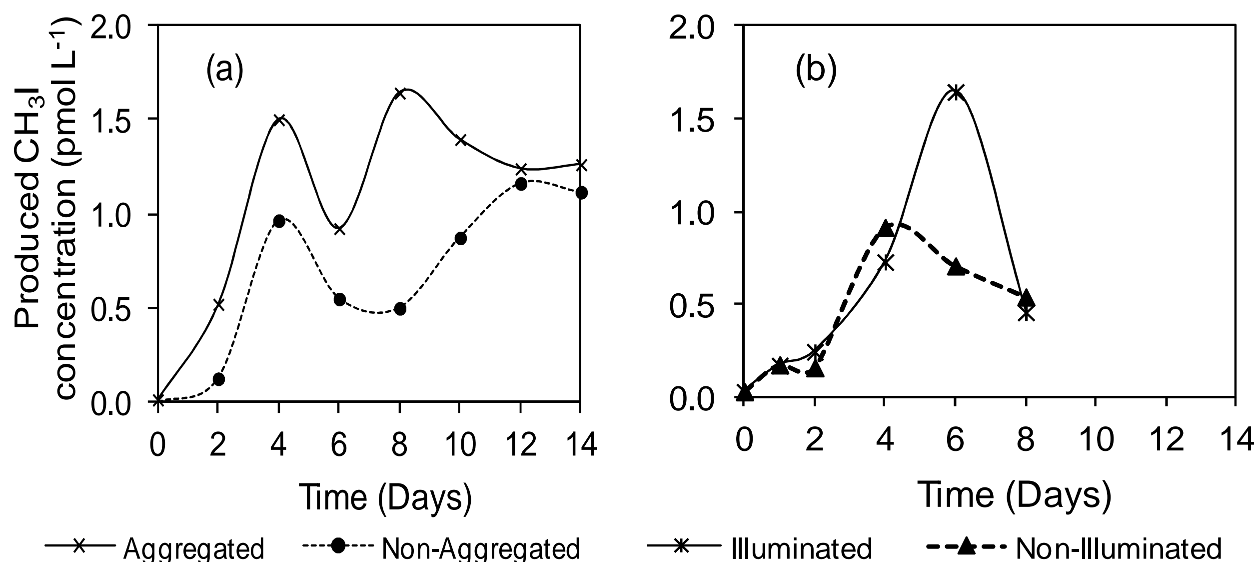

Figure 3 (a & b). Cumulative CH_3I Concentration Difference (pmol L^{-1}) in (a) Aggregated and Non-Aggregated Samples and (b) Illuminated and Non-Illuminated Samples

A test of significant correlation through Pearson's correlation analyses was carried out to ascertain the relationship between bacterial abundance and CH_3I concentration. These analyses yielded statistically

activity and the recorded CH_3I concentration for both the aggregated ($p < 0.05$; Figure 4a) and non-aggregated ($p < 0.001$; Figure 4b) samples. However, the relationship was positive only for the aggregated samples.

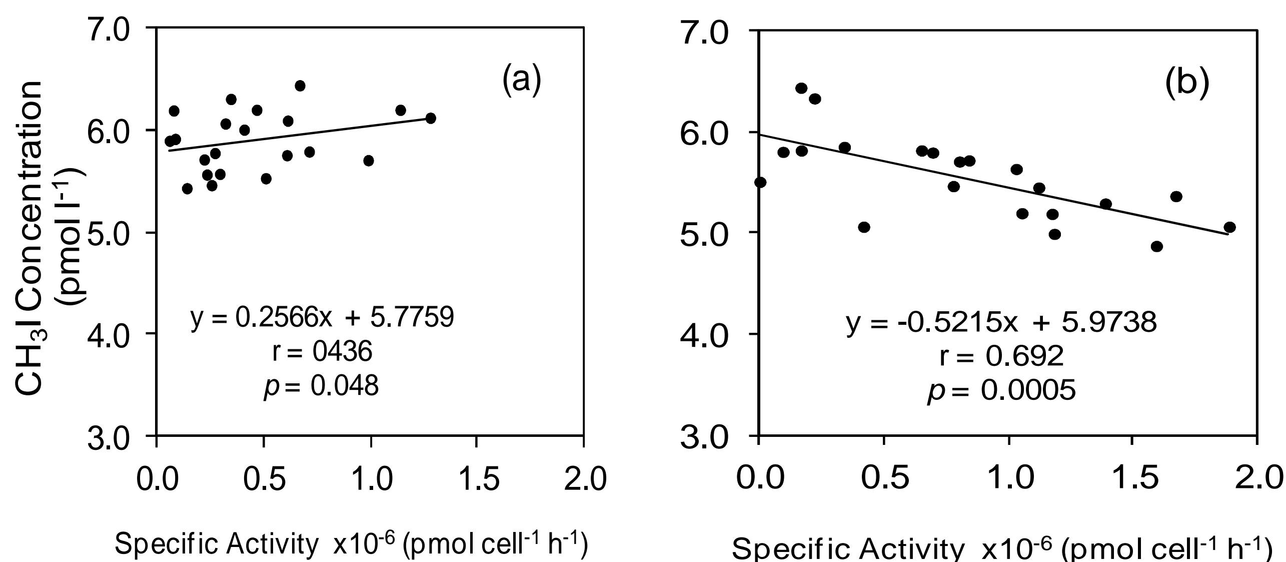

Figure 4 (a & b). Relationships Between CH_3I Concentrations and Bacterial Specific Activity Over the Study Period in (a) *Live-Rolled*, (b) *Live-Unrolled* Samples

3.3. Microbially-Mediated CH_3I Production

Bacterial association with macroaggregates in the aquatic environment until now has never been linked to the production of halocarbons in general and CH_3I in particular. The present study has shown such an association to be a source of aquatic CH_3I. This deduction is based on the comparison between antibiotically treated and non-treated aggregated samples through laboratory simulations.

