Application of Gas Chromatography to Exuded Organic Matter Derived from Macroalgae

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1. Introduction

Macroalgae are the most important primary producers in coastal environments, because their productivity per square community area is comparable to that of tropical rain forests (Mann, 1973; Yokohama et al., 1987). Although a half of the products constitute the organic matter of algal body (Hatcher et al., 1977), considerable part of them would be exuded and dissolved into ambient seawater (Khalilov & Burlakova, 1969; Sieburth, 1969; Hatcher et al., 1977; Abdullah & Fredriksen, 2004; Wada et al., 2007). The organic materials released into ambient seawater induces some alteration of marine and atmospheric environments. For example, some volatile compounds released from macroalgae would escape into air, and play as ozone-depleting substances (Lovecock, 1975; Laturnus et al., 2010). Phenolic compounds are known as a kind of exudates, which acts as a form of defense to the algal body from herbivores (van Alstyne, 1988). A part of the phenolic compounds are likely to dissolve into seawater, and the light shielding role as a component of colored organic matter was suggested in coastal environments (Wada et al., 2007). In addition to these compounds, carbohydrates such as mucopolysaccharides are important component of released organic matter on the body surface, and a part of them is considered to be released into seawater (Wada et al., 2007). It is known that bacterial community acting on the macroalgae is variable depending on the carbohydrate composition of exudates (Bengtsson et al., 2011), and the bacterial community structure probably changes the carbon flux around algal bed. The characterization of organic compounds derived from macroalgae is necessary to elucidate the biogeochemical role of macroalgae, because the effect to biogeochemical processes is variable depending on each organic compound. Thus, quantitative and qualitative approaches have been carried out so far. Application of gas chromatography (GC) would be effective for some of these exuded compounds. In this review, we focused on the analytical methods using GC for analysis of compounds originated from macroalgae.

2. Volatile halogenated organic matter

2.1 Macroalgal release of volatile halogenated organic matter

Depletion of ozone in stratosphere has been focused as one of the most serious global environmental issues, and reaction of halogenated compounds with ozone has been
recognized as the destruction mechanisms of ozone (Farman et al., 1985; Crutzen & Arnold, 1986; Solomon, 1990; Anderson et al., 1991). Such volatile halogenated compounds are not only originated from anthropogenic sources, but also biogenic sources such as macroalgae. Due to such interests, volatile halogenated organic compounds (VHOC) have been intensely studied so far, and development of analytical technique of macroalgal VHOC has been recognized.

2.2 Pre-treatment before injection

When analysis of macroalgal VHOC in seawater samples are carried out, pre-treatment of the sample is important procedure for achievement of high recovery yield. There are several kinds of methods such as closed-loop stripping (CLSP), headspace, liquid-liquid extraction, purge-and-trap (P&T) and solid-phase micro extraction (SPME) methods (Table 1). Within these methods, CLSP had been applied in earlier year (Gschwend et al., 1985; Newman and Gschwend, 1987), but there is a problem that the CLSP method is inappropriate for analyses of extremely volatile compounds (e.g., CH₃Cl and CH₃Br) or relatively involatile compounds (e.g., CBr₄ and CHI₃) (Gschwend et al., 1985). In headspace method, seawater samples were brought into equilibrium with a gas (usually nitrogen) (Lovelock 1975; Manley & Dastoor, 1987; Manley & Dastoor, 1988; Manley et al., 1992). In the case that the concentrations of target compounds were low, cryo-concentration had been also applied (Manley & Dastoor, 1987; Manley & Dastoor, 1988; Manley et al., 1992). Liquid-liquid extraction is that solvent containing internal standard is added into seawater sample, and a part of the solvent phase was injected to GC. This method allows us to make analyses with simple instrumental set-up and the total analysis time is short (Abrahamsson & Klick, 1990; Laturnus et al., 1996; Manley & Barbero, 2001). P&T method have been the most widely-applied method in recent years. Sample seawater is purged with nitrogen or helium gases, and the target compounds in the gas phase were concentrated with cold trap (sometimes with adsorbent; Ekdahl & Abrahamsson, 1997). After degassing, the trapped compounds were transferred into GC instrument by heating (Schall et al., 1994; Nightingale et al., 1995; Laturnus et al., 2004; Weinberger et al., 2007; Laturnus et al., 2010). Recently, SPME technique has been also applied for VHOC originated from macroalgae. SPME fibre is used for trapping of VHOC after purging with pure nitrogen. In case of determination of VHOC in seawater spiked by standards, quantitative quality was confirmed at the concentration level around 100 ng l⁻¹ of VHOC (Bravo-Linares et al., 2010).

