

## ORIGINAL ARTICLE

# Changes in Phosphorus and Sediment Oxygen Demand in Coastal Sediments Promoted by Functionalized Oyster Shell Powder as an Oxygen Release Compound

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## Abstract

In this study, we performed a sediment elution experiment to evaluate water quality in terms of phosphorus, as influenced by the dissolved oxygen consumed by sediments. Three separate model column treatments, namely, raw, calcined, and sonicated oyster shell powders, were used in this experiment. Essential phosphorus fractions were examined to verify their roles in nutrient release from sediment based on correlation analyses. When treated with calcined or sonicated oyster shell powder, the sediment-water interface became "less anaerobic," thereby producing conditions conducive to partial oxidation and activities of aerobic bacteria. Sediment Oxygen Demand (SOD) was found to be closely correlated with the growth of algae, which confirmed an intermittent input of organic biomass at the sediment surface. SOD was positively correlated with exchangeable and loosely adsorbed phosphorus and organic phosphorus, owing to the accumulation of unbound algal biomass-derived phosphates in sediment, whereas it was negatively correlated with ferric iron-bound phosphorus or calcium fluorapatite-bound phosphorus, which were present in the form of "insoluble" complexes, thereby facilitating the free migration of sulfate-reducing bacteria or limiting the release from complexes, depending on applied local conditions. PCR-denaturing gradient gel electrophoresis revealed that iron-reducing bacteria were the dominant species in control and non-calcined oyster shell columns, whereas certain sulfur-oxidizing bacteria were identified in the column treated with calcined oyster powder.

**Key words** : Oyster powder, Oxygen Release Compound (ORC), Phosphorus fraction, Sediment oxygen demand

## 1. Introduction

Oxygen demand in oceanic and coastal areas has been identified as one of the most important factors

determining water quality control. It is not only a sensitive and influential factor with respect to the influx of contaminants but also a limiting factor for marine eco-cycles. Eutrophication promotes the extensive

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proliferation of organisms in marine environments, and the associated increases in photosynthesis and respiration rates promote notable changes in oxygen demand. Furthermore, algal blooms accelerate the sedimentation of particulate matter, and the decomposition of organic matter comprising remnants of dead microorganisms in sediment leads to additional oxygen consumption. This consumed oxygen is referred to as Sediment Oxygen Demand (SOD), which is known to have an influence on a considerable proportion of sediment environment (Bowman and Delfino, 1980; Belanger, 1981).

The SOD characterizing the sediment environment can be classified into two types, namely, biological SOD (B-SOD), which is the oxygen consumed during aerobic mineralization of incoming organic matter and accounts for 65% of SOD, and chemical SOD (C-SOD) which accounts for 10%-30% and is related to the re-oxidization of reductive products derived from anaerobic decomposition (Wang, 1980).

Among the many factors that influence the changes in SOD, the role of phosphorus in sediment is particularly noteworthy. This element is released depending on the oxygen demand in sediment and a deficiency can often limit the growth of phytoplankton (Chapra, 2008). Within sediments, phosphorus can form metal chelates with oxidized iron compounds or can be adsorbed to the sediment, resulting in restricted release into the bulk water in the presence of abundant oxygen. However, under conditions of low oxygen concentrations, reductive reactions facilitate the release of reactive phosphorus salts bound to iron. This release can trigger the development of algal blooms, which in time results in the sedimentation of further large quantities of organic matter that perpetuate the P cycle (Hyun, 2005). Previous studies have demonstrated the regulatory role of SOD in sediments, in that it controls the release of phosphorus salts by limiting excess oxygen consumption in the sediment precipitates.

Recent studies have focused on the use of oxygen release compounds (ORCs) in attenuating sediment contamination. Among these compounds, powdered calcium peroxide has attracted considerable interest, owing to its ability to promote the release of large amounts of molecular oxygen into the water when added to the sediment phase. As a consequence of large pulses of oxygen, the local environment is rendered more aerobic, and, moreover, the soluble calcium ions bind to phosphoric salts, which are eventually removed from the water phase. Further details of the increased application of calcium peroxide can be found elsewhere (Lu et al., 2017; Wang et al., 2019).

