The effect of calcium peroxide originating from oyster shell powder on control of phosphorus compounds in oceanic sediment

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Abstract−This study evaluated the water quality above the marine sediment by inputting oxygen releasing compound (ORC) processed from calcined oyster shells. Presumed vital parameters such as DO, pH, ORP, chlorophyll-a and classified phosphorous compounds were monitored for 20 d. ORP decreased with time in the control bed, while it increased to a positive value as a result of the ORC effect. DO kept showing a relatively high concentration in ORC treated column. We observed an increase of chlorophyll-a and a decrease of dissolved inorganic phosphate (DIP) simultaneously, which meant the released inorganic phosphorus would convert to an organic form in the overlying water. TP rises were the lowest in the ORC column (79%), meanwhile in the control column those went up to 0.304 mg/L (85%). Also, phosphorus fractions were measured in the sediment: Fe-P decreased in control while Fe-P and Ca-P soared greatly in the ORC column. This implies that in more oxidized environment inorganic phosphate bound to Ca-species would be eliminated as solidified precipitates in the sediment pore water, and it consequently suppressed the release of phosphates to the overlying water. The results indicate that the release of phosphorus and resulting eutrophication could be effectively controlled via the local environment improved by calcined ORC.

Keywords: Oyster Shell, Calcined Oyster, Oceanic Sediment, Phosphorus Release, Phosphorus Fractions

INTRODUCTION

Phosphorus is a limited nutritional salt that would provoke eutrophication in various water environments such as oceans and lakes; massive input of phosphorus accumulates in sediment, resulting in algal bloom [1]. The adsorption of P onto sediments and the release of this sedimentary P into water are affected by various physical, chemical and biological factors [2,3]. Some P forms, such as loosely-bound, redox-sensitive and organic P (OP) are bioavailable and can cause eutrophication [4,5]. Different pollutants are adsorbed to the sediments and accumulated on the bottom of the ocean. These sediments may accumulate over long periods and can act as new pollutant sources to the overlying water [6,7].

Different types of phosphorus in sediment are affected by many factors and are closely related to the release of phosphates [8]. Phosphorus can be transferred from water to sediment through biochemical and physical reactions, such as ion exchange, adsorption, and precipitation [9], and also could be released from sediments as the overlying water quality changing [10]. Physical and chemical characterizations of sediments are important for evaluating the P exchange processes between sediments and overlying waters [11]. DO is crucial in the release of phosphorus in that most releases take place in anaerobic conditions [12]. Therefore, this kind of phosphorus release can be controlled by oxygen supply at the sediment-water interfaces. Among different types of phosphorus, ferric oxide bound P, calcium apatite bound P, and metallic/mineral phosphates are of interest. Under the anaerobic condition where ORP is low, reduction dominates, and metal bound phosphates are expelled into the water [13]. The fate of Fe-P can be determined according to the extent of the oxidation-reduction state, whether it is released or remains [14]. Therefore, it is believed that controlling P is the best approach for reducing eutrophication [15].

Recently, ORC application on sediment remediation has drawn much attention. In particular, CaO_2 reacts with H_2O to release oxygen when put in water or in sediment (due to overloaded oxygen molecules) [16,17]. The released O_2 turns the local environment to a more oxidized state as well as Ca species combines with phosphate ions, resulting in an adsorbed complex in the sediment. Two mechanisms let CaO_2 become more vulnerable in sediment remediation [14]. On the other hand, the yearly production of oyster shells on the southern coast of Korea reaches 100,000 tons, most of which is not recycled properly. Some of them are exploited in lime fertilizers and crude $CaCO_3$, but still remain as minor uses [18]. We suggest an alternative way to use oyster shells. The major portion of oyster shells is $CaCO_3$, 94% or more. It can be used directly as raw material for industrial Ca production. Also, it could be converted to calcium oxide when processed at $700\,^\circ C$ or higher. Further oxidation with hydrogen peroxide gives calcium peroxide (CaO_2).

This work investigated the following in a properly set water-sediment column: (1) crude production of calcium-based ORC in a lab, (2) evaluation of water quality with the treatment of calcined oyster shell and raw oyster shell, and (3) phosphate fractions which are critically responsible for phosphorus releases in sediment with ORC treatment.

