Evaluation Microbial Community Structure and Purification Capacity of Tidal Flat Sediment

Udin Hasanudin(1)*, Tadao Kunihiro(2), Masafumi Fujita(3), Hong-Ying Hu(4), Koichi Fujie(5) and Teruaki Suzuki(6)

Summary
Evaluation of changes in microbial community structure and purification capacity in tidal flat sediment with and without clams are important in order to clarify the relationship between clams and microorganisms on the improvement of tidal flat purification function. The objectives of this research are to evaluate the effect of clam on changes in microbial community structure and purification capacity of tidal flat sediments. Mesocosm of artificial tidal flat sediment with and without clams in Aichi Fisheries Research Institute, Gamagori, Aichi, Japan were employed in this experiment. It was found that clams increased diversity, number of quinone species, and density of menaquinone (MK) containing bacteria in tidal flat sediment. Diversity of quinone profile increased from about 9 at initial condition to about 11.6 after 4-month clam acclimations. Number of quinone species also increased from 12 to 15 at the same time. Increases in biodegradability of organic matter and changes in chemicals environment caused changes in microbial community structure in tidal flat sediment with clams. NH$_4$-N concentration in tidal flat sediment with clams was about 3 times higher than in the sediment without clams. This condition was suitable for nitrifying bacteria growth and increase nitrification process in tidal flat sediment with clams. High activity of nitrifying bacteria produced higher concentration of NO$_3$-N. Also, clam activities and high concentration of biodegradable compounds depleted dissolved oxygen (DO) concentration in the sediment. This condition was suitable for MK-containing bacteria growth. High concentration of MK-containing bacteria and low concentration of DO were suitable condition for NO$_3$-N removal from tidal flat sediment. NO$_3$-N removal capacity in upper layer of tidal flat sediment with clams was about 3 times higher than in the sediment without clams.

Keywords:
biodegradability; clams; microbial community structure; nitrate removal; quinone; tidal flat.

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1M.Eng., Department of Ecological Eng., Toyohashi University of Technology, Tempaku-cho 441-8580, Japan. Telp./Fax. +81-532-44-6910, udin@fujielab.eco.tut.ac.jp
2D.Eng., Faculty of Env. and Symbiotic Sciences, Prefectural University of Kumamoto 3-1-100 Tsukide, Kumamoto City 862-8502, Japan. tadao92@mwa.biglobe.ne.jp
3D.Eng., Department of Civil and Environmental Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan. fujita-m@ccn.yamanashi.ac.jp
4Prof. Dr. Eng. Department of Environmental Science and Engineering, Tsinghua University, Beijing, 100084 China (E-mail: hyhu@tsinghua.edu.cn)
5Prof. Dr. Eng, Department of Ecological Eng., Toyohashi University of Technology, Tempaku-cho 441-8580, Japan. Telp. +81-532-44-6905, Fax. +81-532-48-6369, fujie@eco.tut.ac.jp
6D.Eng., Aichi Fisheries Research Institute, 97, Wakamiya, Miya-cho, Gamagori, 443-0021, Japan. teruaki_suzuki@pref.aichi.lg.jp
1. **Introduction**

Tidal flat is a critical transition zone between land, freshwater habitats, and the sea \[^1\]. This place was recognized as important sites for nutrient transformation and sequestration via biogeochemical cycling \[^2, 3\]. Population growth has drastically increased during the last century, and since coastal areas are intensively inhabited, pollution pressure on marine environments has been dramatically enhanced over this time \[^4\]. Overload of nutrient to tidal flat area will cause nutrient accumulation and finally causing malfunction of tidal flat on seawater purification. These conditions promote eutrophication and often cause phytoplankton blooms followed by deposition of organic matter and detritus to seabed sediment \[^5\]. Eutrophication can be regarded as a contributing or enhancing factor, which together with adverse meteorological and hydrographical conditions causes depletion of oxygen and subsequent mortality of benthic communities \[^4\]. The large input of organic matter resulted in anoxia within the sediment, as a consequence of respiration processes, and an enhancement the activities of sulfate-reducing bacteria to produce hydrogen sulfide \[^5, 6, 7\]. Oxygen-deficient water further well up onto surface layer, and moved towards shore, causing a catastrophic impact to the benthic ecosystem of the intertidal flats in the innermost parts of the bay \[^5\]. Therefore, the improvement of natural self-purification potential of tidal flat ecosystem is necessary to enhance nutrient removal from tidal flat area and prevent eutrophication.