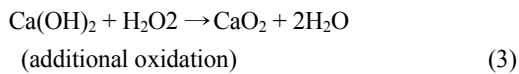
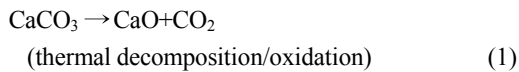
A potential and currently unexploited alternative source of ORCs are oyster shells, one hundred thousand tons of which are discarded annually along the western and southern shores of Korea with little reclamation. Recent progress in shell recycling has, however, found limited markets in the limestone fertilizer and calcium oxide industries. Given their favorable physicochemical characteristics and thermal properties, oyster shells have considerable potential applications. For example, natural shell powder (CaCO<sub>3</sub> 94%) can be used as a raw material for the production of many industrial calcium derivatives. With thermal treatment over 700°C, the majority of this powder is converted to CaO (Lee et al., 2009), whereas the serial addition of hydrogen peroxide results in excess oxidation and the generation of calcium peroxide or higher oxides that can be directly applied as ORCs for water quality control in marine areas.

To evaluate the potential of ORCs in this latter regard, we investigated the behavior of contaminants released from model sediment systems in response to the application of oyster shell-derived ORC via evaluations of environmental indices related water quality and analyses of the correlations between SOD and other internal variables or factors in all test beds.

## 2. Materials and Methods

### 2.1. Preparation of CaO<sub>2</sub> originated from oyster shells

Generally, CaO<sub>2</sub> can be prepared using the following consecutive reactions:



The details of this procedure have been reported previously by Yoo et al. (2018). Briefly, 10 g of calcined oyster powder was heated at 900°C for 10 min, and then left to cool naturally. Thereafter, 4 mL of distilled water was added to the powder in an ice bath and subsequently 5 mL of hydrogen peroxide (30%) was added to the fully mixed slurry to promote further oxidation. Sonicated oyster powder was prepared as follows. Fifty grams of shell powder mixed with 100 mL distilled water was sonicated for 5 min using an ultrasound generator (ULTRA-TURRAX, USA) to fragment and oxidize the calcium carbonate. Thereafter, the resulting moist material was homogenized and dried prior to undergoing the further hydroxylation and oxidation reactions [reactions (2) and (3) above].

### 2.2. Preparation of samples and test columns

The samples of sediment and seawater used in this study were collected from the Donam-dong shore area in Tongyeong City, Gyeongnam Province, South Korea. For collecting sediment and seawater samples, we used Ponar Grab samplers and a water sampler, respectively. All samples were transferred to the laboratory and maintained at 4°C prior to use. As test columns, we prepared four plastic columns (10 cm diameter, 30 cm height) containing sediment and the following oyster shell supplements: a control column

(sediment only), column B [plus 0.5% (w/w) unprocessed (raw) oyster shell powder], column C [plus 0.5% (w/w) calcined oyster shell], and column D [plus 0.5% (w/w) sonicated oyster shell]. To each column, we added 300 g a sediment sample along with GF/C-filtered seawater without disturbance. The top of each column was covered with a lid to minimize oxygen intake. Light of 6300 lux (12L:12D; white LED) and a temperature of 22°C were maintained throughout the experiment. Analyses were performed at 5-day intervals for 20 days.

### 2.3. Analysis

#### 2.3.1. Sediment Oxygen Demand (SOD)

SOD was measured following the procedure described by Wang (1981). Two grams of wet sediment was placed in a 300 mL biological oxygen demand bottle. Fully aerated distilled water was then syphoned into the bottle down the bottle wall, so as not to disturb air within. The initial dissolved oxygen (DO) measurement (YSI 550A) was performed immediately after stirring with a magnetic stirrer. Five hours later, a second DO measurement was made and SOD was calculated using the following equation:

$$SOD = \frac{dC}{dt} \times V \times \left( \frac{1}{M} \times \frac{100}{100 - W} \right)$$

where  $dC/dt$  denotes the change in DO (mg O<sub>2</sub>/L) per unit time,  $W$  is the water content, and  $M$  is the weight of a sample.