MATERIALS AND METHODS

1. Sample Collection and Study Area

Marine water and sediment samples were collected near a marine college in the city of Tongyeong ($34^{\circ}50'28.5''$N$128^{\circ}28'16.4''$E), in the southeast coast of South Korea. The sampling site was characterized by its malicious odor and consistent pollution, such as waste dumping, fishing activity, and especially aquaculture activity. The sediment samples were collected from around 10 m below of water level through a stainless-steel grab sampler at a depth of 0-20 cm. They were quickly packed in airtight cleaned polythene bags and transported to the laboratory for storage at $-20\,^{\circ}$C until quantification and determination of their physicochemical characteristics. The sampler was cleaned with distilled water before use to avoid unnecessary contamination. Seawater was collected as a sample from the water column at a depth of 0.5 m by a water sampler from the same site.

2. Calcined ORC Preparation and Experimental Setup

Generalized procedure of CaO_2 manufacturing is as follows:

$$CaCO_3 \rightarrow CaO + CO_2 \tag{1}$$

$$CaO + H_2O \rightarrow Ca(OH)_2 \tag{2}$$

$$Ca(OH)_2 + H_2O_2 \rightarrow CaO_2 + 2H_2O \tag{3}$$

In this work, the detailed procedure was: (1) 100 g of raw oyster powder was heated at $900\,^{\circ}$C for 2 h, and then cooled before mixing with DW of 40 mL in ice bath (2) The resulting slurry was reacted with hydrogen peroxide (30%) of 50 mL to give calcium peroxide.

In the experimental column, 300 gm of sediment was placed in a 2 L transparent plastic column. Calcined oyster shell powder and raw oyster shell powder were mixed with sediment as of 0.5 wt%. Three columns were used in each column experiment: column A (control), column B (raw oyster shell powder application), and column C (calcined oyster application). And then GF/C filtered seawater (1 L) was added carefully to avoid any disturbance. The column was covered with a lid to prevent extra oxygenation. Incubation was done under 6,300 lux (12/12; LED) at $22\,^{\circ}$C. Analyses were carried out every 5 d.

3. Analysis

3-1. Physicochemical Properties and Chemical Analysis

For phosphates analyses, pore water of sediment was sampled to be centrifuged at 2,000 rpm for 20 min. Water samples were filtered with a GF/C filter before analysis. DTP, DIP, and TP were performed with the molybdenum blue method. For TP, a sample was hydrolyzed with calcium persulfate in an autoclave at 120 oC before spectrometry. DO was measured with a DO probe (YSI 550A, USA). pH and ORP were measured with a multi-mater (Orion 3 star, USA). Chlorophyll-a was determined via the acetone extraction method. That is, 1-L of seawater was filtered and mixed with 90% acetone.

In 24 h, the extract was centrifuged at 30,000 rpm for 10 min. Then, the supernatant was spectrometrically analyzed at 750, 664, 647, and 630 nm. Analysis of Fe followed the ferrozine method [19,20]. Fe(III) was calculated from total Fe and Fe(II).

3-2. SEDEX Method for Phosphorus Fractions

A sequential sediment extraction (SEDEX) method was employed for phosphorus characterization [21]. Five P-fractions for analysis were as follows: loosely sorbed or exchangeable P; ferric iron-bound P; Authigenic apatite+Biogenic apatite+$CaCO_3$ bound P; Fluro apatite P+other inorganic P; Organic P. Fe-P was determined via butanol extraction [22]. All other dissolved P's were spectrophotometrically analyzed at 880 nm via the molybdenum blue method.

3-3. Alkaline Phosphatase Activity (APA) Analysis

One mg of subsurface sediment was captured. Four mL of 1 M Tris-HCl buffer (pH 8.6) was added to be ultrasonicated for 45 s. The enzyme extract was reacted with p-NPP (1 mg/mL) and 0.2 M Tris buffer in a 37 $^{\circ}$C water bath. It was vortexed for 2-3 s and then incubated for 1 h in the bath. The reaction was terminated with 1 mL of 3 N NaOH. The sample was centrifuged at 3,000 rpm for 10 min, and 0.5 mL of the supernatant was diluted to 1 : 10 with 1 M Tris buffer. The absorbance at 405 nm showed p-NP, the yellowish enzymatic product.