2.3 GC Instruments

Analyses of extracted VHOC compounds have been mainly carried out by gas chromatography with electron capture detector (GC-ECD) (e.g., Manley & Dastoor, 1987; 1988; Schall et al., 1994; Laturnus et al., 1996; Manley & Barbero, 2001; Laturnus et al., 2004) or gas chromatography/mass spectrometry (GCMS) (e.g., Gschwend et al., 1985; Newman & Gschwend, 1987; Marshall et al., 1999; Bravo-Linares et al., 2010). Although GC-ECD has been commonly applied, it is necessary to confirm the retention time of each compound using authentic standards (Giese et al., 1999) or GCMS (Manley and Dastoor, 1987) to identify each component. On the other hand, application of GCMS has an advantage that it is reliable to characterize the compounds based on the mass spectrum. Capillary column is also an important part of GC for separation of each compound, and various kinds of
columns (e.g., SE 54, BP-624, Rtx 502.2) have been applied. Most of them have mid-polarity, which have been commercially recommended for analysis of volatile organic compounds.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Target compounds</th>
<th>Sample</th>
<th>Instruments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headspace</td>
<td>CH₃Cl, CH₃Br, CH₃I</td>
<td>Seawater</td>
<td>GC-ECD</td>
<td>Lovelock (1975)</td>
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<td>Closed loop stripping</td>
<td>CHBr₃, CHBr₂Cl, CH₂Br₂</td>
<td>2 Brown algae and 2 Green algae,</td>
<td>GCMS</td>
<td>Gschwend et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>(CH₃)₂CHBr, CH₃CH₂CH₂Br, CH₃(CH₂)₃Br, CH₃I, C₂H₅I, C₃H₇I, C₄H₉I, C₅H₁₁I, C₆H₁₃I, CH₂Br₂, CHBr₃, CH₃₂Br, CHBr₂I, CH₃SC₃H₇, CH₃SSCH₃</td>
<td>1 Brown algae</td>
<td>GCMS</td>
<td>Newman &amp; Gschwend (1987)</td>
</tr>
<tr>
<td></td>
<td>Headspace</td>
<td>CH₃Cl, CHBr, CH₃I</td>
<td>1 Brown algae and seawater</td>
<td>GC-ECD</td>
</tr>
<tr>
<td></td>
<td>Headspace</td>
<td>CH₃I</td>
<td>5 Brown algae</td>
<td>GC-ECD</td>
</tr>
<tr>
<td></td>
<td>Headspace</td>
<td>CHBr₃, CH₂Br₂, CH₃I</td>
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</tr>
<tr>
<td></td>
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<td>1 Brown algae</td>
<td>GC-ECD</td>
</tr>
<tr>
<td>Liquid-liquid</td>
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<td>Seawater</td>
<td>GC-ECD</td>
<td>Klick (1992)</td>
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<td>CH₂Br₂, CHBrCl₂, CH₂CII, CHBr₂Cl, 1,2-C₄H₄Br₂, CH₂I₂, CHBr₃</td>
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<td>Laturnus (1996)</td>
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<td>Seawater</td>
<td>GC-ECD</td>
<td>Abrahamsson &amp; Ekdahl (1996)</td>
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<td></td>
<td>CHBr₃</td>
<td>1 Green algae</td>
<td>GC-ECD</td>
<td>Manley &amp; Barbero (2001)</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Target compounds</td>