#### 2.3.2. Other quality indices

Each pore water aliquot sampled from the sediment was centrifuged at 2,000 rpm for 20 min and then diluted accordingly before determination of concentrations of phosphoric salts. For total phosphorus (T-P) determination, potassium persulfate [K<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>] was added to the original sample and autoclaved at 120°C for hydrolysis. Absorbance was measured at 880 nm using a UV/visible spectrometer

based on  $(\text{NH}_4)_6\text{MoO}_{24}$ . DO and pH/oxidation-reduction potential (ORP) were measured using a DO meter (YSI 550A, USA) and a multi-meter (Orion 3star, USA), respectively. Chlorophyll-*a* was determined following extraction with acetone.

Sediment P was measured using the SEDEX method (Ruttenberg et al., 2009). Ferric iron-bound phosphorus (Fe-P) was determined based on an alcoholic extraction (Watanabe and Olsen, 1962). Other dissolved phosphorus fractions were analyzed by UV/Visible spectrometry as described above.

#### 2.4. Microbial assay using PCR-denaturing gradient gel electrophoresis (DGGE)

Microbial flora were analyzed using a Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). Initially, a fragment of extracted DNA (16S rDNA) was amplified using primers 27F and 1492R, and we subsequently re-amplified the 16S rDNA V3 region by touch down PCR using the GC-341F primer (Bioneer Inc., Daejeon, Korea) with 40 GC-clamps. The PCR conditions applied were as follows: 5 min heating at 95°C, followed by 30 s denaturing along with annealing from 65°C to 55°C and 10 min elongation at 72°C. All PCR products were identified using 1% agarose gel electrophoresis. After purification, DGGE was applied using a Denaturing Gradient Gel Electrophoresis system (C.B.S. SCIENTIFIC, USA). DNA fragments were excised from the gradient gel bands and then washed thoroughly with highly purified distilled water. Following the addition of 25  $\mu\text{L}$  of TE buffer solution, they were centrifuged at  $13,500 \times g$  for 1 min and the supernatant was collected for further analysis. The base sequences were determined and then NCBI BLAST (Basic Local Alignment Search Tool) was applied to determine the "most probable" microorganisms.

#### 2.5. Statistical Analysis

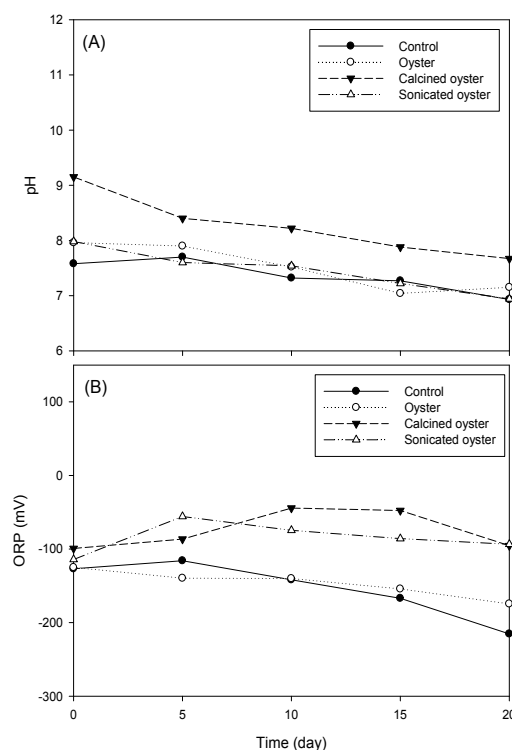
All statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). Significant differences

between columns were assessed via one-way ANOVA. Correlations between variables were determined using Pearson correlation analysis.

### 3. Results and Discussion

#### 3.1. Physicochemical changes in response to ORC treatments

The changes in pH and ORP values in all test columns are shown in Fig. 1. The initial pH of sediments was 7.5 but increased immediately in response to the addition of oyster powders. Columns B and D each showed pH values of 7.9, whereas column C showed a markedly higher value of 9.1, due to the larger quantities of hydroxides formed via Reaction 2, in which the peroxide is converted to hydroxide in a spontaneous hydration reaction (Lu et al., 2017).



**Fig. 1.** Variation of (A) pH and (B) ORP for 20 days in sediment with the treatment.

Natural oyster powder, which is primarily composed of carbonate, has a very low solubility and functions as a buffer ( $\text{CO}_3^{2-}$ ), and thus its addition resulted in a minimal change in pH over the test period (Lee et al., 2009).