It has been known that microbes play an important role in primary production, nutrient cycling and decomposition of organic matter \[^8, 9\]. Also, benthic nutrient regeneration is known as a major driving force in the dynamics of biophilic elements in coastal marine ecosystems. Macrzoobenthos play a fundamental role in nutrient regeneration through their excretory products. One of the important macrobiotic in tidal flat is clam (*Tapes philippinarum*). *Tapes philippinarum* is the major contributor species to the macrozoobenthos biomass in tidal flat \[^10\]. Clams as a suspended feeder were recognized to have filtering capabilities as an important role in removing particulate organic matter (POM) from the water column and consequently reducing the potential for the occurrence of oxygen-depleted water developing in the bottom layer \[^11\]. Clams were efficient at removing particle having diameter between 3 – 10 μm \[^12\]. Also, clam excretion activities produced ammonia nitrogen (NH\(_4\)-N), nitrate (NO\(_3\)-N), nitrite (NO\(_2\)-N), total Kjeldahl nitrogen (TKN), phospate (PO\(_4\)-P), and BOD \[^10, 13\]. Thus, the filtering function of clams might produce carbon and nutrient with higher biodegradability, which useful to enhance microbial growth and accelerate mineralization process in tidal flat sediment \[^14\]. However, uncertainty about the actual contribution of clams to the self-purification capacity of tidal flat sediment may still remain due to insufficient data about the symbiotic relationship between microorganisms and clams in coastal area. Evaluation of changes in microbial community structure and purification capacity in tidal flat sediments with and without clams are important in order to clarify the relationship between clams and microorganisms on the improvement of self-purification function of tidal flat ecosystem.

The objectives of this research are to evaluate the effect of clam on changes in microbial community structure and purification capacity of tidal flat sediments. Moreover, the effect of clams on decomposition and biodegradability improvement of organic matter in tidal flat sediment were also studied to clarify the symbiotic relationship between clams and microorganism on the improvement of tidal flat purification function.
2. Materials and Methods

Research sites

All experimental plots were located in Aichi Fisheries Research Institute, close to Mikawa Bay, Gamagori, Aichi, Japan. The experiment was conducted in four plots of mesocosms of tidal and subtidal sediments. The subtidal sediment was constructed 15 cm lower than tidal sediment to maintain the sediment always under immersion condition. The dimensions of each plot were 2.0 m (W) x 2.5 m (L) x 0.4 m (D) with sand median grain size of 0.95 mm. Clams with 1.9 cm average length and 1.2 g average whole wet weight (included shell) were added to two plots of these sediments corresponding to about 5000 clams per plot on July 23, 2002. Fresh seawater was pumped from about 200 m offshore and supplied to both sediments in the mesocosms. To synchronized water level with natural condition, the water level in Mikawa Bay was online measured and the data was used to arrange flow rate of seawater to the mesocosms.

Sampling methods

One month after adding the clams, pore water quality in all plots, i.e., DOC and NH$_4$-N were monitored for 24 hours to investigate the effect of clams. Pore water sediment also used to analyze molecular weight distribution. About 20 ml of pore water for each analysis was taken from 5 cm depth of sediment using a syringe with capillary tube equipped with double layers of 1.2 μm filter paper (GF/C filter, Whatman).

Sediment cores (n=2) were collected from the 0 – 10 cm depth of each plot using an acrylic tube (5 cm i.d. x 50 cm length) every month over a year from July 2002. The samples from all plots were immediately cooled by ice and then stored at −20°C before quinone analysis. The sediment core sample from 0 – 10 cm was also collected from each plot using the same method and immediately used for biodegradability experiment. In order to evaluated NO$_3$-N removal capacities, the sediment core sample collected from 0 – 2 and 9 – 11 cm depth of tidal sediment with (A1; A2) and without (B1; B2) clams. A replaceable ring of acrylic tube with 4 cm I.D. and 2 cm length was used to collect this sample. Sediment samples for NO$_3$-N removal capacity experiment were used immediately after sampling.