With an estimated CH_3I elevation rate of 0.42 ± 0.09 pmol L^{-1} d^{-1}, microbially-mediated production in the estuarine water samples may be responsible. This production as a result of microbial activity is further enhanced by the association of microbial communities with macro-aggregates present in the experimental media. The results also revealed that microbially-mediated CH_3I productions peaked on Day 8 and 6 for the two experiments respectively after which there were no further increments. This could be attributed to substrate limitation in the experimental media (Ploug et al, 2002) beyond the observed days since microbial assemblages are capable of consuming the aggregates to which they are associated with within a short period (Pomeroy et al, 1984).

Since elevated CH_3I concentration from this study has been attributed to microbial activity, the outcome of a regression analyses between bacterial specific activity and CH_3I concentration exhibited significant correlations, which were positive and negative for aggregated and non-aggregated samples respectively. This observation could be due to 1) the presence of two different groups of microbial communities (one group producing and the other consuming CH_3I) in the same sample; 2) the same microbial community switching between production and consumption depending on changes in physical, chemical and biological conditions in the media at any given period and 3) grazing (Zubkov et al, 2000) by heterotrophic nanoplankton associated with aggregates (Caron et al, 1982) on CH_3I producing bacteria at periods when bacterial abundance and growth are at their threshold.

However, from further studies, it is likely that the removal of CH_3I observed in this study could be attributed to the presence of other microbial assemblages who consume CH_3I (Asare, 2007) in addition to those producing CH_3I in the experimental media. Other studies (e.g. Vannelli et al, 1999 and McDonald et al, 2002) have confirmed that, certain members of the microbial community (methylotrophic bacteria) are indeed capable of oxidizing methyl halides (including CH_3I). This could imply that all the observed concentrations of CH_3I from this study could only represent the net concentrations between the two different microbial processes of production and consumption within the experimental media whereby the absence of macroaggregates in non-aggregated setups translated into a relatively reduced rate of microbially-mediated CH_3I production. It may also, therefore, suggest that there could be a higher rate of CH_3I removal by other processes than production by microbial community in the experimental media with little or no aggregates (Asare, 2007) leading to a decline in net CH_3I concentration with increasing bacterial activity (Figure 4b).

On average, microbial activity from this study elevated CH_3I concentration by about 22% (aggregated) and 15% (non-aggregated) of the total recoverable CH_3I

concentration from the experimental media whilst the process of aggregation (the presence of macroaggregates) had the potential to enhance the microbial CH_3I production process by an additional 7% of the total recovered CH_3I concentration (which translates into approximately 32% of the fraction attributed to microbially-mediated production). From these findings, it can be deduced that, attached bacteria which represent on average about 37% of the total bacterial population were responsible for elevating the overall microbial CH_3I production by an additional 32%. In the aquatic environment, although attached bacteria are more active than their free-living counterparts (Ghiglione et al, 2007 and Grossart et al, 2007), their abundance is generally lower than the free-living forms as was observed in this study. For example, some of the reported percentage constituents of aggregate-attached bacteria range between 6.8% and 22% (Iriberri et al, 1987; Unanue et al, 1992 and Griffith et al, 1994) of the total bacterial abundance. The recorded percentage of 37% from this study although comparatively high, is nevertheless not unusual. On the other hand, being more metabolically active, aggregate-attached bacteria often contribute higher activity than their free-living counterparts (Turley et al, 2000). This supports the observation from this study implying that aggregate-attached bacteria are responsible for a significant portion of the microbially-mediated CH_3I production recorded.

Changes in physical, chemical and biological conditions are known to affect the activity of microbial communities in natural water (Turley, 1993). In order to eliminate the possibilities of any such effects resulting from variations in experimental conditions, samples run both in light and in the dark indicated that variation in light conditions has no significant influence on the pattern of bacterial activity with respect to the overall rate of CH_3I production as observed in this study.

4. Conclusion

In conclusion, microbial association with POM in the estuarine environment results in the net production of CH_3I with the rate of microbially-mediated CH_3I production being enhanced by the process of aggregate formation. There is also evidence that the process of CH_3I production is mirrored by other processes that consume CH_3I present in the experimental media. Based on these findings, microbial activities could be responsible for CH_3I production in estuarine systems and therefore play a crucial role in the global CH_3I cycle whilst having the potential to significantly affect the atmospheric and tropospheric CH_3I concentration. These findings must, however, be applied to the natural estuarine environment with caution since it was entirely based on laboratory simulated experiments.

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