We noted significant differences in ORPs in sediments among different columns ( $P < 0.05$ ). In the control column and column B, the values decreased from -126.8 mV and -125.1 mV to -215.3 mV and -174.6 mV in 20 days, respectively, whereas in columns C and D, the values fluctuated with time. For example, in column C, the initial ORP value was -99.2 mV, which subsequently peaked at -44.4 mV after 10 days and then dropped to -95.5 mV by the final day of the test. In the control column and column B, the ORP underwent a gradual decrease due to the slow transition to more anaerobic conditions (Mort et al., 2010). In contrast, the release of molecular oxygen in columns C and D contributed to maintaining a less anaerobic environment (Wang et al., 2019).

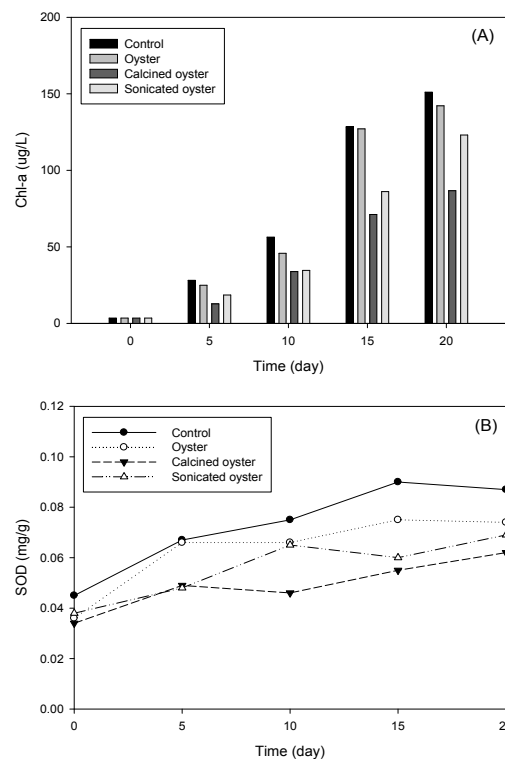
### 3.2. Changes in water quality in response to ORC treatments

#### 3.2.1. Algal growth and SOD

The concentrations of SOD and chlorophyll-*a* in each column were measured in order to correlate oxygen consumption in the sediment with algal growth (Fig. 2). Overall, SOD increased in all columns, including the control column, in which it increased from 0.045 mg/g to 0.080 mg/g. Among the other three columns, the final measured concentration ranged from 0.062 to 0.074 mg/g.

In all columns, chlorophyll-*a* concentrations increased over the entire test period, and was characterized by a steeper increase after 10 days. After 20 days, in all columns with the exception of column C, the final measured concentrations ranged from 123 to 151  $\mu\text{g/L}$  compared with an initial value of 3.45  $\mu\text{g/L}$ , which is indicative of an exponential growth in biomass. Algal growth is dependent on an adequate

source of limiting nutrients such as nitrogen or phosphorus (Hu et al., 2001). To precisely predict algal growth, the intake of nutrients from the sediment and soluble reactive phosphorus (SRP) present in the bulk water should also be considered. At present, however, we have little information regarding physiological aspects of algae in an environment affected by different types of oyster powder. Nevertheless, experimental data are likely to show a negative effect of oyster powder on algal growth regardless of the amounts of oxygen generated.



**Fig. 2.** The variation of (A) Chlorophyll-*a* and (B) SOD for 20 days with treatment.

We found the changing patterns of SOD in response to the different type of oyster powder to be similar to those of chlorophyll-*a*, which would be expected, given that SOD reflects the aerobic degradation of organic matter at the interface between the sediment

and bulk water (Brewer et al., 1977). Excessive proliferation of planktonic biomass at the water surface tends to result in a continual input of organic matter to the sediment. The dead organic mass would be mineralized aerobically, thereby by consuming local oxygen supplies (Biological SOD) (Wang, 1980). Taking into consideration the similar behavior of SOD and chlorophyll-*a* and the microbial flora found in the subsurface sediment, it appears that algal growth was the dominant factor controlling SOD in the present study, as has been reported previously (Park et al., 1991).