<td>Sample</td>
<td>Instruments</td>
<td>References</td>
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<tr>
<td>Purge and trap</td>
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<td>GC-ECD</td>
<td>Laturnus (1995)</td>
</tr>
<tr>
<td>Purge and trap</td>
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<td>GC-ECD</td>
<td>Abrahamsson et al. (1995)</td>
</tr>
<tr>
<td>Purge and trap</td>
<td>CHBr₃, CHCl₃, CH₃CCl₃, CH₂Br₂, CH₂I₂, CH₂Br, CH₂Br₂Cl, CH₂ClI, CCl₄, Cl₂C=CCl₂, Cl₂C=CHCl, C₂H₅I, sec-C₃H₇I, C₃H₇I</td>
<td>1 Green algae</td>
<td>GC-ECD</td>
<td>Mtolera et al. (1996)</td>
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<td>Pedersen et al. (1996)</td>
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<td>GC-ECD</td>
<td>Laturnus et al. (1998)</td>
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<td>Giese et al. (1999)</td>
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<td>GCMS</td>
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<td>Pre-treatment</td>
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<td>Sample</td>
<td>Instruments</td>
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<td>1 Red algae</td>
<td>GC-ECD</td>
<td>Laturnus et al. (2000)</td>
</tr>
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<td>Purge and trap</td>
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<td>1 Red algae</td>
<td>GCMS</td>
<td>Marshall et al. (2000)</td>
</tr>
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<td>GC-ECD</td>
<td>Baker et al. (2001)</td>
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<td>HRGC-ECD/MIP AED</td>
<td>Laturnus (2001)</td>
</tr>
<tr>
<td>Purge and trap</td>
<td>CHCl₃, C₂HCl₃, C₂Cl₄, CHBr₃, CH₂Br₂, CHClBr₂, CH₂Br₂I, CH₂I₂, CH₃I, C₂H₅I, CH₂CII, C₂H₇I, iso-C₃H₇I, C₄H₁₀, sec-C₄H₁₀</td>
<td>1 Brown algae, 4 Green algae and 1 Diatom</td>
<td>GC-ECD</td>
<td>Abrahamsson et al. (2003)</td>
</tr>
<tr>
<td>Purge and trap</td>
<td>C₂Cl₄, C₂HCl₃, CCl₂, CHCl₂I, CH₃Cl₂, CH₂Cl₂, C₂CCl₃, CH₂I₂, CH₂Br₂, CHBr₂I, CHBrCl₂</td>
<td>3 Brown algae, 1 Red algae and 1 Green algae</td>
<td>GC-ECD</td>
<td>Laturnus et al. (2004)</td>
</tr>
<tr>
<td>Purge and trap</td>
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<td>seawater</td>
<td>GCMS</td>
<td>Jones et al. (2009)</td>
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<td>Purge and trap</td>
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<td>1 Brown algae</td>
<td>Not described</td>
<td>Laturnus et al. (2010)</td>
</tr>
<tr>
<td>SPME</td>
<td>CH₃Br, CH₂I₂, CCl₂=CCl₂, CHCl₂Br</td>
<td>4 Brown algae, 2 Red algae and 2 Green algae</td>
<td>GCMS</td>
<td>Bravo-linares et al. (2010)</td>
</tr>
</tbody>
</table>

Table 1. List of studies and methodologies used on VHOC originated from macroalgae
2.4 Estimation of the importance of macroalgal VHOC

