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THE EFFECTS OF MAGNESIUM HYDROXIDE FOR THE MICROBIAL COMMUNITY IN THE SEDIMENTS OF A EUTROPHIC CLOSED BAY

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ABSTRACT: Bench scale laboratory experiments were carried out to evaluate the effects of magnesium hydroxide ($Mg(OH)_2$) on the sediment quality and on the bacterial communities composition of the sediments collected from a eutrophic inner bay. The pH of sediments in $Mg(OH)_2$ addition treatment group has risen up from the initial value of 7.19 to 8.68 after the 10-day incubation, meanwhile the pH of the control group has stayed around 7.16. The acid volatile sulfides (AVS) content of the treatment group was reduced from initial value of 1.28 mg dry-g^{-1} to 1.11 mg dry-g^{-1}, though in the control group it raised to 1.34 mg dry-g^{-1}. The dissolved organic carbon (DOC) content increased from 125.3 mg L^{-1} to 136.5 mg L^{-1} and 183.9 mg L^{-1} in control group and treatment group sediments, respectively. Using a next-generation sequencing (NGS) method, it was revealed that the bacterial communities in sediments of the $Mg(OH)_2$ addition treatment group were different from those of the control group sediments. The number of sulfate-reducing bacteria (SRB) such as genus *Desulfobulbus* in the treatment group sediments has significantly lessened whereas it is elevated in the control group sediments. Redundancy analysis showed that the decrease of AVS in the treatment group was correlated to the reduction of SRB, and the pH increase accelerated the growth of alkaliphilic bacteria (*Fusibacter* and *Alkaliphilus*). Moreover, it was speculated that the increase of DOC in the treatment group was due to the up rise of alkaliphilic bacteria and a decline of SRB.

Keywords: Magnesium hydroxide, Sediment, Next-generation sequencing (NGS), Bacterial communities, Sulfate-reducing bacteria (SRB)

1. INTRODUCTION

The hypoxic condition of submarine has exacerbated the deoxidization and sulfurization of marine sediments not only in enclosed estuaries and inner bays, but also in coastal fish-farming areas [1]. Furthermore, the increase of hydrogen sulfide (H_2S) has inhibited the microbial degradation of protein-rich organic matter [2], and adversely threatened the survival of benthos, fishes and other organisms [3]-[7]. It also accelerated the deterioration of sediment ecosystem that resulted in a serious commercial loss to coastal fisheries [8], [9].

Nowadays, several techniques were used for the inhibition of H_2S production in order to remediate the deteriorated marine sediments [10], [11]. Kanaya *et al.* (2009) [12] demonstrated that the addition of pure iron powder lessened the H_2S in coastal cage culture fields by forming insoluble FeS. Osman *et al.* (2011) [13] and Zhang *et al.* (2008) [14] have examined and controlled the environmental factors on the sewerage system such as dissolved oxygen (DO), temperature and pH in order to eliminate the production of H_2S since it generated some problems on the system before.

Magnesium hydroxide ($Mg(OH)_2$) is a sediment improving agent that had been widely applied in cage aquaculture areas for sediment remediation. Yoshida and Nishino (2005) [15] showed that the direct spraying of $Mg(OH)_2$ on the sediments in areas of cage aquaculture had caused for the pH to moderately rise. On the other hand, the total sulfur or acid volatile sulfides (AVS) has been successfully reduced due to the increase of pH that inhibits the growth of sulfate-reducing bacteria (SRB). Xia *et al.* (2017) [16] have performed an incubation experiments and demonstrated the reduction of SRB by adding $Mg(OH)_2$ through real-time PCR method where the decrease in H_2S was observed, indicating that the loss of activity of SRB were strongly related to the decrease of sulfide (H_2S and AVS) by $Mg(OH)_2$ addition.

Sulfate-reducing bacteria (SRB) are a diverse group of anaerobic microorganisms which are responsible for H_2S production [17]. Many kinds of SRB have been identified and quantified in many anaerobic marine or estuary sediments [18], [19]. Moreover, based on comparative analysis of 16S rRNA gene sequences, SRB have been grouped into 7 known phylogenetic lineages, and most of the SRB were classified in 23 genera within the Delta-

proteobacteria, followed by gram-positive SRB within the Clostridia [20]. These researches mentioned above primarily focused on the relationship between the reduction of H_2S generation and the suppression of SRB growth. However, it is significant to comprehend that the changes of microbial community in the sediment by the addition of $Mg(OH)_2$ was because SRB exist in the community with the complex relationships to other microbes in the sediment.