### 3.2.2. Changes in DO in response to ORC treatments

As shown in Fig. 3, DO underwent changes in the aquatic phase. From an initial value of 7.5 mg/L, DO decreased over time, reaching a final concentration of approximately 4 mg/L. The lowest final DO concentration (3.98 mg/L) was recorded in the control column, whereas the highest value was recorded in column C, which was supplemented with calcined oyster powder.

The factors that affect DO in the aquatic phase are typically diverse and complex. Major oxygen consumers are not only phytoplankton and microorganisms in subsurface of the water but also certain biochemical agents within sediments (Lee et al., 1992). Algae grow via photosynthesis and respiration, during which they consume exogenous oxygen in an alternative manner (Murray and Wetzel, 1987). This implies that the differences in DO concentrations recorded in the different test columns was determined to a large extent by the respiratory rate of algae. A further contributory factor is the consumption of oxygen by sediment constituents (Brewer et al., 1977). The distribution of SOD among columns shown in Fig. 2 may indicate a significant difference in oxygen consumption among different types of sediments. Furthermore, the stabilized sediment found in column B and the control column

may have become more anaerobic with time, with low levels of circulating oxygen, whereas the higher levels oxygen detected in columns C and D may have contributed to lowering the intrinsic value of SOD. In summary, the decrease in DO observed in all columns would have been largely dependent on the levels of organic biomass and the SOD generated by this biomass.

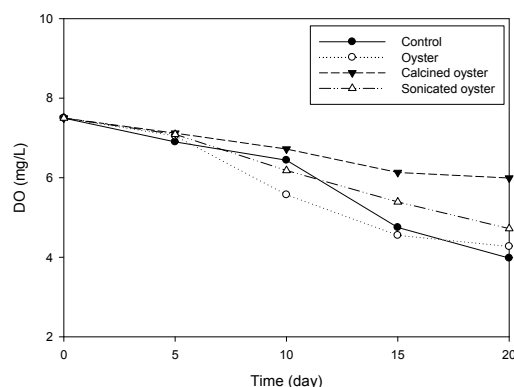
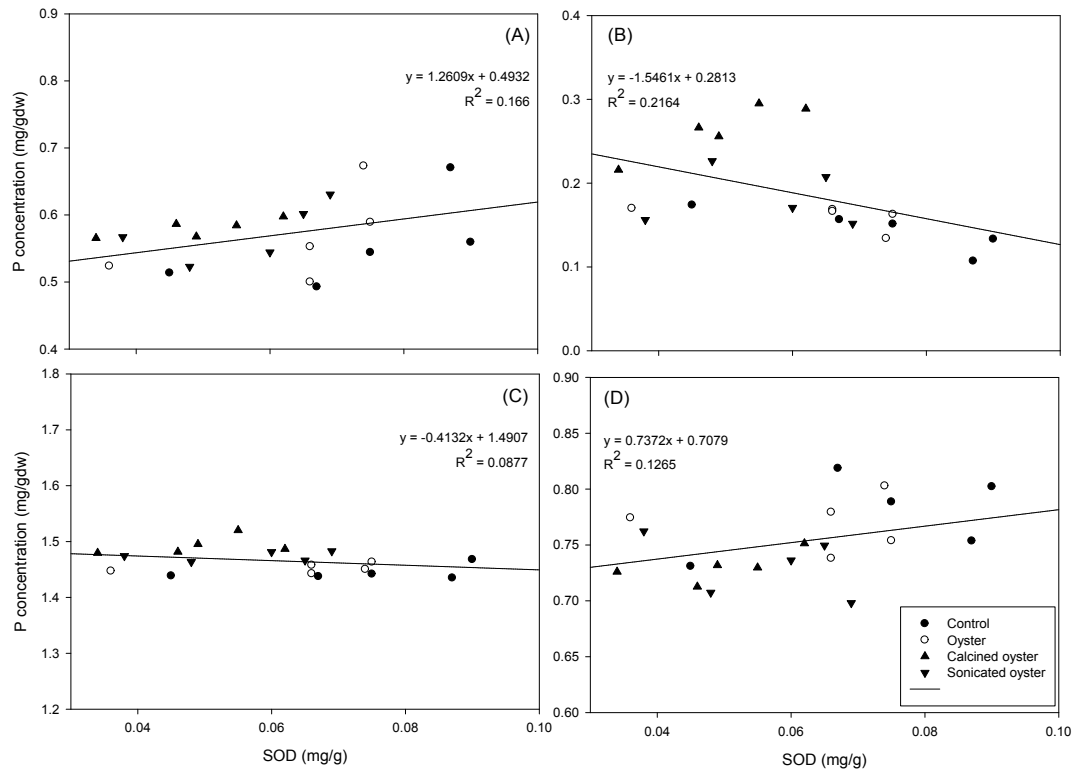