4. Statistical Analyses

Statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The differences between treatments were statistically analyzed via the one-way ANOVA test. Correlations between variables were analyzed using the Pearson correlation coefficient. Mean separations were achieved by using the least significant difference test at $P<0.05$.

RESULTS AND DISCUSSION

1. Physical & Chemical Changes through ORC Treatment

Every change in pH and ORP in sediment is shown in Fig. 1. With the addition of oyster shell powder, pH rose to 9.1 in column C and stabilized around 7.5 at d 20. As shown in Eq. (4), CaO_2 reacts with H_2O to produce $Ca(OH)_2$, which causes a rapid pH rise. In the column treated with plain oyster shell, $CaCO_3$ has very low solubility in the aqueous phase, and a buffering ability to stabilize pH.

For ORP, there were significant differences between columns ($P<0.05$). In columns B and control, those values changed from -125.1 mV and -126.8 mV to -174.6 mV and -215.3 mV, respectively. With time, the values decreased continuously. Meanwhile, in column C, the value increased from -99.2 mV to -44.4 mV in 10 d, and then decreased up to -95.5 mV. Additionally, supplied oxygen from the ORC rebuilt a better oxic environment in column C; meanwhile, in the other columns the anaerobic condition in sediment was maintained or worse [13].

The DO decreased from 7.5 mg/L to 3.9 mg/L, 4.2 mg/L, and 5.9 mg/L in columns A, B, and C, respectively. After 10 d of columns A and B showed a drastic change of oxidation state, i.e., depletion of oxygen due to the propagation of local reductive state nearby sediment.
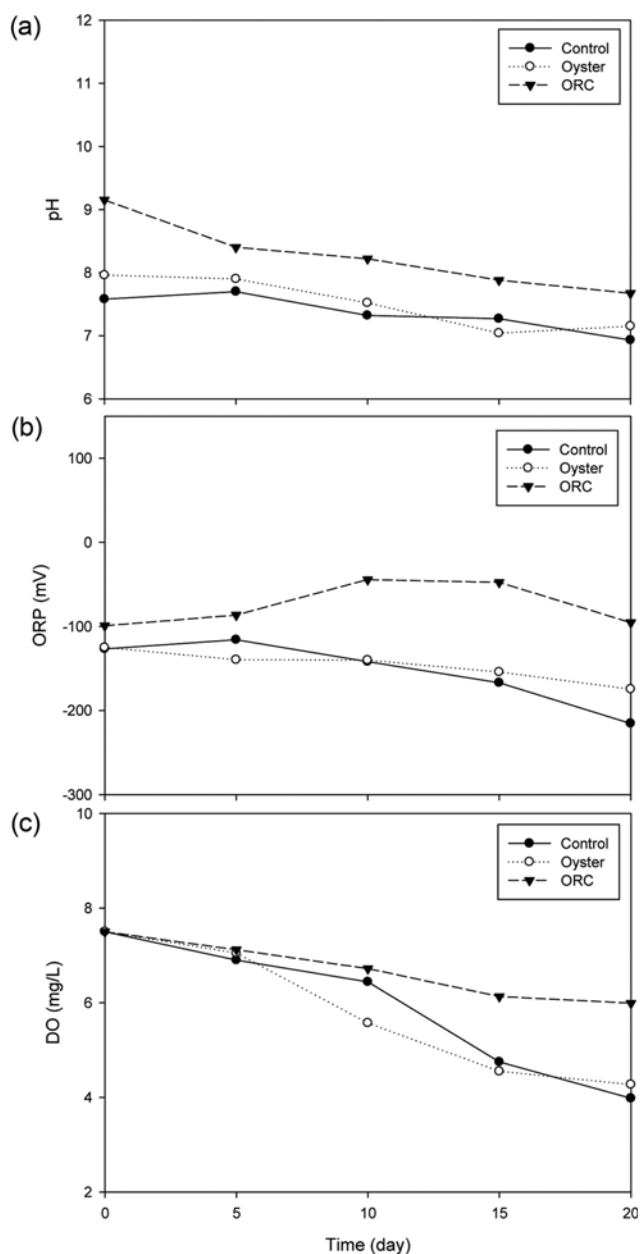
$$2CaO_2 + 2H_2O \rightarrow 2Ca(OH)_2 + O_2\uparrow \tag{4}$$