Hence, this study was done to examine the effect of $Mg(OH)_2$ for the reduction of sulfide on the sediment by a laboratory experiment. Secondly, the next-generation sequencing (NGS) method [21] for analyzing the changes of the microbial communities through spraying $Mg(OH)_2$ was also applied.

2. MATERIALS AND METHODS

2.1 Study Site and Sample Collection

Omura Bay is a typical eutrophic inner bay located in the center of Nagasaki Prefecture (32° 57′ 0″ N, 129° 52′ 30″ E), Japan. The sediment samples were collected from the Togitsu Port Ferry Terminal (32° 50' 0″ N, 129° 50' 0″ E), which located in the southeast water area of Omura Bay. The collection of samples was carried out on August 11, 2015. All sediment samples were stored in cooler boxes and were quickly transported back to the laboratory within 2 hours. After removing the gravel and pebbles, fine sediment (< 2 mm grain size) were frozen (-20°C) for further analysis and DNA extraction.

2.2 Experimental Setup

The collected sediment samples were thoroughly mixed. A total of 170g of sediments were put in a small plastic container (width is 3.5 cm, depth is 3.5 cm, length is 7 cm). For the treatment group, clear water (Ube Materials Co., Yamaguchi, Japan) which contained 2mm diameter $Mg(OH)_2$ granules was mixed thoroughly with the sediments. Meanwhile, there was no $Mg(OH)_2$ added on the control group. Three sediment containers were prepared for each group (three replicates).

All plastic containers, both control group and treatment group, were placed in a rectangular closed plastic reactor (45 cm in length, 30 cm in width and 35 cm in depth) separately. Each reactor was filled with 20 L seawater collected from Omura bay, and aerated with nitrogen gas at 100 mL min^{-1}. The sea water in each reactor was kept at 26ºC using AQUA COOLER SLIM202 constant-temperature water circulator (MARUKAN, NISSO Department, Osaka, Japan) during the incubation which lasted for ten (10) days. The sediments from each group were collected after the incubation and were undergone analysis. The original sediment before the incubation was also analyzed.

2.3 Chemical Parameters of the Sediments

The water content of the sediment was measured before the incubation. Five parallel sediment samples in each container were measured every 4 hours at 105ºC using a laboratory oven (MOV-212F-PE Panasonic, Osaka, Japan). The values were calculated as follows: [(weight of fresh sediment) − (weight of dried sediment)] / (weight of fresh sediment) [22]. The dissolved oxygen (DO) and the pH of the sediment were measured using the same method that was done with Experiment-2 in the previous study [16].

Dissolved organic carbon (DOC) in interstitial water was measured using NPOC (Non-Purgeable Organic Carbon) measurement method in accordance with the standard measurement methods (JIS K0101:1998 Testing Methods for Industrial Water) by using TOC-L equipment (Shimadzu, Co., Kyoto, Japan). The interstitial water samples were injected into the quartz reaction tube and were treated with 20% phosphoric acid, sparged with CO_2-free air at 680ºC for 90 seconds to remove inorganic carbon [23]. The automatically averaged value of DOC in three replicates was used. The acid volatile sulfides (AVS) in the sediment samples were measured in a sulfide detection tube (Detector Tube No. 201H; GASTEC, Kanagawa, Japan), which was used as a surrogate parameter of TS [24]. Five repeating measurements for each sample were carried out.

The pH, DOC and AVS of all the sediment samples were measured before and after the 10th day of incubation.

2.4 DNA Extraction and Quantitative real-time PCR of Bacterial 16S rRNA gene

The sediment samples of three containers from control and treatment groups were mixed into one subsample separately. Total genomic DNAs were extracted from each subsample after the 10th day of incubation with the FastDNA Spin Kit for Soil (MP Bio Japan, Tokyo, Japan) following the instruction of manufacturer. The total genomic DNAs from original sediments were also extracted. Extracted DNA samples were eluted into TE Buffer (Tris-EDTA Buffer, pH 8.0) to a final volume of 50 µL and stored at -20 °C until use.