Fig. 3. Variation of dissolved oxygen in overlying water for 20 days with application of oyster powder.

### 3.3. Correlation between SOD and phosphorus fractions

Fig. 4 depicts the correlations between SOD and the different P fractions determined for each of the oyster powder treatments. The sequentially extracted P fractions from dried sediment are as follows: (A) exchangeable and loosely adsorbed P (Ex-P), (B) ferric iron-bound P (Fe-P), (C) calcium fluorapatite-bound P (CFA-P), and (D) organic P (Org-P).

SOD is known to be closely correlated with P fractions such as Ex-P and Org-P. Ruttenberg (1992) has previously reported that Ex-P is derived from phytoplankton and zooplankton. This correlation leads to an accumulation of organic matter on the sediment surface (earlier algal blooms on the water surface). An increase in the input of organic mass promotes higher levels of oxygen consumption in the vicinity of the



**Fig. 4.** The relation between SOD and sedimentary P fraction (A: Ex-P, B: Fe-P, C: CFA-P, D: Org-P) with treatment of oyster powder.

sediment-water interface (Walker and Snodgrass, 1986). In this regard, we found that SOD values in columns C and D were lower than those in the control column, thereby indicating that lower concentrations of Org-P would lead to a reduction in B-SOD.

With respect to Fe-P, we observed that SOD was negatively correlated with this P fraction in the control column and columns B and D. Fe-P in sediment is related to ORP (Fig.1), as low ORP (under anaerobic conditions) in sediments promotes the conversion of ferrous compounds to ferric ion ( $Fe^{2+}$ ) with the aid of iron-reducing bacteria. These released phosphates dissolve in pore water (Hyun, 2005) and the dissolved phosphate becomes SRP as bioavailable P, which stimulates a growth in biomass (Bostrom, 1982). Therefore, a decrease in Fe-P contributes to an increase

in SOD due to nutrient uptake. In column C, however, we observed a positive correlation, which can be attributed to the fact that the ORC promoted an increase in ORP, thereby facilitating the development of a more aerobic state, in which some ferric ions bind with the DO derived from the bulk water to generate re-oxidized forms, namely, ferrous compounds (Nealson and Saffarini, 1994). The oxygen consumption associated with this re-oxidation is classified as chemical SOD (C-SOD). Concomitant with the chemical oxygen consumption promoted in column C, there was a positive correlation between Fe-P and SOD (Wang, 1980). Furthermore, the lower level of SOD detected in column C compared with that in other columns indicates that the proportion of C-SOD was less than that of B-SOD.

