OYSTER BIO DEPOSITION ENHANCED THE FUNCTIONAL POTENTIAL OF LABILE AND RECALCITRANT CARBON IN SHENZHEN BAY’S SEDIMENTS

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ABSTRACT: Oysters are typical aquatic animals with their special biological property of filter feeding in the water column. However, the biodeposition of oysters may change the sediment chemistry and also may affect the biogeochemical processes. We conducted research work to determine the effect of Oyster Biodeposition (OBD) on the sediments microbes, and their metabolic abilities on carbon at the site rich with oyster culture area of Shenzhen Bay China. After biochemical and Geochip5 analysis, we found that the genes coding the metabolic enzymes pullulanase, glucoamylase, exoglucanase, cellobiase, xylanase, etc. were highly abundant in oyster farming place compared to reference place. Meanwhile, the functional genes cda (6%), pectinase (5%), and xyla (5%) were greatly abundant at the surface oyster area and they were showing significantly higher (P<0.05) normalized gene intensities compared to bottom sediment site. These genes were mostly involved in the degradation of labile and recalcitrant forms of carbon. These detected genes were mainly involved in the carbon degradation process and they were showing more tendency towards the degradation of labile carbon. Small abundance of enzymes such as glyoxal oxidase and phenol oxidase were also found which were involved in the degradation of recalcitrant carbon. Taken together, our results indicated that the OBD enhanced the degrading capacity of both labile and recalcitrant forms of carbon, it has a positive stimulating effect on sediment carbon metabolism.

Key words: Oysters, Biodeposition, Labile carbon, Recalcitrant Carbon, Shenzhen Bay.

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INTRODUCTION

Oysters are nowadays referred to as ecological engineers due to their important role in an aquatic ecosystem (Chowdhury, 2019). Oysters are also known as filter feeders due to their ability to clean water and reduce it’s turbidity (Coen et al., 2007). Oysters are suspended at the surface of bay for aqua culturing purposes. In this way, the fecal material from Oysters enter into the water sediments. The oyster farming area contains a huge deposition of biological organic matter released from the hanging oysters.

Oyster biodeposition (OBD) released from the Oysters get mixed with the sediment underneath and bring about changes in nutrients and physicochemical composition of sediments as well as in the water column (Mangi et al., 2021). There has been a lot of reported work on the organic matter distribution inside water bodies (Painter et al., 2018). Many research have also reported the effect of oyster biodeposition on the composition, and physical, chemical, and biological properties of sediments (Newell, Fisher, Holyoke, & Cornwell, 2005).

Carbon is understood as one of the main components present in sea sediments. From the accumulation and sequestration point of view, there are two types of carbon: Labile and Recalcitrant carbon (Rovira & Vallejo, 2002). The former has the tendency to melt quickly and degrade itself into the water column, whereas the latter is more stable than labile carbon and it can not be degraded easily with water or tidal flux etc (Lian et al., 2018). Shenzhen Bay has an area reserved for Oyster farming which is also a site of interest for researchers who want to explore the effect of Oyster farming on Shenzhen bay’s aquatic ecosystem. Previous studies have reported a significant impact of Oyster farming on Shenzhen bay’s water and sediment portion (Mangi et al., 2021). However, no study has yet provided sufficient and satisfactory knowledge about the effect of OBD on sediment’s labile and recalcitrant form of carbon.

Our key question was to find out the impact of OBD on sediment’s labile and recalcitrant carbon from the perspective of microbial metabolic machinery. It is a well-known fact that the carbon element is supposed to be the main nutrient present in sediments and also carbon is involved in a variety of important functions such as carbon fixation, carbon degradation, the Calvin cycle, etc.
The carbon present in the sediments may affect the metabolic activities of sediment microbial communities. So it is very worthful to determine the effect of oyster biodeposition on sediment’s labile and recalcitrant carbon from a microbial metabolic perspective.

**MATERIAL AND METHOD**

**Study location & Sampling:** The study was conducted on two hydrologically and geographically different sites in Shenzhen Bay (Figure 1). One site was covered with Oyster rafts and farming activity whereas the other site was away from the Oyster culture area with no farming practice (Figure 1). We collected a 100 cm sediment cores in replicates with sediment grabbers from both Oyster and Non-Oyster zones. The samples were kept in an icebox and brought to the lab for Geochip analysis.

**DNA extraction and Geochip microarray:** DNA was extracted from sediment samples using the Powersoil Pro Kit (DNeasy, USA), three times from each sample and pooled together. The DNA extract was checked on a 1% agarose gel, and the DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). An OD260/OD280 >1.8 was used.

The geochip technique was used to analysis carbon related functional genes. The detailed methods as described in reference Zhou et al (2020). We compared the average, standard deviation, and percentage of two distinct groups. We also conducted a t-test to know the level of significance. P-value of <0.05 was set as statistically significant. We labeled the final DNA for hybridizing and scan process. Finally, the gene signal intensities were calculated as mentioned in previous research (He et al., 2018).