The copy number of bacterial 16S rRNA gene was quantified in each extracted DNA sample using real-time PCR TaqMan probe method. Amplification for each DNA samples was carried out in triplicate. The forward primer BACT1369F (5'- CGG TGA ATA CGT TCY CGG -3'), reverse primer PROK1541R (5'- AAG GAG GTG ATC

CRG CCG CA -3'), and the TaqMan probe TM1389F (5'- FAM-CTT GTA CAC ACC GCC CGT C-BHQ-1 -3') (FAM=5-carboxyfluorescein, BHQ=black hole quencher 1) were used to detect the target region of bacterial 16S rRNA gene [25]. The genomic DNA of cyanobacteria *Microcystis* sp. strain NIES843 was used as external standard to determine the copy number of 16S rRNA gene. The thermal cycling condition consisted of an initial denaturation step of 95°C for 30s, followed by 45 cycles each subjected to a denaturation step of 95°C for 10s, as well as the annealing and extension step of 60°C for 30s.

2.5 Next-Generation Sequencing (NGS)

PCR amplification of the extracted DNA from each sediment sample was performed using a barcode tagged primer set for pyrosequencing of the bacterial 16S rRNA genes. This primer set targeted the V4 hyper-variable region [26] of the 16S rRNA genes using the 515F (5'- GTG YCA GCM GCC GCG GTA A -3') and 806R (5'- GGA CTA CHV GGG TWT CTA AT -3') primer set [27], [28]. The PCR was performed at ABI Veriti Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA) with GoTaq® DNA Polymerase (Promega Corporation, Madison, USA). A total of 20 µL PCR reaction system comprised 10µL 2× GoTaq® Green Master Mix Solution (Promega Corporation, Madison, USA), 0.4 µM each of forward and reverse primers (final concentration), and ca. 10 ng of sediment DNA was used as a template. Nuclease-Free Water was added to make a final volume of 20 µL. Each library was amplified using the following protocol: the initial denaturation step of 94 °C for 5 min, followed by 30 cycles each subjected to a denaturation step of 94 °C for 30s, annealing step of 54 °C for 30s and extension step of 72 °C for 40s, and a final elongation step of 72 °C for 5 min. All the amplifications were checked using electrophoresis with 1.5% agarose gels. The bands were extracted and purified with the QIAquik Gel Extraction Kit (QIAGEN (corporate), Venlo, Netherland). DNA was quantified using Qubit® 2.0 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and was then mixed in equivalent proportions. The sequencing of the amplifications was performed on the Roche GS Junior pyrosequencing machine (Roche Diagnostics Corporation, Branford, CT, USA) according to the manufacturer's instructions.

2.6 Analysis of Sequence Data

Bacterial 16S rRNA gene of the original sediments and that of each sediment sample in control and treatment groups after the 10-day incubation was analyzed using Ribosomal Database Project (RDP) pipeline for the investigation of the bacterial community structure. The taxonomic composition was determined using the "Classifier" tool in RDP pipeline. The sequences of the bacterial 16S rRNA gene were aligned using the "Aligner" tool and the aligned data were clustered by using the "Complete linkage clustering" tool in RDP pipeline. The species clusters were defined using the 97% similarity cutoff of the bacterial 16S rRNA gene sequences. The abundance (copy number) of each taxonomic cluster was calculated by multiplying the relative abundance of sequence reads of each taxonomic cluster to the copy number of total bacterial 16S rRNA gene obtained from the quantitative real-time PCR analysis.

2.7 Statistical Analysis

The data were expressed as mean ± standard deviation (SD) and all statistical analyses were carried out in R software (version 3.3.1) (The R Foundation for Statistical Computing, Vienna, Austria). The significant difference (P<0.05 was considered significant) in all sediment samples before and after the 10-day incubation was determined using a student t-test; the Welch's method [29] was adopted to compare two objects which had unequal variance assumption [30]. Redundancy Analysis (RDA) (based on the sequence reads of dominant genera) was performed using the "vegan" package of R, in order to illuminate the relationship among the changes of chemical parameters of sediments by spraying $Mg(OH)_2$, as well as, the bacterial community composition in the sediments.

