

Chemical and Biological Analyses of Bay Sediment Where Magnesium Oxide Compounds Are Applied

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Abstract

Three magnesium compounds, MgO₂, MgO, and Mg(OH)₂, which are supposed to supply oxygen continuously, were applied onto contaminated bay sediment and its ecology in order to activate the local microbial flora. Those compounds were found to reduce chemical oxygen demand (COD), total nitrogen (T-N), and total phosphorus (T-P). Magnesium oxide, in particular, reduced COD by 30% and T-N and T-P considerably. All compounds also suppressed the release of pollutants in the order MgO₂, MgO, and Mg(OH)₂. Analysis of microbial flora showed that the microbial group treated by MgO₂ and Mg(OH)₂ was predictably stable; meanwhile, that treated by MgO increased the number of species, but decreased the total number of microorganisms.

Keywords: Bioremediation, Magnesium oxide, Magnesium peroxide Marine sediment, Microbial community

1. Introduction

Due to growing urbanization and industrialization, bay areas and coastal regions have suffered with lots of inflow of wastewater occurring inland, diminution of mud flats and their biodiversity, and dense fish farming on shore areas. In consequence, a lot of accumulation of remnant food and fish excrement has occurred at the bottom of the sea. Decay of the organic matter in the sediment layer forms an oxygen-deprived water mass, where living creatures find it hard to reside. Moreover, the byproducts of decay, such as H₂S, ammonia, and phosphates, are apt to be released into farms interfering with the spawning migration of fish and causing a massacre of shellfish and fish of limited mobility. Bay areas are of high bioproductivity owing to abundant nutrients originating from the land, waves, and vertical flows in the sea. Also they provide sound, ecological places for diverse sea living organisms with quality food resources containing a high percentage of protein [1]. To prevent bay areas from predictable and contamination, it is desirable to use on-site microbial population, which has long been acclimated to the sea-floor environment, such as its pH and salinity [2]. For restoration of the sea-floor sediment, electrons need to be released for the relief of the inherently high redox potential, through physicochemical actions, such as microbial oxidation of the accumulated organic matter, release of the decomposed intermediates into the sea, and adsorption and desorption of the matter. The physico-

chemical activities can be enhanced by time-controlled releases of electrons via selected oxidants or oxygen release compounds (ORC) [3]. According to the above rationale, this paper has focused on treatments of magnesium-derived ORCs onto the sea-floor sediment and the effects that follow including the changes in properties of the sediment and its microbial distribution.

2. Materials and Methods

2.1. Materials

Sediment samples were collected at 10 cm of depth in marine mud near Namhae. Each one kilogram sample was placed in a reactor bin. Four bins were treated with 5% of magnesium oxides and the fifth was used as a control (Fig. 1). Magnesium oxides, creating an oxic environment, exist in the forms of stable suspensions, which are formed through a surface reaction between proton and hydroxyl ion in the sediment layer. The crystalline structure of the oxides can let oxygen be released long term in accordance with the ratio of hydration. For comparison, magnesium oxide, magnesium peroxide, and magnesium hydroxide were used to estimate the efficiency of sediment decontamination. The magnesium oxide compounds were purchased from



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Sigma-Aldrich (St Louise, MO, USA). The sediments characteristics were measured as shown in Table 1.

2.2. Chemical Analysis

The oxidation reduction potential (ORP) was measured using an ORP probe (ORION model 210A; Thermo Scientific, Waltham, MA, USA). The chemical oxygen demand (COD) was analyzed with potassium permanganate acid and the AVS was measured in a sulfide detection tube (Detector Tube No. 201H; GASTEC, Kanagawa, Japan) as follows: two grams of a sample were mixed with 2 mL of 18 N sulfuric acid, which was continuously pumped into the tube, until the color of the tube changed. The total nitrogen (T-N) was measured by oxidizing a sample with potassium persulfate followed by passing through a cadmium-copper redox column and a spectroscopic absorbance analysis at 543 nm. The total phosphorus (T-P) was quantified by oxidizing a sample with potassium persulfate into inorganic phosphorus followed by ascorbic acid reduction method, in which the absorbance was measured at 885 nm. All spectroscopic analyses were done with a UV-1800 (Shimadzu, Kyoto, Japan).