Laboratory experiment: experimental set up and procedure

Biodegradability of organic carbon in the sediment was analyzed using modified OECD screening test No. 301E \[15\]. 5 gram of sediment was extracted with 60 ml of seawater using warring blender (3000 rpm, 10 min) then filtered using 0.3 μm filter paper (GF-75, Advantec). 40 ml of filtered seawater was filled-in to 200 ml of Erlenmeyer flask. Seed and mineral solutions were also added. The flasks were shake and incubated in the dark condition at 22 ± 2°C. Decomposition was followed by DOC analysis at frequent interval over a 28-day period. A control with inoculation, but without either test material or standard, was run in parallel for the determination of DOC blank.

NO$_3$-N removal capacity was investigated in batch laboratory scale. Tidal sediment with 4 cm diameter and 2 cm depth was put in a 100 ml beaker glass. Seawater was filtered with 0.3 μm filter (GF-75, Advantec) to avoid impurities. Sodium nitrate (NaNO$_3$) was used to enrich nitrate concentration in the filtered seawater. The initial NO$_3$-N concentration was set at about
4 mgN·l⁻¹. The enriched seawater for NO₃-N removal experiment was purged with nitrogen gas for more than 15 min to make anaerobic condition. 60 ml of the treated seawater was added gently to each beaker glass and was incubated at 30°C. The beaker glass for NO₃-N removal experiment was covered with parafilm to prevent oxygen transfer from air to liquid phase. Samples were taken every 3 hours for 12 hours. The flux removal rate (F) was calculated as follow:

\[ F = \frac{(C_t - C_0) \cdot V}{W \cdot t} \]  

where \( C_t - C_0 \) = the differences between NO₃-N concentration at \( t \) time and at initial; \( V = \) volume of seawater; \( W = \) weight of sediment; and \( t = \) time.

**Analytical methods**

The DOC and NH₄-N concentrations of pore water were measured using TOC analyzer (TOC-5000A, Shimadzu) and automatic water analyzer (AACS-III, Bran+Luebbe), respectively. T-test statistical analysis was performed to distinguish the differences between pore water quality in the sediment with and without clams. NO₃-N was measured using an ion chromatography (DX-120, DIONEX) equipped with Ion Pac column AS14 4-mm (10–32) (DIONEX) and a suppressor of ASRS-ULTRA 4-mm (DIONEX).

Microbial quinones in the sediments were analyzed based on the procedure previously reported \[16, 17\]. Quinones were first extracted from the sediments by a mixture of chloroform-methanol (2:1, v/v) and then re-extracted by hexane. The crude extract was purified using solid extraction cartridge (Sep-Pak® Plus Silica, Waters). The types and concentrations of quinones were determined with HPLC equipped with an ODS column (Zorbax-ODS, 4.6 (I.D.) x 250 mm, Shimadzu-Dupont) and a multi-channel UV detector (photodiode array detector, model: SPD-M10A, Shimadzu). A mixture of methanol and di-isopropyl ether (9:2, v/v) was used as the mobile phase at a flow rate of 1.0 ml·min⁻¹. The temperature of the column oven was maintained at 35 °C. Quinone species were identified according to the retention time on the column and the UV spectrum of each peak observed in the multi-channel UV detector. The linearity relationship between the logarithm of the retention times of quinones and the equivalent number of isoprenoid unit (ENIU) was also used to identify the quinone type \[16, 18\]. Ubiquinone with 10 isoprenoid and vitamin K1 were used as the quantitative standards for ubiquinone and menaquinone, respectively. The ENIU can be approximated by the following equation:

\[ \text{ENIU}_k = a + b \log \left( \frac{\text{ET}_k}{\text{ET}_{\text{std}}} \right) + c \left[ \log(\text{ET}_k/\text{ET}_{\text{std}}) \right]^2 \]  

where \( \text{ET}_k \) represents the elution time of a quinone species \( k \), and \( \text{ET}_{\text{std}} \) represents the elution time of standard quinone. The constants are shown as \( a, b, \) and \( c \) which are empirically obtained for each HPLC system \[19\]. The amounts of quinone were calculated from the peak area based on the mole absorption coefficients (ubiquinones: 14.4 mM⁻¹ cm⁻¹, menaquinones: 17.4 mM⁻¹ cm⁻¹ and plastoquinones: 15.3 mM⁻¹ cm⁻¹) \[20\]. The quinone mole fraction was calculated as a ratio of the quinone content in the species \( k \) to the total quinone content. In this paper, the abbreviation of quinone types are ubiquinone: UQ, menaquinone: MK,
plastoquinone: PQ and vitamin K1: VK1.