Fig. 1. Variation of (a) pH, (b) ORP in sediment and (c) DO in overlying water for 20 d.

$$CaO_2 + 2H_2O \rightarrow Ca(OH)_2 + H_2O_2 \tag{5}$$

DO level in column C changed little because of surplus oxygen released from ORC material.

2. Effect of ORC on Water Quality

2-1. Total Phosphorus (TP)

TP and DTP concentrations in overlying water are shown in Fig. 2. Overall, TP increased in all columns ranging 0.304-0.206 mg/L from initial concentration of 0.043 mg/L. Note the lower rate of TP rise in column C. For DTP, the concentrations rose initially, but were stabilized shortly after that. It was found that DIP decreased more than organic species through analysis.

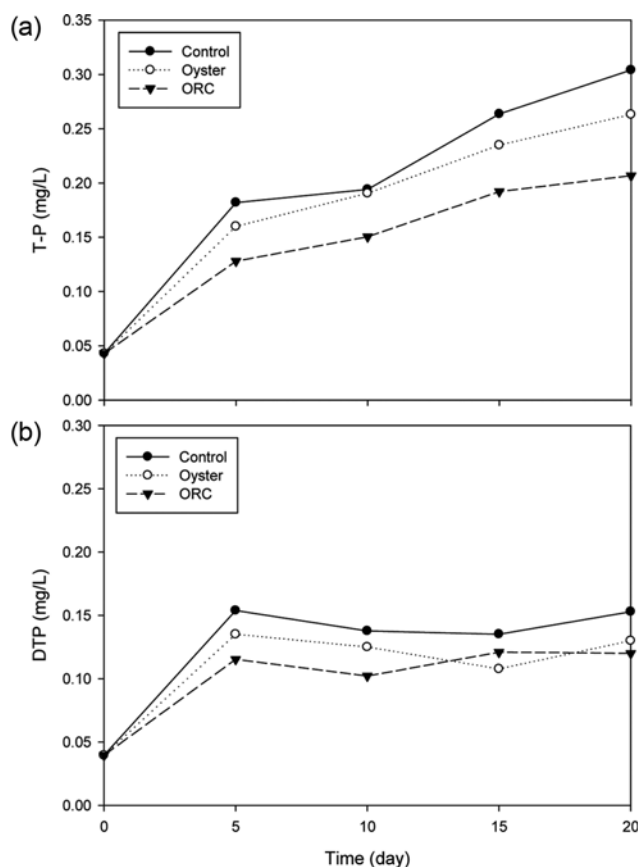The increase of TP can be explained due to the continuous release

Fig. 2. Profile of (a) T-P and (b) DTP in overlying water for 20 d.

of phosphorus compounds in sediment toward the water phase via mechanistic migration (diffusion) [23]. That migration retarded but would not be terminated even in column C, because DO diminished despite the presence of ORC [24]. As shown in Fig. 2, TP concentration subtracted with DTP, which is particulate organic phosphate, which means that the released inorganic phosphorus converts to an organic form. Furthermore, the increasing organic phosphate would exist as DOP in spite of depletion of DIP in 5 d, thus leading to a steady level of DTP (Fig. 2(b)).

2-2. Relation among APA, Chlorophyll-a and DIP

To investigate the effect of limited nutrients on the growth of phytoplankton, we observed Chlorophyll-a vs APA and DIP vs APA in Fig. 3. Chlorophyll-a increased throughout the entire period, particularly with the higher rate in 10 d. Naturally, Chlorophyll-a increased from 3.45 µg/L to 151.0 µg/L while 142.1 µg/L in column B and 86.6 µg/L in column C. For DIP, the concentration rose to 0.123 mg/L in 5 d, and then dropped to 0.08 mg/L in the end. With the treatment of oyster shells, DIP showed lower concentrations.

