Marina Chim. Acta. 19 (1), pp. 20-27, 2018
MARINA CHIMICA ACTA
Jurnal Tiga Bahasa Tentang Kimia Laut
Open Access Journal at : http//journal.unhas.ac.id/index.php/mca
p-ISSN : 1411-2132
e-ISSN : 2620-6145

ANALYSIS OF DIMETHYLSULFIDE AS BIOGEOCHEMICAL CYCLES SULFUR ON REGIONAL ESTUARY SPERMONDE

Rakhmad Armus*[1], Alfian Noor[1], Ahyar Ahmad[1], Muhammad Lukman[2]

[*1]Chemistry Department, Faculty of Mathematic and Natural Science ,
Hasanuddin University, South Sulawesi Indonesia 90245
[2]Merine Science Department, Faculty of Marine and Fisheries, Hasanuddin University,
South Sulawesi, Indonesia 90245
Corresponding author: *lb_humanity@yahoo.com*

ABSTRACT

We developed a more accurate method of analysis for low-concentration volatile compounds used for the determination of free dimethylsulfide (DMS) in marine phytoplankton by using solid phase (SPME) gas chromatography - spectrometry Mass of GC-MS and head space method. DMSP, as a product of bacteria degradation has been scientifically recognized to be affected by climate change due to increased in sea surface temperature, and form DMS compunds.. The present study is to determine the DMSP concentration and degradation pathway in the oligotrophic region of Spermonde waters. The spatial and temporal correlation between DMSP degrading bacterial codes and biological potential controls for DMS formation in the open seas was examined using a statistical factorial analysis of variance and regression against the spatial (or distance) gradients of the Spermonde coastal waters, the concentration of chlorophyll-a, and diatom community structures. Seawater chemical properties, chlorophyll-a, and diatom samples were collected from several sites in the Tallo-Makassar river estuary, and the Pangkep river in September 2017 (transitional wet-to-dry-season). The result shows that the concentration of the Domination Index was higher in the transition season of 35.2 to 85.2 µM than in other seasons. The abundance of diatoms during the transition season reached 16,534 plankter / mL.

Keywords*:* DMS, Tallo rivers, Abundance, SPME, Spermonde

Received : 18 January 2018, Accepted : 27 April 2018, Published online : 30 May 2018

1. INTRODUCTION

Dimethylsulfide (DMS) is the most abundant reduced sulfur gas in the ocean water [1], and it can enter the atmosphere via natural sea-to-air flux, which serve as cloud condensation nuclei, and influence the local and global climate changes [2]. Most of the biogenic DMS emissions come from the marine microalgae, which can biosynthesize dimethylsulfoniopropionate (DMSP), the precursor of DMS.

Because of the different ability to produce DMSP from different microalgae [3], it is very important to study the DMS and DMSP levels from individual microalgal culture to estimate the contribution of DMS from microalgae on the changes of marine environment and global sulfur cycle.

Liquid chromatography-mass spectrometer (LC-MS) can be used to determine DMSP level in culture medium and cells. However, the derivatization processes are tedious [4], and it cannot be used to determine DMS level in solution. An

alternative method is to transform DMSP into DMS by alkaline treatment and then determine DMS level by gas chromatography-mass spectrometry (GC-MS) [5] method. Because live microalgae can rapidly transform DMSP into DMS and the process is affected by many subtle physic-chemical factors even in simple water sample, it is crucial to simultaneously determine DMS and DMSP levels in the microalgal culture with precision. In this study, a sensitive analytical method was established for the determination of DMS and DMSP levels in microalgal culture, and solid phase microextraction (SPME) was coupled with GC-MS (SPME-GC-MS). Solid phase microexatraction or SPME is a green method for extraction of analysts out of a sample. Since SPME is a non-exhaustive extraction technique, some analysts believe that SPME is not quantifiable. This presentation will provide basic information for developing a method to extract and quantify analytes using SPME. Examples will be given on the extraction and quantification of analytes out of various matrices. In this webinar, we will discuss some new SPME technologies such as SPME-OC (over-coated) fibers and Bio SPME that help to isolate and quantify analytes from interfering compounds in the matrix. Guidelines will be provided for enhancement of precision using SPME.