3. RESULTS

3.1 Effects of $Mg(OH)_2$ on pH, AVS and DOC of Sediments

The DO concentrations of seawater in two reactors were stable around 0.4 mg L^{-1} during 10 days of incubation. The water content of all sediment samples ranged from 56.5% to 61.9%.

Chemical parameters of sediments were measured before and after the 10-day incubation, and the results were shown in Fig.1. The pH initial value was 7.19 ±0.05, and the AVS content of original sediment was at 1.28 ±0.12 mg dry-g^{-1}. The DOC content of interstitial water in the original sediment was 125.3 ±0.80 mg L^{-1}. After the 10-day incubation, the pH of the sediments in the $Mg(OH)_2$ treatment group have significantly increased to 8.68 ±0.11 (P=0.0011), while the AVS have significantly decreased to 1.11 ±0.07 mg dry-g^{-1} (P=0.0002). The DOC content of the treatment group (183.9 ±5.65 mg L^{-1}) and control group (136.5 ±6.33 mg L^{-1}) both significantly increased from the initial DOC

(P<0.001 and P=0.013, respectively). Furthermore, the DOC of treatment group after the 10-day incubation was higher than that of the control group (P<0.001).

3.2 Effects of $Mg(OH)_2$ on Bacterial Community

A total of 26561 effective sequences of 16S rRNA gene from all the sediment samples were identified and used for the analyses of abundance, diversity and the taxonomic comparison of microbial community. At 97% gene similarity, 8947 sequence reads were classified into 2396 OTUs in original sediments. After the incubation, 6148 sequence reads were classified into 1803 OTUs in control group and 11466 sequence reads were classified into 2318 OTUs in treatment group sediments. Rarefaction curves were generated by plotting the number of sequences against the number of OTUs observed at the 97% similarity level. The results showed that all curves were unsaturated, and the increase rate of OTUs against the sequences in treatment group was the lowest (Fig.2).

The bacterial community composition before and after the incubation were analyzed at phylum and genus levels. A total of 24 phyla of bacteria were represented amongst the classified sequences, and the top 10 phyla were selected as the dominant phyla. The most major phylum was classified to Proteobacteria, the Delta-proteobacteria and Gamma-proteobacteria class were dominant within the phylum, which covering 36.26-45.85% of the total sequences detected in all sediment samples (Fig.3). Firmicutes was identified as the second major phylum in this study; it covered 18.62% of the total sequence reads detected in treatment group sediments. Chloroflexi and Acidobacteria were also showed higher dominance in treatment group sediments, while Bacteroidetes were much more dominant in original sediments.

Using the real-time PCR, the 16S rRNA gene of total bacteria in the sediments was quantified. The copy number of 16S rRNA gene detected in original sediments was 2.65 ±0.06 $\times 10^{10}$ copy g^{-1}. After the incubation, these increased to 3.93 ±0.37 $\times 10^{10}$ copy g^{-1} in the control group, and decreased to 1.01 ±0.07 $\times 10^{10}$ copy g^{-1} in the treatment group. Based on the copy numbers of 16S rRNA gene of total bacteria and the relative abundance of the classified bacterial genera by NGS analysis, the copy number of 16S rRNA gene in each dominant genus was calculated (Fig.4).

In Figure 4, the significant decrease of SRB was evidently showed; *Desulfobulbus*, *Desulfopila* and *Desulfsarcina* belong to Delta-proteobacteria in the treatment group sediments after the incubation. Meanwhile, *Haliea* and *Thioprofundum* that belong to Gamma-proteobacteria, and *Acidobacteria* Gp10 and *Acidobacteria* Gp23 that belong to Acidobacteria have decreased in the treatment group. However, these genera mentioned above have increased in the control group sediments. On the contrary, *Fusibacter*, *Alkaliphilus* and *Tindallia* that belong to Firmicutes were significantly increased in the treatment group sediments after the incubation.