In order to evaluate macroalgal release of VHOC, an effective approach is seawater sampling in and out of algal bed in the field or incubation experiment in closed system. Analysis of seawater sample often showed higher concentration of VHOC around algal bed compared with offshore region, strongly suggesting the significant release of VHOC from macroalgae (Lovecock, 1975; Manley & Dastoor, 1987; Klick, 1992; Manley et al., 1992; Nightingale et al., 1995). The incubation experiment can provide production rate of VHOC for each macroalgal species (Gschwend et al., 1985; Manley & Dastoor, 1987). Assuming that the estimated production rate of VHOC is comparable with other macroalgae, some researchers estimated global VHOC production rates (Gschwend et al., 1985; Manley & Dastoor, 1987). Although they multiplied the production rate per algal biomass which is experimentally defined by standing crop in global ocean, the most serious problem is that no detailed investigation on algal biomass in global ocean has been reported yet. Calculation of algal biomass in global ocean was estimated based on coastal length by De Vooys (1979) who also provided an estimation of primary production in global ocean as 0.03 PgC y\(^{-1}\). No estimate of biomass has been published by other researchers, but primary production was revised by Charpy-Roubaud & Sournia (1990). They made estimation of primary production as 2.55 PgC y\(^{-1}\) based on algal community area, which is two orders of magnitude higher than the values of De Vooys (1979). Such discrepancy with the previous estimates implies that there is no reliable value of macroalgal parameters such as biomass and productivity in global ocean. In addition to the development of analytical technique, estimates of algal biomass in global ocean will be also required for understanding the macroalgal contribution to ozone-depletion.

3. Phenolic compounds

3.1 Macroalgal phenolic compounds

Macroalgae synthesize phenolic compounds, and a part of them is likely exuded (Paul et al., 2006). Considering that the phenolic compounds accumulated in the outer cortical layer of the thalli (Shibata et al., 2004; Paul et al., 2006), these materials would be actively released. Dissolution of phenolic compounds was also supported by in situ field experiment (Wada et al., 2007), in which absorption spectra of macroalgal excretion were relatively similar to those of the materials containing aromatic ring (lower exponential slope of the absorption spectra) (Blough & Del Vecchio, 2002). The UV absorbing property of phenolic compounds (Łabudzińska & Gorczyńska 1995) suggests the attenuation of UV radiation to seawater by phenolic compounds originated from macroalgae. Since biological activity in surface seawater is affected by UV penetration (Blough & Del Vecchio, 2002), analysis of phenolic compounds shows the interaction between macroalgae and other marine organisms.

3.2 Cupric oxide oxidation

Analytical procedure for macroalgal phenolic compounds is cupric oxide (CuO) oxidation method, in which polymeric compounds are degraded to small molecules that can be quantified by GC instrument. Although this technique has been mainly applied to lignin which also contains phenolic structure, some researchers had applied it to macroalgal materials (Goni & Hedges, 1995). Generally, the samples were reacted with CuO at 150-
170°C for 3 h under alkaline conditions, and acidified after the reaction. The oxidized fraction was extracted with ethyl ether, and the solvent was evaporated. The products were dissolved in pyridine, and they are derivatised to trimethylsilyl derivatives for gas chromatographic analysis. Molecules containing aromatic ring were identified using GC or GCMS (Goni & Hedges, 1995), but a possibility that some of them originated from protein, because 3 kinds of amino acids (phenylalanine, tryptophan and tyrosine) have aromatic group in their molecule. Since \( m \)-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid has been suggested as non-amino acid derived from organic compounds containing phenolic structure such as tannin, these products are likely useful as an indicator of macroalgal materials when we use CuO oxidation method.