The location of research is in the southern part of Makassar Strait or in the southwest side of the South Sulawesi Peninsula (Spermonde Shelf), especially the waters around the estuaries of big rivers, i.e.,Tallo estuary 05°57 S, 119°26 E – 05°11 S, 119°25 E, 04°59 S (5 stations), and Pangkep estuary 04°52 S, 119°30 E – 04°49 S, 119°29 E with 10 stations.

2. MATERIAL AND METHODS

2.1 Reagents

DMS (purity > 99.0 %) and DMSP Dimethylpropiothetin hydrocloride was analytical standard (purity > 99%) were purchased from Sigma Andrich (Milwaukee, MI, USA), and Helium (chromatography-grade) was purchased from Berca (USA).

2.2 Preparation of DMS stock solution

The stock solution of DMS was prepared by dissolving accurately weighed quantities in methanol to give a concentration of 1 g L^{-1}, and the solution was stored at –20 °C protecting it from light. The calibration curve was obtained for the DMS sample by diluting the stock solution with freshly boiled cool seawater.

2.3 Synthesis of DMSP

Dimethylsulfoniopropionate hydro-chloride was synthesized according to the method described by Chambers et al (1987) [6]. About 1.0 mL (14.5 mmol) of acrylic acid and 2.5 mL (34 mmol) of DMS solution were dissolved in 15 mL of methylene chloride. Hydrogen chloride gas was bubbled into the solution in excess at room temperature until the precipitation was completed. The resulting solid was isolated by filtration and recrystallized in methanol/diethyl ether (1:1, *V/V*). The purity was determined above 95% using hydrogen 1-nuclear magnetic resonance spectrometer (Advance 400 MHz; Bruker, Germany).

2.4 Plankton sampling and analysis

Mesozooplankton was collected through vertical tow from 1.0 m above the sediment

to the water surface with a 80 µm- size plankton net (0.3 and 1.45 min mouth diameter and length, respectively). Samples were fixed and preserved with formalin in 5% final concentration, and the abundance was counted under an inverted microscope. Micro phytoplankton was collected using a 55 µm mesh-size plankton net (0.37 and 1.3 min mouth diameter and length, respectively) and preserved with 1% Lugol's iodine solution. Phytoplankton species were further identified and enumerated under a microscope (Leica,DM2000). No flow meter was fitted in the net mouth to measure the quantity of sea water, thus the total abundance of phytoplankton and zooplankton was unavailable, and only a draft relative abundance of dominant species (described as high, medium, and low abundance in a given volume, such as 5 and 1mL of concentrated sample for zooplankton and phytoplankton, respectively) was used in later analysis.

2.5 SPME-GC-MS conditions

The SPME needle with 75 µm DVB/CAR/PDMS (divinylbenzen/Carboxen /Polydimethylsiloxan) absorption fiber coating (Supelco, Bellefonte, PA, USA) was maintained at 250°C for over 30 min (Kremr 2015). About 1 mL of the sample was transferred into a 15-mL crimp top vial containing 12 mm × 4.5 mm polytetrafluoroethylene magnetic stirrer (CNW, Berlin, Germany). After adding 4.0 mL of seawater and 0.81 g of solid NaCl or 0.5 mL of 10 M NaOH, the vials were immediately sealed with a teflon-lined, butyl rubber septum (CNW) and incubated in a 30 °C water bath with continuous magnetic stirring. The SPME needle was injected through the septum, and the fiber was exposed to absorb the DMS in the headspace for 30 min before injection into the GC-MS (Agilent 7890 A GC.A.01.10.3).