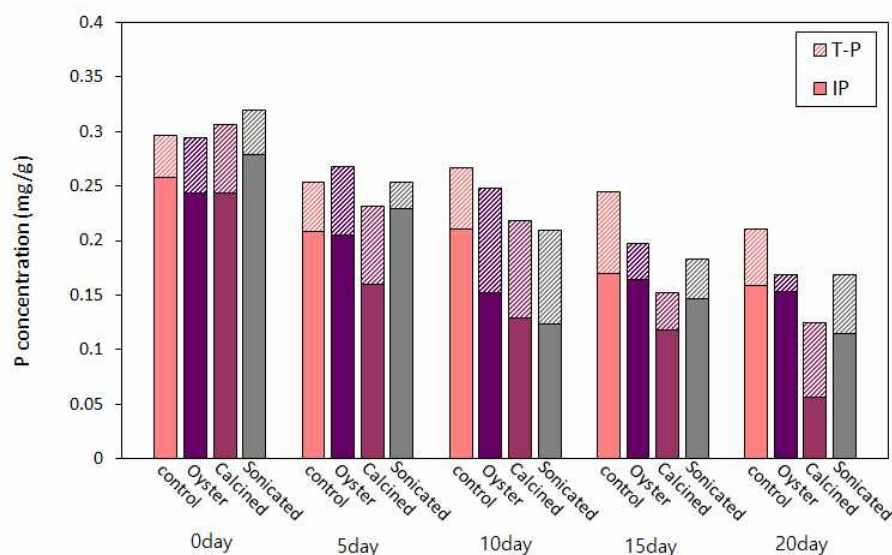


Fig. 5. Variation of TP, IP in sediment pore water for 20 days with treatment.

We found that CFA-P and SOD tended to be relatively poorly correlated. Phosphorus bound to calcium in sediment is generally recalcitrant to separation (Ruttenberg and Berner, 1993). CFA-P forms only in the presence of abundant calcium compounds in sediment, and thus may have little influence on changes in SOD. However, we found that in columns C and D, the overall CFA-P was higher than that in the control column. The generation of CFA-P is partially attributable to the formation of an insoluble complex comprising SRP in the pore water of sediment bound to calcium (Yang et al., 2015). This control of the release of limiting nutrients should have an indirect influence on the reduction of B-SOD.

#### 3.4. Phosphate releases with ORC treatments

Our measurements of the concentrations of T-P and dissolved inorganic phosphorus (DIP) in the sediment pore water over time are shown in Fig. 5. In all columns, T-P was observed to decrease continuously with time: control, 0.296 mg/g to 0.210 mg/g; column B, 0.294 mg/g to 0.169 mg/g; column C, 0.306 mg/g to 0.125 mg/g; and column D, 0.320 mg/g to 0.168 mg/g.

The concentrations of DIP were found to decrease in manner similar to those of T-P.

When phosphate concentrations in the pore water of sediments are higher than those in the bulk water, there is a diffusive migration of phosphate across the interface between sediment and water, the rate of which depends on the concentration gradient (Froelich et al., 1982). Simple diffusion explains the gradual decrease in T-P in pore water and its subsequent increase in the water layer. This migration of T-P was more clearly observed in the control column than in any of the other columns. Soluble phosphates in pore water tend to bind to metal oxides or calcium components under certain conditions to form complexes, which may be readily adsorbed within the sediment matrix (Cha et al., 2005). In column D, supplementation with oyster powder promoted the formation of Ca-P, which eventually removed a proportion of the pore water-borne phosphate, whereas in contrast, in column C, the existing Fe-P as well as Ca-P largely remained within the pore water due to the relatively high oxidative or low reductive state



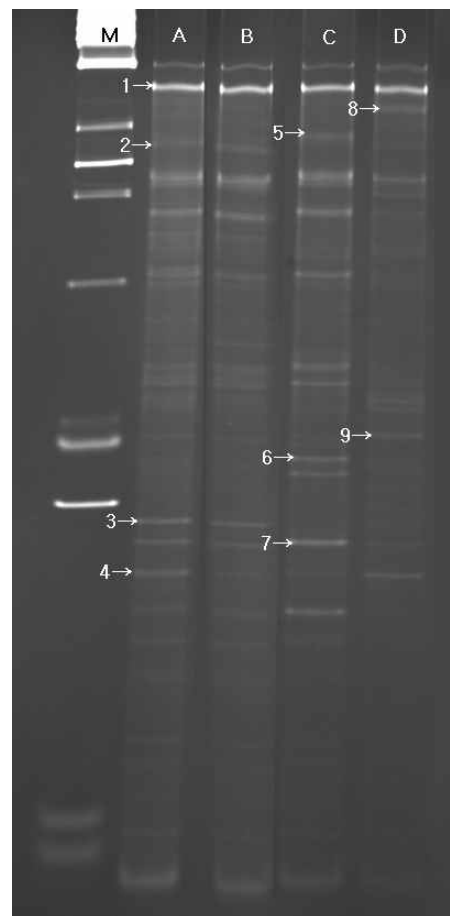
**Table 1.** Base sequences of 16S rDNA read from DGGE band