In order to evaluate the changes of microbial community structure in the sediments with and without clams, the diversity \( (D_Q) \) and dissimilarity \( (D) \) indices of respiratory quinone profile were calculated $^{[18]}$. These indices were calculated by the following equations:

\[
D_Q = \left( \sum_{k=1}^{n} \left( \sqrt{f_k} \right) \right)^2 \tag{3}
\]

\[
D_{(i,j)} = \frac{1}{2} \sum_{k=1}^{n} |f_{ki} - f_{kj}| \tag{4}
\]

where, \( f_k \) is the mole fraction of quinone species \( k \), \( n \) is the number of quinone species with the mole fractions higher than or equal to 0.001, \( f_{ki} \) and \( f_{kj} \) are the mole fractions of quinone species \( k \) for \( i \) and \( j \) samples, respectively.

### 3. Results and Discussion

#### Organic matter degradation

The average concentrations of DOC, DTN and NH\(_4\)-N in pore water of tidal and subtidal sediments with and without clams are shown in Table 1. In tidal sediment with clams, concentrations of DOC and NH\(_4\)-N were about 1.34 mg/l and 1.14 mg/l higher than in the sediment without clams, respectively. Clams consumed POM and excreted their feces as a source of DOC and NH\(_4\)-N. Most bivalves were efficient at removing particle having diameter between 3 – 10 μm and total suspended solid removal rate of clams were 0.4–4.9 (mg·l\(^{-1}\)·TSS (g-clams)\(^{-1}\)·day\(^{-1}\)), depend on shell size $^{[12]}$. The difference between DOC concentration in the sediment with and without clams was not so high. This strongly suggests due to the low concentration of particulate organic carbon (POC) in the seawater. POC concentration in the seawater was only about 2.3–4.9 mg·l\(^{-1}\). Also, sediment microorganisms consumed the DOC produced from clam excretion rapidly. DOC was continuously released from POC and followed by a rapid cycling of DOC during particle decomposition $^{[21]}$. NH\(_4\)-N concentration in the sediment with clams was about 80% of DTN. This result is consistent with the results of previous research.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>DOC (mg/l)</th>
<th>DTN (mg/l)</th>
<th>NH(_4)-N (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with clams</td>
<td>without clams</td>
<td>with clams</td>
</tr>
<tr>
<td>Tidal Sediment</td>
<td>6.97</td>
<td>5.63</td>
<td>2.15</td>
</tr>
<tr>
<td>Subtidal sediment</td>
<td>6.47</td>
<td>5.67</td>
<td>0.51</td>
</tr>
</tbody>
</table>

The excretion rate of *T. philippinarum* is about 50 mg.(kg.clam)\(^{-1}\)·day\(^{-1}\) and 80 mg.(kg.clam)\(^{-1}\)·day\(^{-1}\).
1. day\(^{-1}\) for ammonia nitrogen and total Kjeldahl nitrogen (TKN), respectively\(^{[13]}\). DOC, DTN and \(\text{NH}_4\)-N concentration in the sediment with clams also higher at low water level. During emersion, the penetration of oxygen into sediments may increase\(^{[22, 23]}\) and lead the production of DOC, DTN and \(\text{NH}_4\)-N due to clam excretion. This result indicated that clams could be used to accelerate degradation of organic matter and nutrients in tidal flat sediment.

A different pattern was found in subtidal sediment. DOC and \(\text{NH}_4\)-N concentrations in pore water of sediment with clams were about 0.8 and 0.1 higher than in the sediment without clams. This indicated that the effect of clams was relatively low. Since subtidal sediment is always under immersion conditions, it is expected that DO concentration should be low, lowering the activity level of clams. Therefore, there was no significant difference in DOC and \(\text{NH}_4\)-N concentrations in subtidal sediment with and without clams. \(\text{NH}_4\)-N concentration in pore water of subtidal sediment with clams was only about 27% of DTN. This also indicated that clam activities were low.