Gas chromatographic analysis was carried out using a DB. wax capillary column (30 m × 250 mm x 0,25 mm, Supelco). The temperature of the injector was 250 °C. The Helium (99.999%) was used as the carrier gas with column flow rate of 2.0 mL min^{-1} linear velocity 32,434 cm sec^{-1} and a precolumn pressure of 85.0 kPa. After injection, the oven temperature was set at 40 °C for 3 min, then programmed at a rate of 3 °C min^{-1} to 40 °C and kept for 1 min, and then programmed at a rate of 4 °C min^{-1}, and then to a final temperature of 220 °C at a rate of 20 °C min^{-1} and kept for 10 min. The injection volume was 1 µl in nonsplit mode.

The mass spectrometer was operated in electron compact mode with electron energy of 70 eV. Ion source temperature was set at 220 °C, and interface temperature was set at 250 °C. The mass spectrometer scanned from *m/z* 40 to 400. The solvent cut-off time was set at 0.6 min. The determination was carried out in selective ion mode (SIM) mode, *m/z* 62 was selected as target ion, and *m/z* 47 as reference ion.

2.6 Calculation of DMS, DMSPd and DMSPp

The content of free DMS, dissolved DMSP (DMSPd), and particulate DMSP (DMSPp) were determined after conversion of DMSP into DMS with alkaline treatment and calculated as follows: (1) DMS_1 is equal to the content of free DMS in 1.0 mL culture; (2) DMS_2 is equal to the sum of DMSPd and free DMS in 1.0 mL culture, so that DMSPd = DMS_2 – DMS_1; and (3) DMS_3 is equal to the sum of DMS_2 in 1.0

mL culture and DMSPp in 2.0 mL culture, so that DMSPp = [DMS_3 – 2 × DMS_2]/2.

3. RESULT AND DISCUSSION

3.1 Sample collection method

To quantitatively determine the content of DMSPp within the microalgal cell, it is necessary to separate the cells from the culture medium. The two common cell harvesting methods, centrifugation and filtration, were compared; the experiments indicated that DMSP cound dissolve into the solution after cell membrane rupture during the filtration process. However, the analysis results showed that the contents of DMSP in the upper, middle, and lower layers of the culture medium after centrifugation at 8000 rpm were almost the same, only slightly higher in the lower layer. This indicates that centrifugation process cound not change the DMSPd content in the culture medium by choosing the upper layer supernatant even if the cell cupture of a small amount of cells happens. In fact, by comparison of DMS content in the supernatant between the samples obtained from filtration and centrifugation, the higher result appeared in the former group. Jiao (2003) and [7] Turner (1988) also suggested not to choose filtration as the cell harvest method [7]. Therefore, a simple and reliable sample preparation method was established for the simultaneous determination of free DMS, DMSPd and DMSPp, as described in the Materials and Methods section. After centrifugation, the upper half of the supernatant solution was used for the determination of DMS_1 and DMS_2, the other half of the solution containing the microalgal cells was collected for the determination of DMS_3.

3.2 Effects of solute on the quantitative analysis of DMS and DMSP

The sample preparation methods for volatile sulfur compound determination by GC-MS are usually including headspace direction injection [8], SPME [9], and purge trap [10]·[11]. SPME was chosen because it easily couples with GC-MS. To achieve reliable recovery of DMS in this step, salinity and pH the most important factors, if constant temperature and speed of electromagnetic stirring, shound be maintained. Inorganic salt may increase the ionic strength of the solution and accelerate the release of DMS from liquid phase to the headspace [12]. The results indicated that the addition of NaCl to the DMS solution lead to an obvious increase of DMS signal detected by SPME-GC-MS (as shown in Fig.1). Similarly, the addition of NaOH also increased the DMS signal intensity significantly.