2.3. Sediment Releases Resulted with Treatments

Five reactors (one for control and four for tests) were prepared for sediment releases experiments. Each reactor was filled with 4 L of sea water and 1 kg of sediment with a caution. The experimental scheme is shown in Fig. 1. To prevent air-in, the reactor top was completely sealed off and the sides were blocked from the sunlight to prevent algal growth and possible pH change. The reactors were maintained in a still condition during the experiments. The temperature in the reactor was kept at room temperature and analyses were sampled at the middle height of the reactor every week. All samples were filtered via GF/C (47 mm) before being analyzed. Release rates were estimated by the method that Hieltjes and Lijklema [4] proposed. The rates were evaluated under an assumption that the concentrations of species *i* depend only on flow-in, flow-out, and horizontal surface area over the sediment, and with the material balance over the reactor.

$$r = \frac{V_1(C_n - C_0) + \sum V_2(C_{n-1} - C_a)}{A t} \quad (1)$$

where *r* is releases rate (g/m²/day), *V*₁ is volume of core supernatant (m³), and *C*_{*n*} is nutrient (salt) concentration at the *n*th sampling (mg/L).

2.4 Analysis of Microbial Community

The microbial community was analyzed via FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). First, 16S rDNA out of the extracted DNA sample were identified using primers 27f and 1492r. The second touch-down polymerase chain reaction (PCR) was run through the 16S rDNA V3 region and re-amplified with primer GC-341F equipped with 40-bp GC-clamps (Bioneer Inc., Daejeon, Korea). In PCR, a sample was heated at 95°C for 5 min and was denatured for 30 sec. Then it was annealed starting at 65°C decreasing by 0.5°C per cycle, 15 more cycles at 55°C, and 45 sec of elongation at 72°C with 10 min. The products of PCR were identified in 1% of agarose gel.

DNA fragments were cut from bands on the denaturing gra-

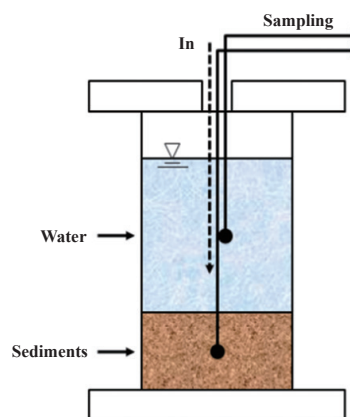


Fig. 1. Lab-scale reactor for the release experiments.

dient gel and were washed with highly purified distilled water. After being added with TE buffer (25 uL), the DNA samples were centrifuged at 13,500 g for 1 min. The collection was frozen (-70°C) and thawed (50°C) three times for 5 min each. After centrifugation, the supernatant was collected for further analysis. The finalized collections were amplified again for an NCBI BLAST (Basic Local Alignment Search Tool) search designated for the most probable, phlemonic similarity. Principle component analysis (PCA) was run for floral similarity through SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Treatment of Magnesium Oxides for the Decontamination of Sediment

For COD (Fig. 2), magnesium oxide with 31.8% seemed to more actively contribute to reducing COD than MgO (19%) and Mg(OH)₂ (14.1%) in 20 days. The results imply that magnesium oxide can inherently generate more oxygen in the sediment, so as to help form a more favorable, oxidative environment for residing microorganism than the others.

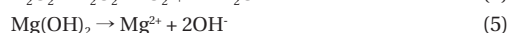
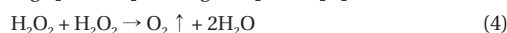
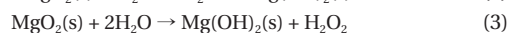


Table 1. Characteristics of the sediment

| Parameter | Concentration |
|---|---------------|
| Ignition loss (%) | 33.48 |
| COD (mg/g DS) | 28.9 |
| AVS (mg/g DS) | 0.0014 |
| Total phosphorus (mg/g) | 1.57 |
| Total nitrogen (mg/g) | 0.045 |
| Dissolved oxygen (mg/L O ₂) | 5.45 |
| pH | 7.85 |
| ORP (mV) | -283.3 |

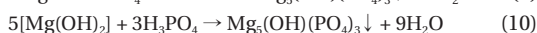
COD: chemical oxygen demand, DS: dissolved solids, AVS: acid volatile sulfide, ORP: oxidation reduction potential.