DGGE band	Description	Identity (%)	NCBI accession no.
1	<i>Marinobacter</i> sp.	97%	KF201612.1
2	<i>Shwanella</i> sp.	94%	FJ169987.1
3	<i>Ferrimonas</i> sp.	98%	AB682169.1
4	<i>Pseudomonas</i> sp.	95%	MK007373.1
5	<i>Thiobacillus</i> sp.	97%	HQ674835.1
6	<i>Bacillus</i> sp.	98%	KR095405.1
7	<i>Klebsiella</i> sp.	95%	KP209262.1
8	<i>Halomonas</i> sp.	98%	EU375038.1
9	<i>Sulfuriferala</i> sp.	97%	NR_114805.1

promoted by the calcined oyster shell powder. Consequently, a larger amount of phosphate was eliminated in column C than in the other three columns (Jensen and Thamdrup, 1993). Inputs of any type of oyster shell powder can suppress the rate of phosphate release, thereby contributing to an improvement in water quality, characterized by less eutrophication and an oxygen balance within sediments.

### 3.5. Microbial community

For assessment of the sediment microbiota, we collected sediment samples for PCR-DGGE analysis. Table.1 lists all the microorganisms subsequently identified. We selected nine bands in the DGGE gel (Fig. 6). In the control column and column B, we identified four major species, namely *Marinobacter* sp., *Shwanella* sp., *Ferrimonas* sp., and *Pseudomonas* sp., with a high probability. In column C, there were marked differences in the detected microbial species, with *Thiobacillus* sp. and *Bacillus* sp. being the major components, whereas *Halomonas* sp. and *Sulfuriferala* sp. were dominant in column D. The band A1 shown in Fig. 6 was found to be common to the microbial communities in all columns. This common species, identified as *Marinobacter* sp., is known as decomposer of organic matter and a primary contributor to an increase in SOD on the sediment surface (Beaudoin et al., 2016). In the control column,



**Fig. 6.** Denaturing Gradient Gel Electrophoresis (DGGE) analysis of surface sediment at 20 day (A: Control, B: Oyster, C: Calcined oyster, D: Sonicated oyster).

the low ORP and high oxygen deficit contributed to the dominance of diverse anaerobes. *Shewanella* sp. and *Klebsiella* sp. are representative heterotrophic anaerobes and Fe<sup>3+</sup>-reducing bacteria, such as *Ferrimonas* sp. and *Pseudomonas* sp., reduce ferric to ferrous ions by oxidizing molecular hydrogen. Furthermore, species of *Shewanella* reduce iron or manganese by using oxygen, ferrous ions, manganese, nitrate, or sulfur as electron receptors (Nealson and Saffarini, 1994), whereas *Thiobacillus* sp. found in column C oxidizes various sulfur and ferrous compounds (Tuovinen and Kelly, 1973). The presence of sulfur oxidizers is indicative of the presence of other oxidizers, which may utilize a range of reductive compounds in sediment to increase C-SOD (Wang, 1980).

#### 4. Conclusion

In this study, we investigated environmental factors, including limiting nutrients, SOD, and microbial flora, in a model system comprising sediments and overlying water. Four different test columns were used to determine the effect of oyster shell powder prepared using thermal or ultrasound treatment. Calcined and/or ultrasonicated oyster shell powder was found to increase sediment pH via hydration and enhanced the "aerobic mood" due to an increase in oxygen supply, whereas the control column and that supplemented with raw oyster shell powder were characterized by the development of anaerobic conditions. We also found SOD and chlorophyll-*a* (simulating algal growth) to be positively correlated. Supplementation of sediments with oyster shell powders was found to reduce the rate of DO reduction. The observed relationships between SOD and P fractions provided insights into some of the underlying physical/biological mechanisms occurring this sediment-water incubation experiment, including diffusive migration, insoluble complex formation, dissolved molecular oxygen generation, and the

growth of biomass. Moreover, PCR-DGGE analyses identified the major microbial species in each of the test columns. Overall, the treatments with oyster shell powders were found to contribute to the control of nutrient release and effectively reduced oxygen consumption in sediments.

#### Acknowledgements

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