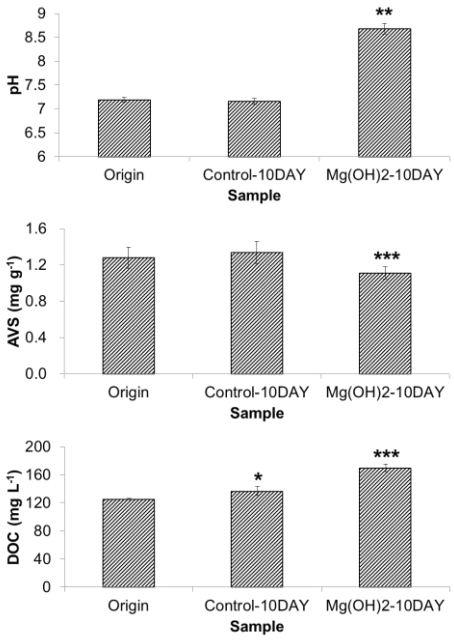

Fig.1 Changes of pH, AVS and DOC of sediments before and after the incubation. Statistical significant level as follows: * P<0.05, ** <0.01, *** P<0.001.

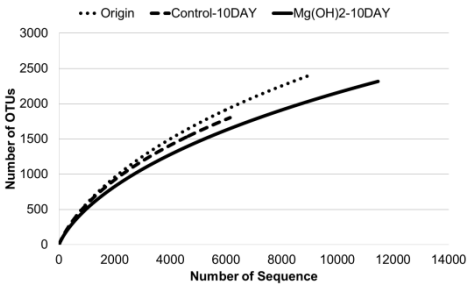

Fig.2 Rarefaction analysis of bacterial 16S rRNA gene from all sediment samples.

3.3 Relation among pH, AVS, DOC and Bacterial Communities

The pH, AVS, DOC and each copy numbers of 16S rRNA gene of bacterial species were used to conduct the redundancy analysis (RDA) to illuminate the relation among them. In Figure 5, the first two axes (RDA1 and RDA2) by three parameters (pH, AVS, DOC) explained the 88.44% and 11.55% variation of the bacterial communities, respectively. AVS showed the positive correlation (r=0.954, P=0.011) with the RDA1 axis, while pH

and DOC negatively correlated with the RDA1 axis (r=-0.966, *P*=0.004 and r=-0.890, *P*=0.001), respectively. The AVS has been gradually increasing whereas the pH and DOC have been gradually decreasing from left to right in RDA1 axis.

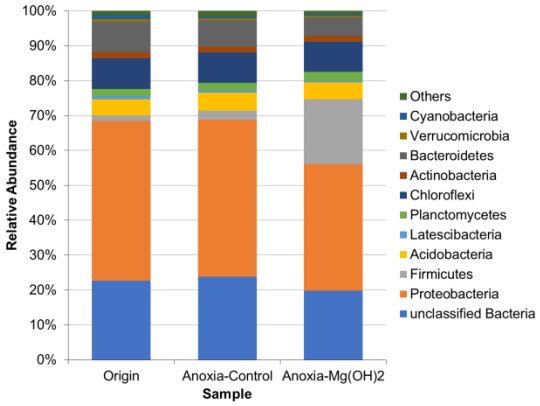

Fig.3 Bacterial composition at the dominant bacterial phyla level. Origin: initial sediment samples; Control-10DAY and $Mg(OH)_2$-10day: sediment samples of control and treatment group after the 10-day incubation.

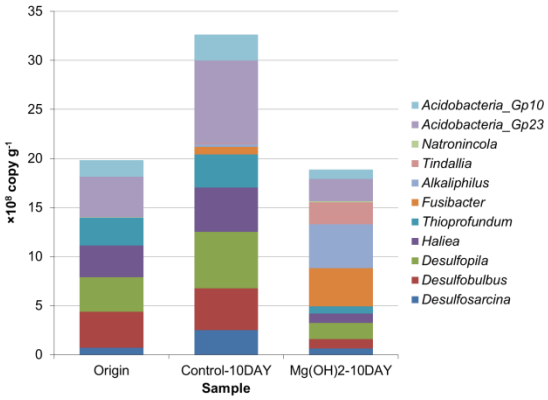

Fig.4 Bacterial composition at the dominant bacterial genera level. Y axis is the copy number of bacterial 16S rRNA gene of dominant genera. Origin: initial sediment samples; Control-10DAY and $Mg(OH)_2$-10DAY: sediment samples of control and treatment group after the 10-day incubation.