### 3.3 Application of CuO oxidation method for macroalgal exudates

There are just a few studies on analysis of phenolic compounds using CuO oxidation method (Goni & Hedges, 1995), but they examined various species belonging to brown (Nereocystic luetkeana, Fucus fardneri, Costaria costata, Desmarestia viridis and Sargassum muticum), green (Ulva fenestrate and Codium fragile) and red algae (Opuntiella californica, Odonthalia floccose). In their study, there were 11 kinds of aromatic products after CuO oxidation, and \( m \)-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid were determined as non-amino acid derived materials as described above. The contents of these two products in body weight of algae were the highest in the brown algae (1.5-2.3 and 4.3-14 times than red and green algae for \( m \)-hydroxybenzoic acid and 3.5-dihydroxybenzoic acid), suggesting that production of phenolic compounds by brown algae is larger than other macroalgal groups.

### 4. Carbohydrates

#### 4.1 Release of carbohydrates

Macroalgal body is covered by sticky mucus due to excretion of materials containing mucopolysaccharides for protection of their body from external stress such as desiccation (Percival & McDowell, 1981; Painter, 1983) and changes in ambient ion condition (Kloareg & Quatrano, 1988). Since the monosaccharide composition of mucopolysaccharides is different among brown, red and green algae, monosaccharide analysis is effective to understand the original source of mucopolysaccharide. Here we described the chemical characteristics of carbohydrate species originated from these algae, and reviewed the methodological aspects of monosaccharide analysis.

#### 4.1.1 Brown algae

The major mucopolysaccharide of brown algae is alginate, which is made up of two kinds of uronic acids (mannuronic acid and guluronic acid). The molar ratio of mannuronic acid to guluronic acid (\( M/G \)) ranges from 0.25 to 2.25 (Kloareg & Quatrano, 1988). Another well-known mucopolysaccharide is fucan, which comprises L-fucose and sulphate as major constituents. Actually, fucans are heterogeneous group of polysaccharide, and the fucose content ranges from 55 to 96% of total monosaccharides (Marais & Joseleau, 2001; Bilan et al., 2004; 2006). Considering these monosaccharide composition, quantification of uronic acids and fucose would be available for characterization of mucopolysaccharides of brown algae.
4.1.2 Red algae

Carrageenans are mainly extracted from *Chondrus*, *Gigartina*, *Eucheuma* and *Hypnea*, and they are highly sulphated galactans (De Ruiter & Rudolph, 1997). These polysaccharides are categorized into several families based on the position of the sulphate groups. Agars are also a kind of galactan, but this is a low sulphated polymer, which is often extracted from *Gelidium*, *Gracilaria*, *Ahnfeltia*, *Acanthopeltis* and *Pterocladia* (Kloareg & Quatrano, 1988). These polysaccharides are commonly found in red algae, and galactose is the major constituents.

4.1.3 Green algae

Mucopolysaccharides originated from green algae are highly branched sulphated heteropolysaccharides such as xylogalactoarabinans, glucuronoxylorhamnans and rhamnoxylogalacto-galacturonan (Kloareg & Quatrano, 1988).

4.2 Depolymerization and derivatization of carbohydrates

4.2.1 Depolymerization

As mentioned above, monosaccharide composition reflects the original sources of the mucopolysaccharides released into extracellular region. For GC analysis of monosaccharide composition, it is necessary to depolymerize the polysaccharides and derivatize the monosaccharides in volatile forms. Well-known depolymerization and derivatization procedures are 1) hydrolysis-alditol-acetate or -trimethylsilyl and 2) methanolysis-trimethylsilyl methods. In this section, we describe the features of the methods, and introduce the application to macroalgal carbohydrates.