DMSP is converted to DMS and acrylate under alkaline condition. After adding NaOH to the sample solution, the measured DMS include the background DMS and DMSPd. Meanwhile, the background of DMS was stronger after adding NaOH to increase pH and ion strength of the solution. Hydrochloric acid can be added to neutralize the solution after alkali conversion to obtain similar pH condition as in the initial sample [13]. However, in our experiment, the alkali conversion rate of the standard DMSP to DMS in non-DMS seawater after neutralization was lower 85.7% ± 4.3% than that of non-neutralization 102.3% ± 5.6%. It is indicated that neutralization cound reduce the DMSP conversion rate.

To obtain an accurate result, the medium condition shound be controlled to

produce the same proportion of DMS into the headspace before and after the alkali conversion. In this experiment, the signal intensity of DMS after adding 0.5 mL of 10 M NaOH was equal to that after adding 5 mL of 0.81 ± 0.03 g NaCl in the range of 0.1–10 μg l^{-1} of standard DMS solution (Fig.1). Therefore, 0.81 g of NaCl was added in 5 mL to one sample to measure the background DMS (DMS_1), and 0.5 mL of 10 M NaOH was added to other sample for over 12 h to measure the sum of background DMS and conversional DMS from DMSP (DMS_2 or DMS_3).

3.3 Determination of DMS and DMSPp content

DMS and DMSPp were analyzed according to the method described in Turner etal.(1990). DMS in seawater (20mL) was directly purged using Hidrogen gas for 11min, concentrated in Tekmar 3000 system, and analyzed usinga Hewlett Packard Gas Chromatograph (QP-5000)-Mass Spectrum (HP5973) fitted with a Selected Ion Monitor (SIM). The chromatographic column was a fused silica capillary DB-17 column (0.25mm x30 m). For the filtered DMSPp samples, 2.0 mL of 5M NaOH was added followed by 2.0 mL n-hexane to recove rthe solution. After12-h hydrolyzing (to DMS) at room temperature in darkness, approximately 1.0 mL of n-hexane-DMS extraction (supernatant) was collected and DMS concentration was measured using the GC–MS (HP6890-HP5973) fitted with SIM as the method described above. Calibration was based on the addition of known amounts of standard DMS in an ethylene glycol solution to degassed sea water, that was Subsequently subjected to the same procedure as seawater samples. The analytical precision was generally better than 5% in routine sample analysis, and the detection limit for DMS was 0.1 nM.

DMS fluxes were calculated using the simplified equation given in ping. et.al.(2016), which is based on the air–sea gas exchange equationof Liss and Slater (1974):

$$F = K\left(\frac{Cg}{H} - Cl\right) \dots\dots\dots\dots\dots\dots\dots\dots (1)$$

where F is the sea-to-air fluxes of DMS (mmol m^{-2} d^{-1}), K is the DMS transfer velocity, H is the Henry's law constant, and Cg and Cl are DMS concentrations (nmol L^{-1}) in the atmosphere and seawater phase, respectively. The atmospheric DMS concentration is negligible, hence the flux can approximated using the following equation:

$$F = -KCl \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

where K can be estimated based on wind speed and sea temperature according to the formula of Liss and Merlivat (1986).

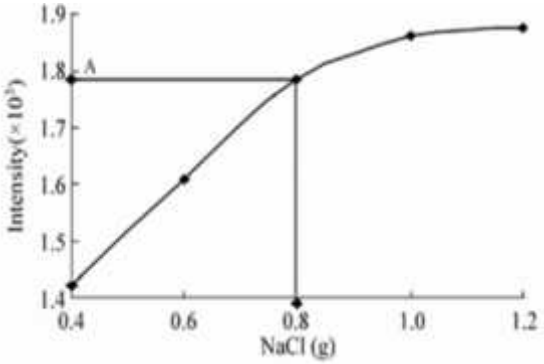

Figure 1 Effect of NaCl content on DMS signal intensity at 5 $\mu g l^{-1}$.