Acid volatile sulfide (AVS) is one of the most affecting factors, not only for sedimental organism, but for aquatic living matter. MgO_2 decreased AVS by 77.3% (Fig. 3). A small decrease in AVS for the control sample might be a natural volatility. Also, note that $Mg(OH)_2$ removed AVS as much as MgO_2 . An acidic condition and abundant presence of sulfate reducing bacteria are known to produce more H_2S [5-6]; and MgO_2 , an excellent oxygen producer, seemed to suppress this anaerobic action for reducing AVS matter. Another mechanism might be applied to explain the high performance of $Mg(OH)_2$: as proposed in Eq. (6), generated hydrogen sulfide (active in pH 6.5–7.5) can be easily transformed to magnesium sulfate in the alkaline environment (pH should be higher than 8.5), thus lowering residing AVS matter. The less effective ORC, MgO was found to show the lower AVS removal efficiency.



Fig. 4 indicates that nitrogen compounds can be eliminated through ORC treatment. T-N concentrations were diminished by 44.4%, 28.38%, and 10.45% through treatments of $Mg(OH)_2$, MgO_2 , and MgO , respectively. Shifting to oxic mood by addition of the ORCs could facilitate nitrification via activation of ammonia- and nitrite-oxidizing microorganism, therefore resulting in releases of the formed nitrates [7]. Releases of ammonia into air may depend on the surrounding pH.

Fig. 5 shows that phosphorus compounds also decreased significantly with treatments of MgO_2 and $Mg(OH)_2$ by ranges of 58.6%–65.2%. The effect of MgO was relatively trivial. Metal associated phosphorus is naturally released from the sedimental, anaerobic environment, a highly reducing state, and the associated metal ions undergo detachment from the complexes to leave inorganic phosphorus. Added $Mg(OH)_2$ may dissociate into Mg^{2+} and OH^- , which react with the inorganic phosphates to form insoluble phosphorus compounds like $Mg_5(OH)(PO_4)_3$ as shown in equations [7, 8]. High pH, such as 9 or higher, generated by the addition of $Mg(OH)_2$, could accelerate the formation of insoluble precipitates of phosphorus [8].



3.2. Evaluation of Releases Rates from Sediment

Fig. 6 shows that five percent of ORCs injection on the sediment was found to be effective on the suppression of releases in different organic matter. For example, COD (representing carbonaceous organics) was decreased by 73% compared to the control with treatment of MgO_2 . Accordingly, the ORP dropped from -280 to -40 mV with the swapping of oxygen. Obviously, organic compounds seemed to be decomposed by the activity of reviving microorganism.

Nitrogen compounds were also less released by ranges of 17.9% to 4.7%. However, the efficiencies were not remarkable in spite of the elevated pH and ORP values, because of possible re-suspension of the magnesium particles interfered with by other pollutants in the surroundings.

In most cases of ORC treatments for T-P, phosphorus was rather dissipated away. The phosphorus salts sneaked into the

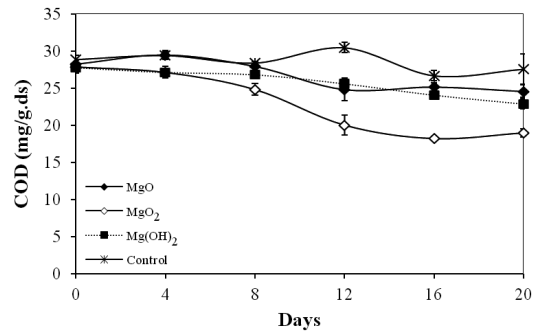


Fig. 2. Changes of chemical oxygen demand (COD) on sediment treated by control (no addition), 5% MgO , 5% MgO_2 , and 5% $Mg(OH)_2$ for 20 days.

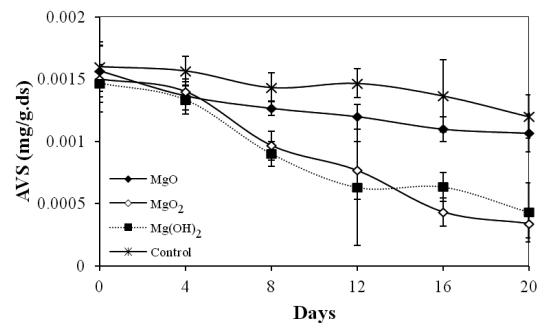


Fig. 3. Changes of acid volatile sulfide (AVS) on sediment treated by control (no addition), 5% MgO , 5% MgO_2 , and 5% $Mg(OH)_2$ for 20 days.