**Data Analysis:** The data was calculated as mean, standard deviation, and percentage. The comparison between the two groups test and control was determined and their significance was measured by applying a t-test. To test for differences among more groups, we applied One-Way ANOVA with single factor or independent variable. All statistical analysis was done by using SPSS version 21 and the P-value of <0.05 was set as a statistically significant value.

![Figure 1: The sampling sites of Shenzhen Bay.](image)

**RESULTS**

**Textural Characteristics of Sediments:** We found that the sediments had about 47% fine sands at the depth of 0-30 cm of Oyster area compared to 40% at the reference area (Table 1). We found that the sediments had about 29% coarse sands at the depth of 0-30 cm of Oyster area compared to only 26% at the reference area (Table 1). We found that the sediments had about 25% fine gravel at the depth of 0-30 cm of Oyster area compared to only 33% at the reference area. We found that the sediments had about 42% fine sands at the depth of 30-60 cm of Oyster area compared to only 32% at the reference area (Table 1).
Table 1: Textural characteristics of Shenzhen Bay’s sediments.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>Fine gravel</th>
<th>Coarse sand</th>
<th>Fine sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster site</td>
<td>0-30 cm</td>
<td>25%</td>
<td>29%</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td>30-70 cm</td>
<td>28%</td>
<td>30%</td>
<td>42%</td>
</tr>
<tr>
<td></td>
<td>70-100 cm</td>
<td>34%</td>
<td>31%</td>
<td>35%</td>
</tr>
<tr>
<td>Reference site</td>
<td>0-30 cm</td>
<td>33%</td>
<td>26%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>30-70 cm</td>
<td>38%</td>
<td>30%</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>70-100 cm</td>
<td>33%</td>
<td>34%</td>
<td>33%</td>
</tr>
</tbody>
</table>

We found that the sediments had about 30% coarse sands at the depth of 30-60 cm of Oyster area compared to only 30% at the reference area. We found that the sediments had about 28% fine gravel at the depth of 30-60 cm of Oyster area compared to only 38% at the reference area. We found that the sediments had about 35% fine sands at the depth of 70-100 cm of Oyster area compared to only 33% at the reference area. We found that the sediments had about 31% coarse sands at the depth of 70-100 cm of Oyster area compared to only 34% at the reference area. We found that the sediments had about 34% fine gravel at the depth of 70-100 cm of Oyster area compared to only 33% at the reference area (Table 1).

Comparison of Labile and Recalcitrant carbon between Surface and Bottom Oyster site: We compared the surface site of the oyster farming region (TO) with the bottom portion of the same site (BO). We noticed that the surface sediment portion had more abundance of functional genes compared to the bottom sediment portion (Figure 2). The change between surface and the bottom area was statistically significant (P<0.05). The gene named cda which is responsible for starch degradation showed the highest normalized gene intensity (6%) at the surface sediment portion of the oyster area compared to the bottom sediment area. Like the cda gene, pectinase (5%) and xyla (5%) were also greatly abundant at the surface oyster area and they were showing significantly higher normalized gene intensities compared to the bottom sediment site (Figure 2). The labile carbon enriched compounds were Starch, Pectin, and hemicellulose at the surface oyster site. All three functional genes cda, pectinase, and xyla involved in the degradation of labile carbon (Figure 2). As far as the degradation of recalcitrant carbon was concerned, we found two main genes CsoS1_CcmK (7%) and GAPDH_Calvin (6%) which exhibited higher normalized gene intensities. The two genes CsoS1_CcmK and GAPDH_Calvin were coding the enzymes which were used in the biological processes related to the bacterial microcompartments and Calvin cycle (Figure 2). The degradation pattern of recalcitrant carbon was similar to the labile carbon and it showed significant variation calculated at a p-value of (P<0.05).

![Figure 2: Comparison of Labile and Recalcitrant carbon between Surface and Bottom Oyster site](image-url)
The other genes involved in carbon fixation, methanogenesis, and methane degradation were also found but their abundance were not as much higher as that of the genes related to the degrading labile and recalcitrant carbon. In our data results, we found more activity of labile and recalcitrant carbon degradation at the surface oyster farming site compared to the bottom oyster farming site (Figure 2).