The dominant genera *Desulfobulbus*, *Desulfopila*, *Haliea* and *Thioprofundum* have showed a significant positive correlation with AVS (*P*<0.001) while it showed a significant negative correlation with the pH (*P*<0.001). On the other hand, *Fusibacter*, *Alkaliphilus* and *Tindallia* have a positive correlation with the pH (*P*<0.001) and negative correlation with AVS (*P*<0.001), this showed a clear distinction between the characteristic of the other bacteria (Fig.5). Moreover, the genera *Fusibacter*, *Alkaliphilus* and *Tindallia* only dominated the treatment group sediments rather than in original sediments (Fig.4). However, only *Natronincola* showed a negative correlation with pH (r=-0.042, *P*=0.914) and AVS (r=-0.008, *P*=0.984) simultaneously, varied from other genera within Firmicutes. The genera *Acidobacteria* Gp23 and Gp10 were also have a negative correlation with pH (*P*<0.05) and a positive correlation with AVS (*P*<0.05).

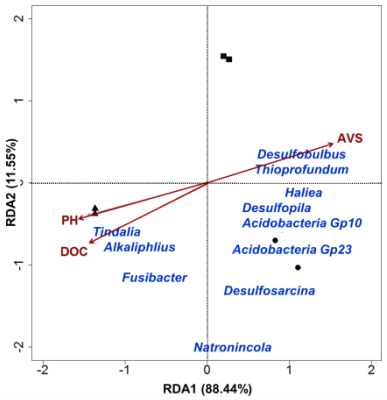

Fig.5 Redundancy analysis (RDA) plots of the relationship between chemical parameters and bacterial community in sediment. Red arrows, physicochemical variables; Blue letters, dominant genera; ■ , Origin; ● , Control-10DAY; ▲ , $Mg(OH)_2$-10DAY.

4. DISCUSSION

This study clearly demonstrated that spraying $Mg(OH)_2$ on the sediments not only inhibited the generation of sulfide in the sediment, but also altered the composition of bacterial communities in the sediments. These results were consistent with previous researches as mentioned in the introduction [14], [15].

Xiong *et al.* (2012) [31] demonstrated that pH was the dominant factor influencing the alkaline sediment community structure, the phylotype richness and the phylogenetic diversity. The results showed that the relative abundance of Delta- and Gamma-proteobacteria have lessen while abundance of Firmicutes have surge as the pH risen. Moreover, some ions correlated with pH, such as Mg^{2+}, showed a significant positive correlation with the relative abundance of Firmicutes [31]. Similar results were obtained in treatment group sediments after the incubation due to the alkalization by spraying $Mg(OH)_2$. The relative abundance of Proteobacteria in the treatment group has lessened except Firmicutes which abundance has risen. This showed the difference of bacterial composition with original sediments as presented in Figure 3. As shown in Figure 4, the reason of the Firmicutes increase was primarily due to the genera *Fusibacter*, *Alkaliphilus* and *Tindallia*, which are alkaliphilic bacteria.

Moreover, the RDA analysis clearly demonstrated that these genera have multiplied as the pH increased (Fig.5).

The genera *Desulfobulbus* and *Desulfopila* showed the same increasing tendency with AVS along the RDA1 axis. This positive correlation between SRB and AVS was due to the reduction of sulfate to sulfide through SRB. The genus *Thioprofundum*, which is a chemolithoautotrophic sulfur-oxidizing bacteria (SOB) belongs to Gamma-proteobacteria, has also showed a positive correlation with AVS in RDA plot. This result indicated that SRB and SOB identified in this study have existed symbiotically in the sediments. *Thioprofundum* can use oxygen or nitrate as an electron acceptor, and the reduced inorganic forms of sulfur (such as Thiosulfate ($S_2O_3^{2-}$), elemental sulfur (S^0) and tetrathionate ($S_4O_6^{2-}$)) as electron donors for their chemolithotrophic growth. Moreover, this genus of bacteria uses carbon dioxide (CO_2) as a sole carbon source [32]. In this experiment setup, the DO content of seawater above the surface of the sediments was kept around 0.4 mg L^{-1}, that could supply the oxygen for SOB to maintain the respiration activity. On the other hand, the oxidized forms of sulfur produced by SOB, such as sulfate, could be used by SRB as an electron acceptor [33]. The electron donors of SRB such as dissolved organic carbon (DOC) and hydrogen (H_2) were taken up from sediments. Furthermore, products of fermentation such as organic acids from the sediments that accumulated during anaerobic metabolism would provide the SRB with an ideal energy source. Van den Ende *et al.* (1997) [34] have demonstrated that the cycling of oxidized and reduced sulfur compounds between the sulfate-reducing and sulfide-oxidizing symbionts were the major factor for continuous cultures with free-living SRB and SOB.