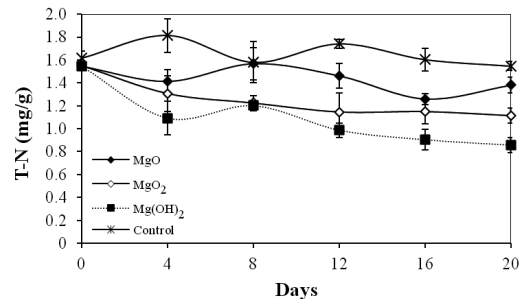


Fig. 4. Changes of total nitrogen (T-N) on sediment treated by control (no addition), 5% MgO , 5% MgO_2 , and 5% $Mg(OH)_2$ for 20 days.

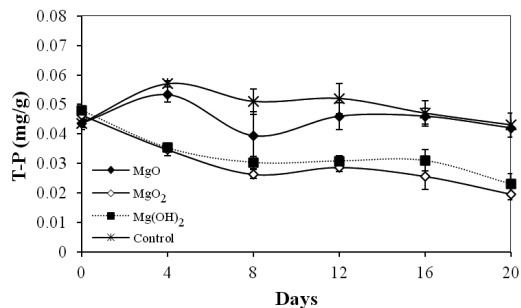


Fig. 5. Changes of total phosphorus (T-P) on sediment treated by control (no addition), 5% MgO , 5% MgO_2 , and 5% $Mg(OH)_2$ for 20 days.

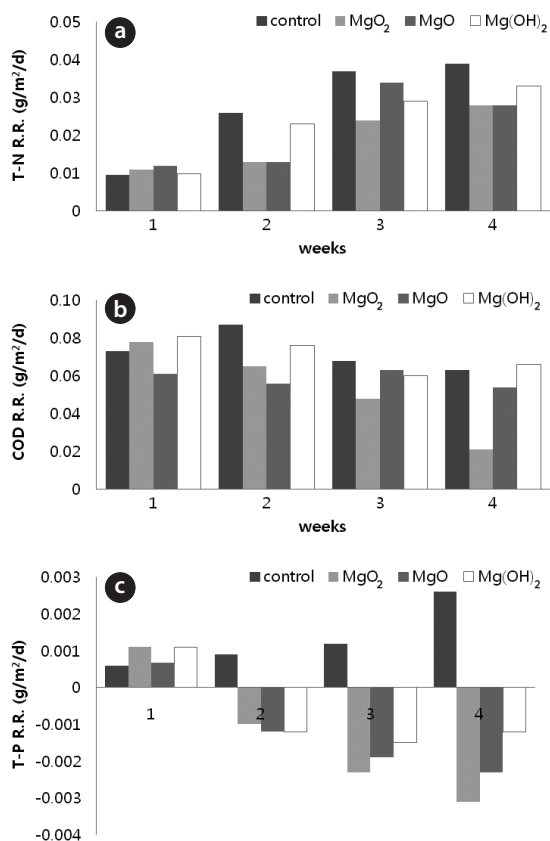


Fig. 6. Release rates (RR) of MgO₂, MgO, and Mg(OH)₂ at sediment: (a) chemical oxygen demand (COD), (b) total nitrogen (T-N), and (c) total phosphorus (T-P).

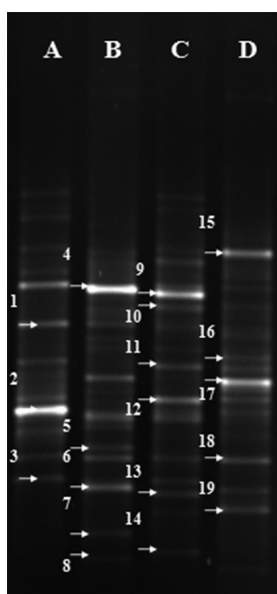


Fig. 7. Denaturing gradient gel electrophoresis (DGGE) analysis of sediments treated by treatment: A, control (no treatment at initial time); B, MgO₂ (treatment after 20 days); C, MgO (treatment after 20 days); and D, Mg(OH)₂ (treatment after 20 days).

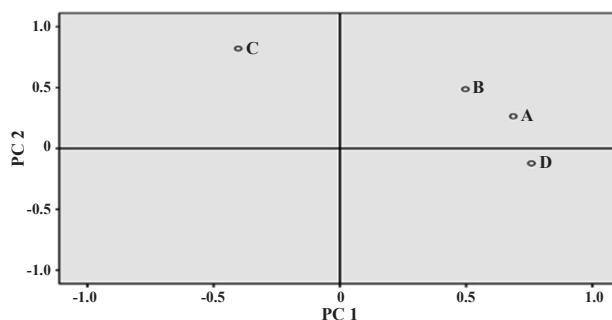


Fig. 8. Similarity test for microbial community after treatment: A, control (no treatment at initial time); B, MgO₂ (treatment after 20 days); C, MgO (treatment after 20 days); and D, Mg(OH)₂ (treatment after 20 days).

oxide crystalline structure, thus crystals were quickly hydrated and finally fixed as insoluble complexes or up-taken by aerobic microorganism via the phosphorus uptake mechanism. [9].