Clams also affected biodegradability of organic matter in tidal flat sediment (Fig. 1). Almost 90% of DOC from tidal sediment with clams was removed during biodegradability test (within 28 days), while in the sediment without clams DOC removal was only about 44.2%. This indicated that clams promoted the production of readily biodegradable substances in tidal flat sediment. Clams consume particulate organic matter, digest that compounds in their body, and excrete their feces as sources of dissolved and readily biodegradable substances. In the case of without clams, the degradation of particulate organic matter is likely due to an ecto-enzymatic activity of attached bacteria which renders the particles soluble through macromolecular hydrolysis and produces dissolved organic compounds and small molecules which are subsequently taken up by attached and free-living bacteria\(^{[21, 24, 25]}\). Degradation rate through microbial hydrolysis process is lower than degradation rate through clam activities. Therefore, DOC concentration and biodegradability of organic matter in the sediment with clams were higher than in the sediment without clams.

![Figure 1. DOC concentration during biodegradability test in tidal flat sediment with and without clams](image-url)

Figure 1. DOC concentration during biodegradability test in tidal flat sediment with and without clams

In subtidal sediment, biodegradability of organic matter in the sediment with clams also much higher than in the sediment without clams. All of DOC from subtidal sediment with clams was removed during biodegradability test, while in the sediment without clams; only about 60% of DOC was
removed. Fig. 1 also shows that DOC concentration in subtidal sediment was lower than in tidal sediment and DOC concentration in subtidal sediment with clams was only about 42% of DOC concentration in the sediment without clams. This result indicated that transfer rate of organic matter from overlying water to subtidal sediment was lower than that in tidal sediment. Immersion and emersion cycle in tidal sediment might be increased accumulation of organic matter in tidal sediment. This phenomenon also shown that clams accelerated organic matter degradation in subtidal sediment, but the contribution of clams was relatively low. This result is consistent with the results of NH$_4$N concentration in subtidal sediment, as has been previously described.

Changes in microbial community structure

The changes of chemicals environment in the sediment with clams, as has been described, promoted changes in microbial community structure, which indicated by changes in microbial quinone profile. Fig. 2 and 3 show that clam increase quinone content in tidal and subtidal sediments. Quinones exist in almost all microorganisms and play an important role in the electron transport for respiration. In general, one species or genus of bacteria has only one dominant type of respiratory quinone. So, the quinone profile can be used as an index to characterize the microbial community $^{[17, 26, 27]}$. Also, quinone content can be used as an index for the amount of biomass $^{[17, 28]}$. Therefore, both microbial community structure and the concentration of biomass were simultaneously quantified by analyzing quinones in a microbial community.

During July 2002 – July 2003, the average quinone content in tidal sediment with clams was about 26% higher than in the sediment without clams. More detail, the concentrations of UQ, MK, and PQ+VK1 in tidal sediment with clams were about 34, 55, and 15% higher than in the sediment without clams, respectively. This indicated that through their excretion activity, clams improved the growth rates of microorganism in tidal sediment, especially MK containing bacteria. High biodegradable compounds from clam excretion activities are easier to consume by microorganism for growth and their metabolism. Clam activities and mineralization of biodegradable organic matter by microorganisms consumed DO and caused DO concentration in tidal sediment with clams was lower than in the sediment without clams. This condition was suitable for MK-containing bacteria growth, and caused UQ/MK ratio in the sediment with clams was lower than in the sediment without clams. UQ/MK ratios in the sediment with and without clams were 0.8 and 1.1, respectively. This indicated that clams changed the dominant group of bacteria and caused MK-containing bacteria become dominant in tidal sediment with clams.