In previous study [16], the significant increase of DOC content in $Mg(OH)_2$ addition treatment group after a 20-day incubation was attributed to the decrease in the abundance and activity of SRB. In this study, a similar result was observed in which the DOC content after the 10-day incubation in the treatment group sediments was higher. Meanwhile, the population of Firmicutes (the genus *Fusibacter*, *Alkaliphilus* and *Tindallia*), which are mostly alkaliphilic anaerobes, had significantly increased. These bacteria can produce organic acids with low molecular weight as the major fermentation products [35]. As a result, abundance decrease and the activity of SRB accelerate the accumulation of DOC in the sediments [16], also the increase of alkaliphilic anaerobes such as Firmicutes which can produce low molecular weight organic matters, might be another major reason for the increase of DOC observed in the treatment group in this study.

In addition, *Acidobateria* Gp10 and *Acidobacteria* Gp23 have demonstrated that anaerobic bacteria dominantly inhabiting marine sediments with wide range of pH from 6 to 8.5 [36]. They can utilize a variety of sugars, amino acids, alcohols, cellulose and chitin as the carbon sources for fermentation to produce organic acid [37]. Consequently, the increase of *Acidobacteria* Gp23 observed in the control group contributed to the accumulation of DOC in the sediments [37], [38]. The observed increment of DOC in control group sediments might be primarily due to the continuous supply of DOC by Acidobacteria, despite the consumption of organic carbon due to the increase of SRB in the sediments.

On the other hand, the rarefaction curves of all sediment samples failed to reach a saturation level as the richness of bacterial species in the sediments collected from Omura bay were comparatively high. In addition, the rarefaction curve of the treatment group was located below than that of the original group, implying it to have a lower bacterial richness compare to the original sediments. This was consistent with the result of Torsvik *et al.* (1998) [39] and Horner-Devine *et al.* (2004) [40], which suggested that the higher nutrient availability leads to a decrease in species diversity. The increased DOC content in the treatment group implied that there is a high carbon or energy resources existed in the sediments. At this high resources availability, the space and resource in the sediments became less patchy and the species diversity was reduced due to the competitively superior dominant species.

The discussion above involved a variety of biological processes demonstrating that the addition of $Mg(OH)_2$ in an anaerobic sediment will make it more microbially diverse. Still, the impact of $Mg(OH)_2$ to the microbial community among sediment remains to be elucidated. Moreover, further study is needed regarding on the interplay between sediment microbial community and its chemical and nutrient characteristics.

5. CONCLUSION

After the 10-day incubation, the pH value of the sediments and DOC content of interstitial water in treatment group have increased, while the AVS content have decreased. The NGS analysis revealed that the changes in the composition of bacterial community. The addition of $Mg(OH)_2$ in sediments showed a decrease in number of Delta-proteobacteria and increase number of Firmicutes.

The RDA analysis also revealed that SRB within Proteobacteria showed negative correlation (P<0.001) with pH and positive correlation (P<0.001) with AVS. However, Firmicutes has a positive correlation (P<0.001) with pH, and negative correlation (P<0.001) with AVS. Moreover, the genera *Fusibacter*, *Alkaliphilus* and *Tindallia* within

Firmicutes have also showed a positive correlation with DOC (P<0.001).

The results of this study indicated that adding $Mg(OH)_2$ to the sediment can inhibit the activity of SRB and can reduce the generation of sulfide, and at the same time it can influnce the composition of bacterial communities by accelerating the growth of alkaliphilic bacteria.

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