From Fig. 3, APA correlated fairly with Chlorophyll-a because the hydrolase was induced from phytoplankton. However, DIP correlated with APA negatively. Algae grow on the consumption of limited nutrients [25]. SRP (soluble reactive P) depicted as DIP can be taken in directly by the plant. In that case, we assumed that SRP in the water phase could be utilized first rather than any other phosphates like organic phosphate [1]. As bioavailable P is rich, the growth of the plankton increases. The massive biomass triggered
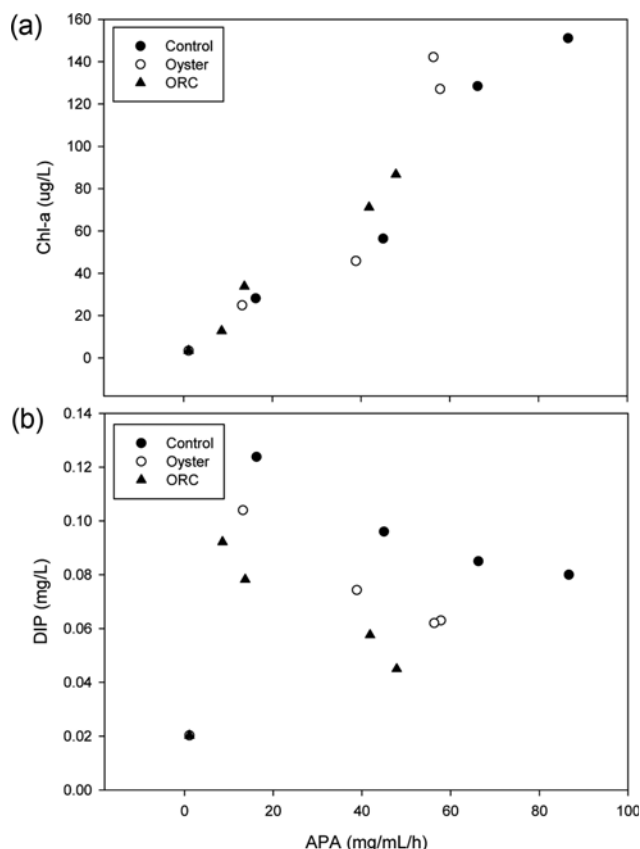
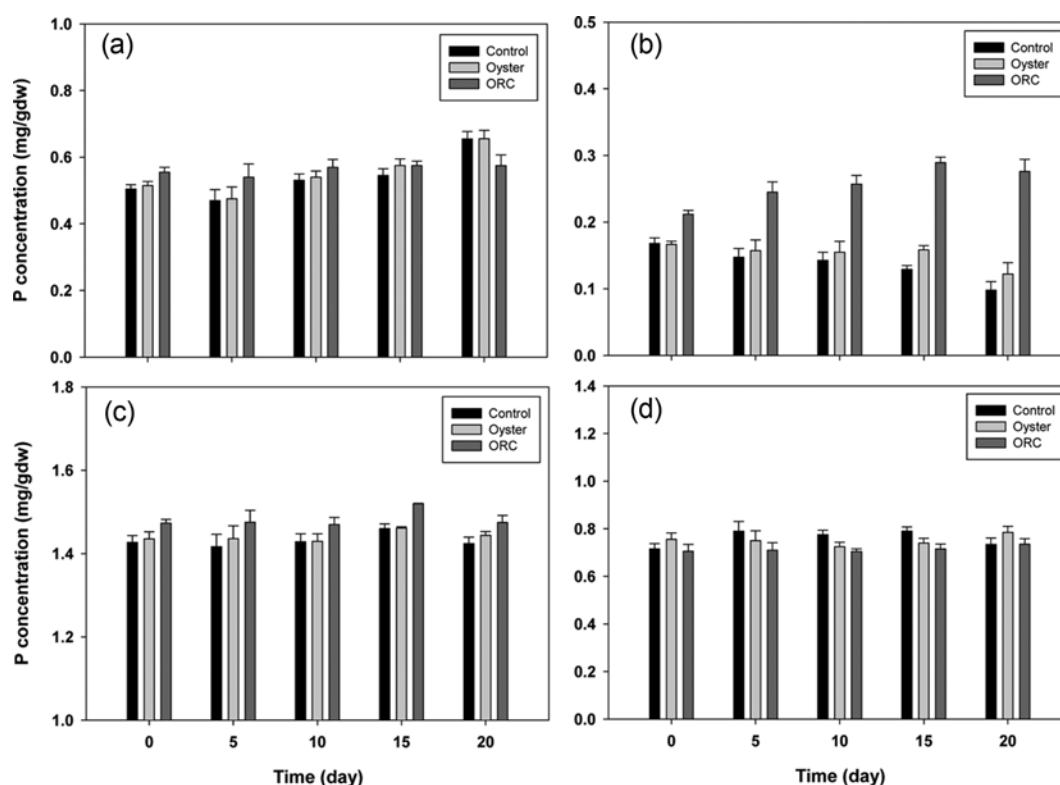**Fig. 3. (a) Chlorophyll-a and (b) DIP in overlying water for 20 d.**