3.3. Analysis of Microbial Community

Fig. 7 shows different DNA combinations of the habitat microbial community in accordance with ORC treatments. The overall population showed no significant diversity with such treatments.

BLAST (Genebank) results for 16S rDNA sequences can be found in Table 2. *Vibrio* sp. (band 2) and *Pseudomonas* sp. (band 3) were found to be diminished in all three treatments; meanwhile, *Psychrobacter* sp. and *Desulfosporosinus* sp. (band 16) increased for MgO₂ and Mg(OH)₂ treatments, respectively.

Using DGGE band profile, the position and clarity of all bands were checked in number for PCA analysis (Fig. 8). One party included A (control), B (MgO₂), and D (Mg(OH)₂); and the other had C (MgO). Y values were found to be lower than X's, which meant little change in the total population between microbial groups. However, the population of some specific species considerably increased. Considering the similarity of B, D, and A, we can assume a sort of "microfloral stability" by means of the two treatments. In contrast, group C revealed an increased diversity of species, but a decrease in total population.

4. Conclusions

This paper demonstrates the effectiveness of treatments with the ORCs MgO₂, MgO, and Mg(OH)₂ over contaminated bay sediment. Treatment with MgO₂ reduced COD by as much as 31% in 20 days. With the same treatment, AVS decreased by 77%. T-N also decreased by ranges of 44.4%–10.45% possibly because of facilitated nitrification with generated oxygen. T-P was also greatly removed by as much as 58%–65% in the form of insoluble inorganics for MgO₂ and Mg(OH)₂. Four-week releases experiments showed that all three ORCs, including MgO₂ as the best candidate, were effective in the suppression of the main contaminants out of the sediment environment. PCA along with denaturing gradient gel electrophoresis (DGGE) and BLAST summarized no significant changes in microbial populations, except for one or two species with ORC treatments.

Table 2. Base sequences of 16S rDNA read from DGGE bands

| DGGE band | NCBI accession no. | Description | Identity (%) |
|-----------|--------------------|---|--------------|
| 1 | GQ351352_1 | <i>Alkalibacterium</i> sp. G4 | 91 |
| 2 | YZ62533 | <i>Vibrio</i> sp. | 94 |
| 3 | HG20054 | <i>Pseudomonas</i> sp. | 91 |
| 4 | AB016056_1 | <i>Psychrobacter pacificensis</i> | 98 |
| 5 | GQ169113_1 | <i>Psychrobacter</i> sp. HTGH13 | 96 |
| 6 | U85880_1 | <i>Psychrobacter immobilis</i> | 96 |
| 7 | Y15345_1 | <i>Acyrtosiphon pisum</i> PRLIST08 | 91 |
| 8 | HQ204300_1 | Bacterium GG49E | 97 |
| 9 | GU253361_1 | Bacterium GG49E | 100 |
| 10 | HQ836471_1 | <i>Olgasolaris</i> sp. associated epsilon proteobacterium | 90 |
| 11 | AB474389_1 | <i>Pseudorhodobacter</i> sp. OL31 | 87 |
| 12 | DQ649446_1 | Carbazole-degrading bacterium OC13S | 91 |
| 13 | AJ294338_1 | <i>Shewanella</i> sp. YACN-12 | 98 |
| 14 | U53824_1 | <i>Halomonas variabilis</i> | 98 |
| 15 | AY082482_1 | Aurantimonasmanganooxydans SI85-9A1 | 99 |
| 16 | FJ875954_1 | <i>Desulfosporosinus</i> sp. 44a-T3a | 97 |
| 17 | EU346548_1 | Marine bacterium SIMO-4479 | 93 |
| 18 | JF738027_1 | Marine sponge bacterium PLATERafhis+(1)-11 | 93 |
| 19 | HQ333016_1 | <i>Rhodobacter azotoformans</i> | 93 |

DGGE: denaturing gradient gel electrophoresis, NCBI: National Center for Biotechnology Information.

Acknowledgments

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