Point A indicates the same DMS signal intensity by adding 0.5 ml 10 M NaOH equals to the addition of (0.81 ± 0.03) g NaCl in 5mL DMS sample solution

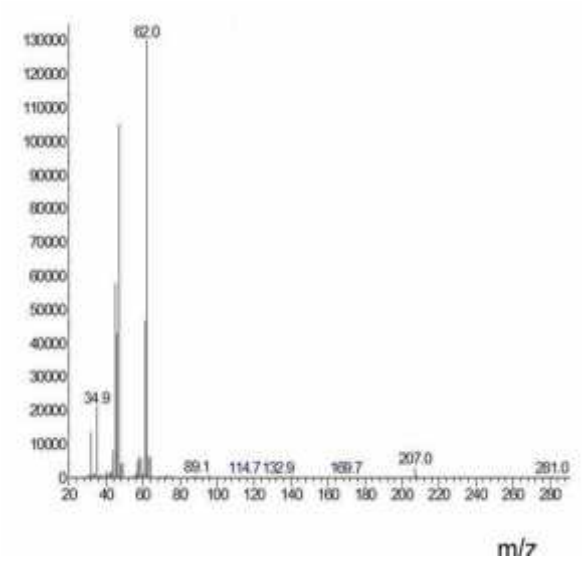

Figure 2 TIC chromatogram of DMS on SPB-1, retenrion time 34.9 min Z^{-1}

The calibration curve (fig.1) for DMS was linear in the range of 0.01–100 μg l^{-1}, and the linear regression equation was $Y = 130152.3x - 5798.3$ with the correlation coefficient (r) of 0.9999. For sample with concentration higher than 10.0 μm l^{-1}, the signal was expressed as $Y = -7680.5x^2 + 170118.6x - 17269.7$, with correlation coefficient (r) of 0.9768.

The absolute recoveries were 93.1% ± 4.3%, for spike concentration of 0.02, 0.20 and 2.0 μg l^{-1}, respectively. The RSDs were less than 5%.

3.4 Analysis of phytopalnkton samples

In the process of analyzing the phytoplankton samples, cooled boiled seawater was used to dilute the sample because the DMS concentration after conversion from DMSP to DMS was much higher than the linear range.

Table 1. Contens of plankter dan abudance in different Phytoplankton and zooplankton

Species	Organism	Plankter/ mL	Abudance/L
Phytoplankton			
Pleurosigma sp	f112	0,112	3360
Leptocylindricus sp	*1232*	1,232	36960
Rhizosolenia sp	*534*	0,534	16020
Coscinodiscus sp	*16534*	16,534	496020
Ceratium tripos	*174*	0,174	5220
Ceratium furca	*678*	0,678	20340
Ceratium longipes	*1857*	1,857	55710
Peridinium sp	*nd*	*nd*	*nd**
Zooplankton			
Temora sp	*1437*	1,437	43110
Calanus sp	*532*	0,532	15960
Unidentified Larva Polichaeta	*nd*	*nd*	*nd*
Unidentified Larva Bivalvia	*nd*	*nd*	*nd*
Unidentified Larva Echinodermata	*nd*	*nd*	*nd*

4. CONCLUSION

In conclusion, an accurate and reliable analytical method for the simultaneous determination of DMS, DMSPd, and MSPp in the microalgae was established using modified SPME-GC-MS, the major improvement in the procedure was that free DMS kept equal for the treatment with 0 DMSP content.81 g NaCl or 0.5 mL of 10 M NaOH, so that in the microalgae sample can be accurately measured.

ACKNOWLEDGMENT

The HS-SPME coupled with GC-MS is a rapid, simple, sensitive and solventless method. Results presented in this work show that phytoplankton species include relatively wide range aroma profiling compounds. For

the extraction of sulphur volatile compounds the optimisation using Central Composite Design has been carried out. The DMS as volatile compounds have been extracted using a DVB/CAR/PDMS fiber that has shown the best efficiency for the extraction of those compounds.

We are especially grateful for the support from DIKTI who helped us for studed.