4.2.1.1 Acid hydrolysis

Various acid solutions have been examined for hydrolytic depolymerization of polysaccharides in marine samples, and detail of the results was previously reviewed (Panagiotopoulos & Sempere, 2005). Briefly, acid solutions which are mostly examined are HCl and H$_2$SO$_4$, and the recovery has been evaluated. Mopper (1973) compared these two acid solutions at same concentration (2 N), and suggested that HCl efficiently depolymerizes carbohydrate in ancient sediments but it led destruction of those in anoxic sediment (Mopper 1977). Acid strength is also important factor controlling the recovery yields. Two step of hydrolysis reaction, in which pre-treatment was carried out in 72% H$_2$SO$_4$ solution at ambient temperature and diluted sample (1-2 N) was heated at 100°C, are often used in order to achieve complete hydrolysis. These two steps reactions would be relatively strong, and sometimes induce loss of recovery of pentoses (Mopper, 1977), but the total yields of aldoses tend to be higher (Skoog & Benner, 1997). Mild hydrolysis was performed in solutions of dilute H$_2$SO$_4$, HCl, CHCl$_2$COOH, H$_3$PO$_4$, (COOH)$_2$ and trifluoacetic acid. In some cases, their recoveries are comparable with those of strong hydrolysis reaction (Panagiotopoulos & Sempere, 2005). However, they would be inappropriate for refractory species of carbohydrates such as cellulose (Skoog & Benner, 1997).

4.2.1.2 Derivatization and GC analysis

Since carbohydrates are polyhydroxy compounds, it is essential to convert them into the volatile derivatives. Commonly used derivatization methods are trimethylsilylation and
Alditol-acetate derivatizations. Generally, hexamethyldisilazane and trimethylchlorosilane have been used as derivatizing agents and pyridine as solvent for trimethylsilylation. Analytical procedures of this technique are simple and rapid (Sweeley et al., 1963), and appropriate volatility is obtained. This technique would be also applicable to nonreducing sugars. However, monosaccharide with free carbonyl groups can be present as different tautomers, and each tautomeric form occurs as different peak. Consequently, such derivative method would provide complicated chromatogram in GC analysis. When complex sugar mixture is analyzed, reduction of the monosaccharides should be considered to avoid the overlapping of peaks (Ruiz-Matute et al., 2011). In alditol-acetate method, the carbonyl group of monosaccharide is reduced using reducing reagent (e.g., KBH₄), and hydroxyl group of generated alditol is acetylated. This technique has several advantages, that alditol-acetate derivative produces single peak for each monosaccharide, and that the derivative is stable allowing clean-up for analysis (Knapp 1979). However, this method needs the large number of steps in the experimental procedures, and it is laborious and time consuming (Ruiz-Matute et al., 2011).

4.2.1.3 Methanolysis

Although hydrolysis is commonly used for analysis of neutral aldoses, the method is inappropriate for some kinds of carbohydrates such as uronic acid because of instability of uronic acid in acid hydrolysis reaction (Blake and Richards, 1968). Considering that uronic acid is also an important component of macroalgal mucopolysaccharides as mentioned above, alternative methodology should be examined. To overcome this problem, methanolysis reaction, which provides high recovery yields for uronic acid at 95-100% (Chambers and Clamp, 1971), is available.

Condition of methanolysis reaction commonly accepted is in 0.4-2 N methanolic HCl at 80°C for 5-24 h (Chambers & Clamp, 1971; Doco et al., 2001; Mejanelle et al., 2002). Under this condition, the recoveries of both neutral aldoses and uronic acid are stable (Chambers and Clamp, 1971). Since methanolysis reaction is interfered by water, this reaction should be carried out after drying the samples completely. After depolymerization of polysaccharides by methanolysis reaction, trimethylsilyl derivatization has been usually applied (Dierckxsens et al., 1983; Bleton et al., 1996; Doco et al., 2001; Mejanelle et al., 2002). Since water in the sample interferes in the trimethylsilyl reaction as well as methanolysis reaction, drying of the sample is an essential procedure (Chamber & Clamp, 1971).

4.2.1.4 GC analysis for methanolysis-trimethylsilyl derivatives

In the methanolysis-trimethylsilyl method, several kinds of isomers are generated from one monosaccharide, and the chromatogram is often complicated due to the presence of a large number of peaks. Quantification of all of the isomers would be ideal, but detection of minor isomer peaks is sometimes difficult. In such a case, quantification is generally achieved by picking up major peaks, because the isomer composition would be constant regardless of initial chemical form of the monosaccharides if the methanolysis reaction was performed under same conditions (Mejanelle et al., 2002). Wada et al. (submitted) actually showed similar isomeric composition using seawater samples from natural environment and authentic standards.