the related enzymes to lead the production of SRP from organic phosphate. With the treatment of oyster shell powder, growth of algae was suppressed owing to reduced intake of DIP from sediment.

3. P-fractions in Sediment with ORC Treatment

3-1. P-fractions in Sediment

P fractions extracted from the sediment are important entities in a sediment-water system. Four P fractions are shown in Fig. 4: exchangeable and loosely adsorbed P (EX-P); ferric iron-bound P (Fe-P); calcium fluorapatite bound P (CFA-P); Organic P (Org-P).

In the curve of Ex-P, this P fraction increased with time. The concentration level was in the order of column C, column B, and control. Ex-P consists of $CaCO_3$-P and organic phosphates from phytoplankton and zooplankton, which are easily biodegradable [21]. The input of oyster shell powder accelerates the binding of soluble calcium ions and dissolved phosphates to form Ex-P in short times. In the latter period, the concentration of Ex-P in column B, 0.673 mg/g·dw was higher than that in column C, 0.597 mg/g·dw because of higher flow-in of organic phosphates originating from the plankton mass shown in Fig. 2.

Fe-P values were statistically significant among columns ($P < 0.05$). The concentrations in columns A and B dropped; meanwhile, that increased in column C by 35%. Fe-P is regarded as one of the most important factors determining the releasing rate of phosphorus which depends on the local redox condition [26]. Especially, phosphates separated from ferric iron(III) under a reductive mood could be taken by algae in the first place [27].

Generally, in an O_2-rich mood, Fe exists as FeOOH by some oxidation. This FeOOH binds to phosphates to be accumulated in

Fig. 4. The sedimentary P fraction (a) Ex-P, (b) Fe-P, (c) CFA-P, (d) Org P for 20 d.

sediment [28]. In columns A and B where oxygen is depleted with time, Fe-P decreases under more anaerobic conditions. Once Fe(III) turned into Fe(II) by reduction, Fe-P would be dissociated into Fe(II) and PO_4^{3-}. Then those ions were dissolved in porewater. The accumulated phosphates in pores of sediment tend to diffuse out by concentration gradient [29]. In contrast, the Fe-P increase found in column C well correlated with ORP increase given in Fig. 1. More oxidative condition via ORC treatment facilitated the binding of phosphates in porewater with ferric oxides; the increase of Fe-P throughout the whole period as CFA-P was also different statistically between control column and the other ones (P<0.05), although CFA-P remained all the way to the end with 1.43-1.48 mg/g·dw in all columns. With ORC or $CaCO_3$, phosphates in porewater could form Ca-P so that diffusible phosphates reduced in sediment [14].

$$3Ca^{2+}+2PO_4^{3-}\rightarrow Ca_3(PO_4)_2\downarrow \qquad (6)$$

Eq. (6) depicts the non-degradable phosphate complex as Ca-P, which accompanies the elimination of SRP in porewater [30]. Plain oyster shell powder in column B provided a much smaller concentration of Ca^{2+} because of its extremely low solubility, which leads to less formation of Ca-P. Organic phosphates have shown up and down in columns A and B (10-20%), meanwhile that changed little in column C.