The effect of clams on changes in microbial community structure in subtidal sediment was relatively low. The average of quinone content in tidal sediment with clams was only about 3% higher than in the sediment without clams. In subtidal sediment, clams increased UQ containing bacteria growth. The existence of clams increased pore size in the sediment and caused transport DO from overlying water to pore water become easier. Higher DO concentration in the sediment with clams promoted UQ containing bacteria growth and inhibited MK containing bacteria growth. The concentrations of UQ and PQ+VK1 in subtidal sediment with clams were about 33% and 6% higher than in the sediment without clams, respectively. While the concentration of MK in subtidal sediment with clams was about 26%
Figure 2. Monthly variations of quinone content in tidal flat sediment with and without clams

Figure 3. Monthly variations of quinone content in subtidal flat sediment with and without clams
lower than in the sediment without clam. These results indicated that clams have no significant effect to accelerate microbial growth in subtidal sediment. The changes of microbial community structure in subtidal sediment was not caused by clams activities, but might be caused by changes in physical properties of the sediment.

The fluctuation of organic matter and nutrient concentrations in seawater also affected the abundance and density of microorganism in the sediment. Nutrient concentration due to bivalve excretion was also fluctuated within a year \[10\]. It was observed that quinone content, number of quinone species, and diversity of quinone were fluctuated within a year. Accumulation of organic matter during summer season is usually followed by increase of abundance and density of microorganism at early autumn.

During one-year observation (July 2002 – July 2003), the diversity of quinone was fluctuated from 6.1 to 8.7 and from 5.0 to 8.8 in tidal sediment with and without clams, respectively. While in subtidal sediment, the diversity of quinone was fluctuated from 4.0 to 8.4 and from 4.5 to 8.7 in the sediment with and without clams, respectively. Variations of seawater quality and environmental condition influenced excretion activities of clams and biodegradability of organic matter in the sediment. Finally, the microbial community structure in the sediment was affected by these variations. Fig. 4 shows that clams decreased fluctuations of diversity and fraction dominant quinone. This indicated that clams improved the stability of microbial community structure in tidal and subtidal sediment over a year. Clams also tend to increase the diversity of quinone especially in tidal sediment. These results indicated that the effect of clam on microbial community structure in tidal sediment was more significant than in subtidal sediment.

Figure 4. Changes in diversity of quinone (DQ) correspond to fraction dominant of quinone (fd) in tidal and subtidal sediment with and without clams.
According to heterotrophic bacteria, the diversity of quinone was calculated from the composition of respiratory quinones (including UQ and MK). Fig. 5 shows that clams increased the diversity of respiratory quinones ($DQ_{uq+mk}$) in tidal sediment, but not in subtidal sediment. Fraction dominant quinone in tidal sediment with clams also more stable than in tidal sediment without clams or subtidal sediment. The diversities of respiratory quinones were fluctuated from 8.3 to 11.6 and 6.9 to 11.3 in tidal sediment with and without clams, respectively. While in subtidal sediment, the diversities were fluctuated from 8.0 to 10.4 and 9.4 to 12.1 in tidal sediment with and without clams, respectively. These results give more evident that clams affected microbial community structure especially heterotrophic bacteria in tidal sediment.

Figure 5. Changes in diversity of respiratory quinones ($DQ_{uq+mk}$) correspond to fraction dominant of quinone ($fd$) in tidal and subtidal sediment with and without clams.

The dissimilarity between quinone profiles in the sediment with and without clams ($D_{\text{with, without clams}}$) is shown in Fig. 6. Results show that the dissimilarity was fluctuated within a year observation. Probably, it is due to the fluctuation of organic matter concentration in seawater. The annual chlorophyll-a concentration in Mikawa Bay fluctuated from 83 to 149 mg chl-a·m$^{-2}$ for microphytobenthos (at 0–1 cm of the surface layer of sediments) and from 0.5 to 15.3 mg chl-a·m$^{-2}$ for phytoplankton (at a water depth of 1 m in the intertidal flat) [29]. In enclosed coastal sea near large city, such as Mikawa Bay, eutrophication of the water column occurs, and, consequently, there is excessive organic loading on the seabed, especially during summer months [5]. Microorganism and clams (if any) will degrade these biomasses to produce higher biodegradable compounds several times after. Therefore, during summer till beginning of autumn, the abundance and density of microorganism in the sediment with clams were higher...
than that in the sediment without clams. This condition caused high level of dissimilarity index between microbial community structure in tidal and subtidal sediment with and without clams. Others environmental factor, such as temperature and DO are also influencing microbial community structure in tidal flat sediment. It was observed that the highest effect of clams on the microbial community structure occurred at the beginning of autumn (September 2002). Moreover, in this time microbial concentration (quinone content) in the sediment with clams also much higher than in the sediment without clams. This strongly suggested that the utilization of clams on the improvement of tidal flat purification capacity is effective during summer till early autumn. $D_{\text{with, without clams}}$ in tidal sediment was higher than in subtidal sediment. From a year observation, the average of $D_{\text{with, without clams}}$ indices in tidal and subtidal sediment were 20% and 14%, respectively. The presence of clams increased DOC and NH$_4$-N concentrations in tidal and subtidal sediment as described previously, causing changes in chemical environments. Clam activities also increased DO consumption rate in the sediments. These conditions might lead to change in microbial community structure in the sediments.