The detail of GC or GCMS detection of methanolysis products has been already reviewed elsewhere (Mejanelle et al., 2004), and here we have simple explanation. When the analysis
is carried out for less than 10 neutral and acidic monosaccharides, GC analysis will provide reliable identification of monosaccharide components. However, we should consider the presence of contaminant which is present in natural environment, because ambient seawater contains not only macroalgal exudates but organic constituents existing in seawater. In case that the contaminant peaks overlap with those of target monosaccharides, selective ion monitoring mode of GCMS instrument will be available (Wada et al., submitted). Using electron impact mode, some fragment ions are generated, and base peaks were m/z 73, 204 and 217 in the most cases. Considering that the peaks at m/z 204 and 217 are often found for pyranosides and furanosides, respectively (Mejanelle et al., 2004), the composition of fragment ions will provide useful information about not only quantification, but also identification of the isomers generated by methanolysis reaction.

4.3 Analysis of carbohydrates released from macroalgae

Mucopolysaccharides which are known as extracellular carbohydrates have been analyzed using GC or GCMS after some purification treatments (Lee et al., 2004; Mandal et al., 2007; Karmakar et al., 2010; Rioux et al., 2010; Stephanie et al., 2010). On the other hand, direct analysis for carbohydrates released into ambient seawater is limited (Wada et al., 2007; 2008). In their studies, in situ field bag experiment was developed, and the carbohydrates released from a brown alga, *Ecklonia cava*, were obtained by SCUBA diving. By hydrolysis and alditol-acetate derivative method, it was shown that fucose is the major monosaccharide component (36-44% of total carbohydrates), and that values had no significant seasonal variation. Since fucose is the major constituent of fucan derived from brown algae, constant dissolution of extracellular mucopolysaccharides into ambient environments from brown algae was suggested.

5. Conclusion

Macroalgae releases various organic compounds extracellularly and their role and dynamics in aquatic environments partly depend on the chemical composition. Since VHOC has been measured by numerous researchers, the VHOC analysis has been well improved in a few decades. On the other hand, there are possibilities of other unapplied option for phenolic compounds and carbohydrates analysis. For phenolic compounds, pyrolysis-GCMS is also considered as another potential tool, and Van Heemst et al. (1996) had tried to show the macroalgal contribution to marine organic matter pool. However, contamination of proteinaceous phenol under the process of pre-treatment was not as easy (Van Heemst et al., 1999). If this issue can be resolved in future, this instrument would also become a powerful tool for analysis of macroalgal phenolic compounds. Analysis of carbohydrates has been carried out for the extracts from algal body, but there is limited information on direct analysis of carbohydrates released to seawater as described above. Particularly, analysis using methanolysis method for seawater sample has been examined, yet. Thus, examination on the applicability of methanolysis method for seawater sample may be an important issue in the future.

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7. References


Progress in agricultural, biomedical and industrial applications' is a compilation of recent advances and developments in gas chromatography and its applications. The chapters cover various aspects of applications ranging from basic biological, biomedical applications to industrial applications. Book chapters analyze new developments in chromatographic columns, microextraction techniques, derivatisation techniques and pyrolysis techniques. The book also includes several aspects of basic chromatography techniques and is suitable for both young and advanced chromatographers. It includes some new developments in chromatography such as multidimensional chromatography, inverse chromatography and some discussions on two-dimensional chromatography. The topics covered include analysis of volatiles, toxicants, indoor air, petroleum hydrocarbons, organometallic compounds and natural products. The chapters were written by experts from various fields and clearly assisted by simple diagrams and tables. This book is highly recommended for chemists as well as non-chemists working in gas chromatography.

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