3-2. Fe in Sediment

As shown in Fig. 5, the behavior of Fe(III) and Fe(II) in sediment. Fe(III) and Fe(II) interacted complementarily as predicted.
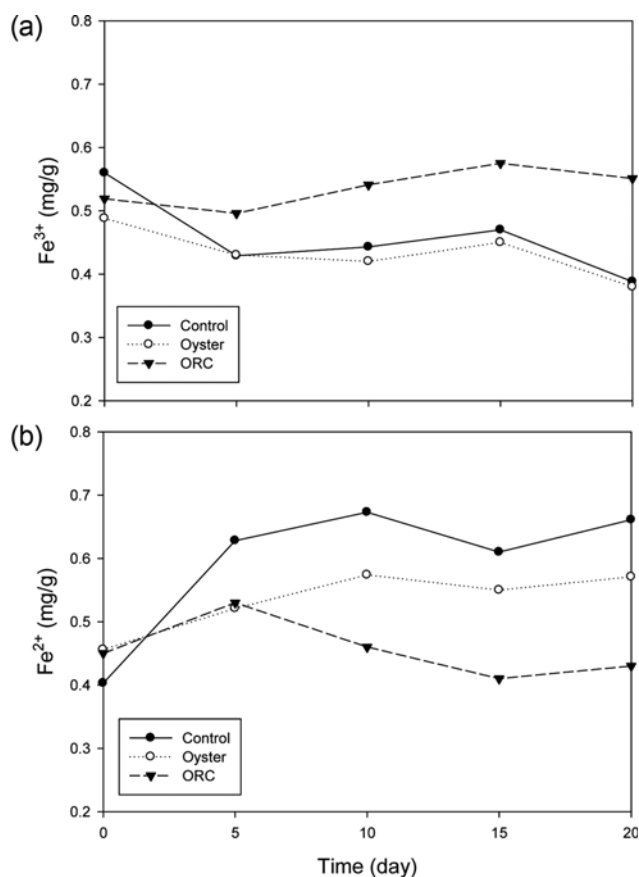
For example, Fe(III) changed 0.56 mg/g to 0.38 mg/g, while Fe(II) increased to 0.66 mg/g from 0.40 mg/g in column A. With ORC, Fe(III)/Fe(II) became less fluctuating, which meant some difference between oxidative states of the two columns. In anaerobic mood in sediment, Fe(III) reducing bacteria (FeRB) turn Fe(III) (insoluble) into Fe(II) (soluble) [29].

The reductive environment due to depletion of oxygen enforces FeOOH to Fe(II), which is separated from 'metal-salt' precipitate. As a result, the diffusion of soluble phosphates enhances algal growth at the subsurface of water [31]. Our results agreed with other research in which ferric oxides and Fe-bound phosphates decreased at the same time [32]. Oxygen supply through ORC treatment suppresses dissociation of 'metal-phosphate' complex and furthermore slows the release of soluble phosphates from a sediment environment.