REFERENCES

[1] C.-X. ZHOU, J.-L. XU, X.-J. YAN, Y.-D. HOU, and Y. JIANG, 'Analysis of Dimethylsulfide and Dimethylsulfoniopropionate in Marine Microalgae Culture', *Chin. J. Anal. Chem.*, vol. 37, no. 9, pp. 1308–1312, Sep. 2009.

[2] M. O. Andreae and W. R. Barnard, 'The marine chemistry of dimethylsulfide', *Mar. Chem.*, vol. 14, no. 3, pp. 267–279, Feb. 1984.

[3] J. Li, B. E. Carlson, and A. A. Lacis, 'El Niño-Southern Oscillation correlated aerosol Ångström exponent anomaly over the tropical Pacific discovered in satellite measurements', *J. Geophys. Res. Atmospheres*, vol. 116, no. 20, p. 1M,2M,3M,4M,5M,6M,7M,8M,9M,10M,11M,12M, 2011.

[4] P. H. Wiebe, S. B. Schnack-Schiel, and S. Nishida, 'Introduction to species diversity of marine zooplankton', *Deep Sea Res. Part II Top. Stud. Oceanogr.*, vol. 57, no. 24–26, pp. 2061–2063, Dec. 2010.

[5] T. Niki, T. Fujinaga, M. F. Watanabe, and J. Kinoshita, 'Simple Determination of Dimethylsulfide (DMS) and Dimethylsulfonio propionate (DMSP) Using Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry', *J. Oceanogr.*, vol. 60, no. 5, pp. 913–917, Oct. 2004.

[6] R. Kumar and K. M. Kumari, 'Aerosols and trace gases characterization over Indo-Gangetic plain in semiarid region', *Urban Clim.*, vol. 12, pp. 11–20, Jun. 2015.

[7] S. M. Turner, P. D. Nightingale, W. Broadgate, and P. S. Liss, 'The distribution of dimethyl sulphide and dimethylsulphoniopropionate in Antarctic waters and sea ice', *Deep Sea Res. Part II Top. Stud. Oceanogr.*, vol. 42, no. 4, pp. 1059–1080, Jan. 1995.

[8] W. Han, C. Li, X. Miao, and G. Yu, 'A Novel Miniature Culture System to Screen CO2-Sequestering Microalgae', *Energies*, vol. 5, no. 11, pp. 4372–4389, Nov. 2012.

[9] M. L. Melamed, P. S. Monks, A. H. Goldstein, M. G. Lawrence, and J. Jennings, 'The international global atmospheric chemistry (IGAC) project: Facilitating atmospheric chemistry research for 25 years', *Anthropocene*, vol. 12, pp. 17–28, Dec. 2015.

[10] G.-P. Yang, M. Levasseur, S. Michaud, and M. Scarratt, 'Biogeochemistry of dimethylsulfide (DMS) and dimethylsulfonio propionate (DMSP) in the surface microlayer and subsurface water of the western North Atlantic during spring', *Mar. Chem.*, vol. 96, no. 3–4, pp. 315–329, Sep. 2005.

[11] Gui-Peng Yang and S. Tsunogai, 'Biogeochemistry of dimethylsulfide (DMS) and dimethylsulfonio propionate (DMSP) in the surface

microlayer of the western North Pacific', *Deep Sea Res. Part Oceanogr. Res. Pap.*, vol. 52, no. 4, pp. 553–567, Apr. 2005.

[12] S.-H. Ho, C.-Y. Chen, D.J. Lee, and J.-S. Chang, 'Perspectives on microalgal CO2-emission mitigation systems — A review', *Biotechnol. Adv.*, vol. 29, no. 2, pp. 189–198, Mar. 2011.

[13] J. Wang *et al.*, 'El Niño-Southern Oscillation in Tropical and Midlatitude Column Ozone', *J. Atmospheric Sci.*, vol. 68, no. 9, pp. 1911–1921, Sep. 2011.