2.4 Tidal flat purification capacity

In this study, NO$_3$-N removal capacity was used to evaluate the purification capacity of tidal flat sediment. Figure 7 shows that high concentration of microorganism (quinone content) in the sediment has correlation with high removal rate of NO$_3$-N from the sediment, especially in the sediment with clams. The increases of carbon and nutrient concentration with higher biodegradability promoted microbial growth in the sediment with clams, which is indicated by higher concentration of quinone. Quinone content in the sediment with clams (A) was higher than that in the sediment without clams (B). Also, quinone content in 0-2 cm depth of sediment was higher than that in 9-11 cm depth of sediment. This could be related with DO and substrate limitation in the deeper sediment. High concentration of microorganism in the
sediment with clams was followed by higher NO$_3$-N removal rate. While, in the case of tidal sediment without clams, higher concentration of microorganism in 0-2 cm depth of sediment was not followed by higher NO$_3$-N removal rate proportionally. This indicated that microbial communities in the sediment with and without clams are different. NH$_4^+$-N concentration in tidal sediment with clams was about 3 times higher than in the sediment without clams (Table 1). This condition was suitable for the growth of nitrifying bacteria and increase nitrification process in tidal sediment with clams. The availability of NH$_4^+$ is one of major factors regulating nitrification in the coastal marine sediment [30]. Higher activity of nitrifying bacteria produced higher concentration of NO$_3$-N. Also, the existence of clams consumed DO and decreased DO concentration in the sediment. Low oxygen concentration or anaerobic condition is suitable for the growth of denitrifying bacteria [30]. NO$_3$-N removal capacity in 2 cm of the top layer of tidal flat sediment with clams was about 3 times higher than that of tidal flat sediment without clams. High concentration of denitrifying bacteria and low oxygen concentration were suitable conditions for NO$_3$-N removal from tidal flat sediment. High concentration of photosynthetic (photoheterotrophic) bacteria, which indicated by PQ and VK1, also increased NO$_3$-N removal rate through NO$_3$-N utilization as a terminal electron acceptor in their metabolism [31]. The increased of sediment depth decreased NO$_3$-N removal capacity. This could be related with low concentration of microorganism and the absence of photoheterotrophic bacteria in the lower sediment.

4. Conclusions

Clams appear to be a suitable organism to accelerate the decomposition of organic matter and nutrient removal in tidal flat sediment by improved biodegradability and promoted microbial growth in the sediment. This condition caused changes in microbial community in the sediment. The existence of clams increased quinone content, number of quinone species, and diversity of quinone in tidal sediment. Variations of seawater quality and environmental condition also influenced dissimilarity between microbial community structure in the sediment with and
without clams. Changes in microbial community structure increased nitrate removal capacity in the sediment with clams. Results of this study are useful as additional information to improve tidal flat purification function and prevent eutrophication in the coastal area.

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TOYOHASHI UNIVERSITY OF TECHNOLOGY
DEPARTMENT OF ECOLOGICAL ENGINEERING
FUJIE-GOTO LABORATORY
1-1 Hibarigaoka, Tenpaku-cho, TOYOHASHI – 441-8580, JAPAN
Telp./Fax.: +81-532-44-6910
http://fujielab.eco.tut.ac.jp

Main Research Themes:

- Development recycling techniques using sub and supercritical water processes
- Environmental information analysis on material cycle processes for environmental preservation
- Development environmental preservation technology by utilization of microbe functions
- Design and maintenance of waste water treatment systems which have high performance and low energy consumption
References