3-3. Phosphates in Porewater

TP and IP in porewater are shown in Fig. 6. TP changes were remarkable among columns. Especially, reduction in column C was 59% (0.306 to 0.125 mg/g), the highest among all. IP changes were also alike to TP. As confirmed the concentration gradient between pores and bulk water drives phosphate's release (seen as phosphate reduction in porewater phase) [31]. This redistribution of phosphates is shown in Figs. 2 and 4. Also, the reduction of phosphates in porewater greatly counted on P-fractions (Fig. 4). TP in porewater, Ex-P, Fe-P, and Ca-P correlated with each other significantly (P<0.05). ORC input made a great contribution to the reduction of phosphates in porewater of sediment by forming Ca-P and Fe-
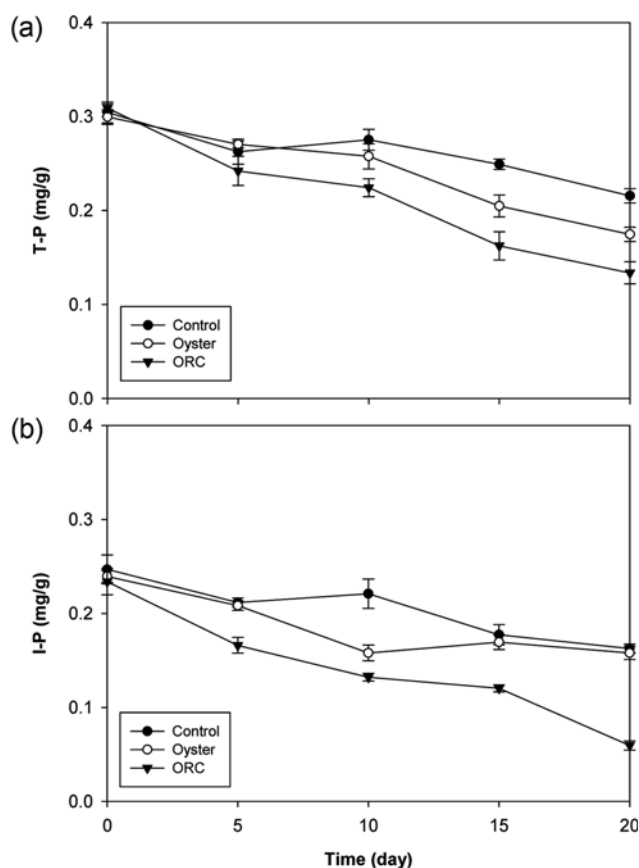

Fig. 5. Variation of (a) Fe(III), (b) Fe(II) in the sediment for 20 d.

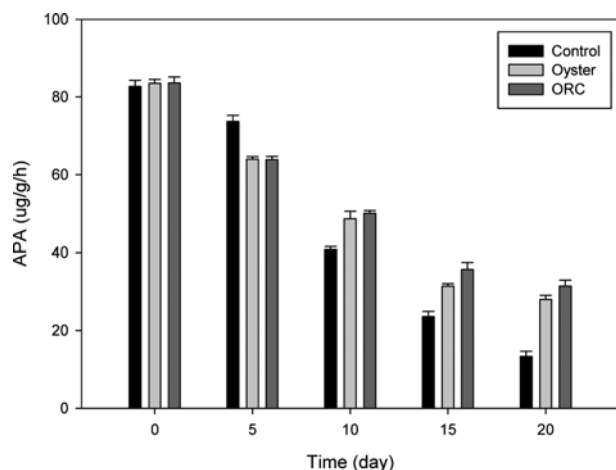Fig. 6. The profile of (a) T-P, (b) IP in sediment pore water for 20 d.

Fig. 7. Variation of APA on the surface of sediment for 20 d.

P in particular.

3-4. Effect of ORC on Enzymatic Activity in Sediment

APA change in sediment with time is shown in Fig. 7. APA decreased in all columns. In control, the value changed from 81.6 µg/g/h to 30.3 µg/g/h; meanwhile, that dropped to 28.77 in columns B and C. Theoretically, bioavailable P links to less APA because of the lower need of phosphate synthesis. In column C, oxygen triggers Fe-P formation to result in the elimination of soluble phosphates. Consequently, microbes in sediment activate the hydrolase production in early times as 84.7 µg/g/h of enzymatic activity, the highest of all. Also, the overall reduction of APA with time might be due to a cumulation of organic phosphates on the sediment surface, which implies no longer need for additional hydrolase production.

CONCLUSIONS

This work comprises phosphate behavior in a sediment-water column system along with various additives such as plain oyster shell powder and calcined oyster shell powder as an oxygen supplier. ORC treatment enhanced ORP, more oxidative environment and alkaline mood with the maintenance of steady DO. Also, ORC interfered with growth of phytoplankton by almost 43% as observed in terms of Chlorophyll-a. TP also was significantly reduced by 32% with the treatment of ORC. Through P fraction analysis, Fe-P and CFA-P are the most crucial factors affecting the release of phosphates in sediment via Fe- or Ca-binding with phosphates (ion-salt complexation) depending on local redox condition. Column experiments strongly suggest the effectiveness of calcined oyster shell powder as an oxygen provider for remediation of various sediment.

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