**Comparison of Labile and Recalcitrant carbon between Oyster and Reference site:** After compared the site of oyster farming region (TO) with the control site (TN), we noticed that the sediment portion of oyster site had a significant abundance of functional genes compared to the control site sediment portion (Figure 3). The change between Oyster farming and the non-oyster oyster farming area was statistically significant at a p-value of (P< 0.05). The gene pectate_lyase_oomycetes (10%) which is responsible for starch degradation showed the highest normalized gene intensity at the oyster area compared to the control area. The labile carbon enriched compounds were Pectin at the oyster site. Apart from pectate_lyase_oomycetes, another gene such as pel_Cdeg (10%) detected by Geochip5 in oyster area exhibited the degradation of labile carbon (Figure 3). The pectin degradation was significantly higher at the oyster farming portion compared to the control site. The genes responsible for coding the enzymes involved in pectin degradation and the degradation of labile carbon were showing greater average normalized gene intensities. As far as the degradation of recalcitrant carbon was concerned, we found one key gene ccmM (29%) coding enzymes for microbial compartments showed the highest average normalized gene intensity at Oyster site compared to the reference site (Figure 3).

![Figure 3: Comparison of Labile and Recalcitrant carbon between Oyster and Reference site](image)

DISCUSSION

**Sediment differences and reasons at two sites:** As far as the textural characteristics of the sediments at both oyster and non-oyster place are concerned, we found that the oyster area had more sand compared to the control area. The fine sand in the Oyster area was about (47%) whereas the fine sand in the reference area was just (40%). Not only the fine sand but also the coarse sand was found higher at the oyster site as it was (29%) at the oyster site and just (26%) at the control site. The fine gravel was just (25%) at the oyster site whereas it was (33%) at the control site. We here just mention the surface sediment portion (0-30 cm ) deep regarding the discussion on textural characteristics of Shenzhen Bay sediment. However, we have mentioned the complete horizontal and vertical distribution of sand and gravel in tabular form (Table 1). The reason behind more sandy appearance of sediment cores at an oyster site may be due to location. Our studied oyster site was near to pearl river...
estuary so it is possible that the oyster might have received some inputs from the pearl river estuary (Qi-Jie, Bo-Sun, Yong-Jun, & Ming-Guang, 2003). According to one study, estuaries are also one of sources of organic matter and sandy components (Eyre, Maher, & Squire, 2013). Another reason for less sand at the control site may be the location factor as the control site was near the beach with more human activities and land-based inputs. The land-based inputs may be comprised of mud according to one research (Jarvie, Neal, & Tappin, 1997).

**Carbon metabolism functional genes and their significance at two sites:** The surface sediment portions receive direct input from the water column. The surface sediment layer is a bit soft and labile due to the fresh input of biological deposition. According to some studies surface sediment layers with aquaculture activities receive more organic matter (Holmer, Wildish, & Hargrave, 2005). Our study is also in agreement with other studies which have reported that the higher gene intensities are a big indicator of particular metabolic enzymes encoded by genes (Bai et al., 2013). The cda has been reported many times regarding it’s role in starch degradation. Similarly, pectinase and xyla have also been involved in labile carbon degradation. CsoS1_CcmK and GAPDH_Calvin were found involved in the biological processes related to the Bacterial Microcompartments and Calvin cycle. These both genes are also responsible for the degradation of recalcitrant carbon. Our overall results also suggested that the carbon degradation was higher compared to carbon fixation and the Calvin cycle. Our results are also in collaboration with the results matched from other studies (Mangi et al., 2021). As far as the comparison of Labile and Recalcitrant carbon between Oyster and Reference site was concerned, we found significant variations between the two distinct sites. The possible reason for such distinct variation was the presence of OBD input at oyster site. The input received from the sediment portions at the oyster site had more labile carbon and organic matter. The degradation of labile carbon at the oyster site can be considered due to the mix-up of oyster biological deposition with sediment layers as reported in other studies (Hoellein, Zarnoch, & Grizzle, 2015). However, the degradation of recalcitrant carbon is not frequent but in our case study, we found genes involved in the degrading process of recalcitrant carbon.

**Oyster farming has a positive stimulating effect on carbon metabolism in sediments, or has ecological significance for accelerating carbon metabolism:** The effect of biological deposition on the sediment portion of oyster farming site became quite visible after employing the Geochip5 advance microarray technique. We observed that the carbon compounds with labile nature and the carbon compound with recalcitrant nature were under degradative effect. The high presence of functional genes responsible for coding the enzymes involved in the degradation of labile and recalcitrant carbon was sufficient proof to conclude that they were under direct effect of OBD. We have seen similar studies where the greater abundance and normalized gene intensity is considered as the specified role of such a group of genes in a variety of metabolic events (Bai et al., 2013). In this way, we concluded this very interesting research after getting some important findings.

**Conclusion:** Our study confirmed that the OBD to the water column and subsequent sediment layers changed the physicochemical and biological nature of the sediment portion present underneath oyster rafts. Thus the sediments degrade more Labile and Recalcitrant Carbon and shape the microbial metabolic abilities in such a way that they facilitate more carbon degradation instead of carbon fixation. Oyster farming has a positive stimulating effect on carbon metabolism in sediments, which has important ecological value for improving carbon metabolism in marine sediments.

**Conflict Of Interest:** There is no conflict of interest to declare

**Author’s Contribution:**

All authors have contributed equally.

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