Microbial bioavailability of dissolved organic nitrogen (DON) in the sediments of Lake Shankou, Northeastern China

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ABSTRACT

Dissolved organic nitrogen (DON) extracted from Lake Shankou sediments using KCl was isolated into hydrophobic and hydrophilic fractions. The bioavailabilities of the hydrophobic and hydrophilic fractions to three types of bacterial communities collected from sediments, activated sludge and compost products were examined. The DON recoveries obtained by DAX-8 and cation exchange resins treatment were 96.17% ± 1.58% and 98.14% ± 0% for the samples obtained from N4 and N14 stations, respectively. After 25 days of incubation at 25°C, most DON (59% to 96%) was degraded. Hydrophilic DON exhibited a higher reduction rate than hydrophobic DON during the growth phase. Untreated wastewater from Changshuihe town was the main degradable DON source to station N4, and 93% of hydrophilic DON and 80% of hydrophobic DON were degraded. Station N14 received a large amount of refractory DON from forest soils and exhibited DON degradation rates of 82% and 71% for the hydrophilic and hydrophobic fractions, respectively. Amino acid contents and fluorescence intensities were also analyzed. Approximately 27% to 74% of amino acids were taken up by day 5, and their concentration gradually increased in the following days due to the decomposition of dissolved proteins. Parallel factor analysis resulted in identification of tryptophan-like proteins, tyrosine-like proteins and FA-like substances. During the growth phase, 40%–51% of the tryptophan-like proteins were taken up by bacteria, and the accumulation of tyrosine-like proteins was attributed to the release of biotic substances. The concentration of the FA-like substances decreased due to microbial decomposition.

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Introduction

Dissolved organic nitrogen (DON) is a major nitrogen species in estuaries, oceans, rivers and soils and has been found to account for 12% of the total dissolved nitrogen (TDN) in ocean water, with higher concentrations in surface layers than in deep layers (Ogawa et al., 1999). In estuarial waters, DON concentrations average 12–35 μmol/L (Lønborg and Søndergaard, 2009) and account for 17%–92% and 22%–69% of the TDN pool in April and August, respectively (Veugler et al., 2004). The DON concentrations in soils from Germany and Taiwan vary from 2.00 to 3.21 mg/L and 0.57 to 4.47 mg/L, respectively (Schmidt et al., 2011). In addition, DON is

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constantly produced and used by microorganisms in aquatic systems, especially during the summer (Zhang et al., 2015), which may increase their primary production levels (Antia et al., 1980). In aquatic systems, bottom sediments often serve as an important assembly area for DON degradation and circulation, which influences the nitrogen dynamics in the overlying water column. In addition to the direct absorption of DON by microorganisms, DON mineralization and immobilization occur in the sediment layer to provide a source of inorganic nitrogen (Wang et al., 2015). The released inorganic nitrogen is available for uptake by phytoplankton, which increases the risk of eutrophication.

The DON pool is associated with a mixture of complex compounds. Urea, dissolved free amino acids, proteins, nucleic acids, amino sugars, and humic substances have frequently been observed in water (Antia et al., 1991). Most of these species are directly bioavailable or can be mineralized to ammonia to support microbial growth in aquatic systems. Bioavailable DON reportedly accounted for 43% and 28% of the DON in two coastal water samples during 150-day laboratory incubations (Lønborg et al., 2009). Asmala et al. (2013) reported that 5.5% to 21.9% of DON in Finnish boreal estuaries with different land use patterns was bioavailable and that the 5.5% to 21.9% of DON in Finnish boreal estuaries with different land use patterns was bioavailable and that the bioavailable DON in the Arbutus Lake watershed ranged from 12% to 43% (Kang et al., 2013). Approximately 28%–57% of wastewater effluent DON has been reported to be bioavailable (to algae and bacteria) and biodegradable (by bacteria) (Sattayatewa et al., 2009).

Some researchers have focused on the effects of DON compounds with different molecular weights (MWs) on bacterial growth (Huo et al., 2014). Most amino acids and proteins in freshwater systems have MWs of lower than 5 kDa, whereas most dissolved proteins are high-MW compounds (Berman and Bronk, 2003). Humic acid-like components are associated with high MWs, aromatic structures and hydrophobic properties (Tadanie et al., 2000; Sarathy and Mohseni, 2007), whereas fulvic acid (FA)-like substances have low MWs and are byproducts of microbial decomposition (Chen et al., 2003; Ishi and Boyer, 2012). Whether bacteria and phytoplankton can use DON fractions depends on the environmental conditions, chemical composition and microbial species present. Generally, the bacterial abundance is much higher when lower-MW DOM is added (Fagerberg et al., 2009).

Natural and anthropogenic inputs to watersheds are two important DON sources in water systems. Seitzinger et al. (2002) suggested that 0 to 73% of the DON from natural (forests) and anthropogenic (animal pastures, urban/suburban storm water runoff) sources is used by estuarine plankton communities. DON components in pasture soils can also be decomposed and are bioavailable (Ghani et al., 2013). During periods of incubation, DON is immobilized by microbes and mineralized in the soil, and its biodegradation is not affected by inorganic nitrogen input (Schmidt et al., 2011). However, few studies have investigated the bioavailability of DON in sediments; thus, it is necessary to investigate the microbial bioavailability of DON in sediments. The main objectives of this study were as follows: (1) to use DAX-8 resin coupled with cation exchange resin to separate the DON components in sediments; (2) to investigate the bioavailability of hydrophilic and hydrophobic DON fractions; and (3) to discuss amino acid and fluorescence variations during periods of incubation.

### 1. Materials and methods

#### 1.1. Study area

Lake Shankou (126°50′46.86″–126°50′48.34″E, 48°31′40.75″–48°31′53.50″N) is located between Xiao Xingan Mountain and the Nenjiang Plain, China. It is a deep, artificial reservoir with a surface area of 84 km² and a mean annual runoff of 820 million m³ (Fig. 1). Before the artificial reservoir was constructed, water flowed through the valley for a distance of more than 20 km. Several projects were implemented in the catchment area during its construction, including land clearing, forest burning and farmland submerging. Three major rivers flow into Lake Shankou, including the Nanbei, Changshui and Tulumu Rivers. The major types of land in this lake region include forestland (68.13%), everglades (14.46%), grassland (8.29%), dry cultivated land (7.08%) and construction land (0.13%).

#### 1.2. Sediment sample collection and pretreatment

Surface sediment samples (0 to 10 cm) were collected using a grab sampler at two stations that represented two different DON-polluted regimes of Lake Shankou in October 2013 (Fig. 1). N4 was located in the Changshui river branch, which receives large amounts of untreated municipal wastewater from the town of Changshuihe, and N14 was located in the Nanbei river branch, which receives runoff water from sloping farmlands and woodlands. The samples were placed into sealed polyethylene tubes and temporarily stored in iceboxes at 4°C. After immediate transfer to the laboratory, the samples were stored frozen below −20°C and then freeze-dried at −50°C using FD-1D-50 freeze-dryers. The dried samples were homogenized using an agate mortar and pestle and passed through a 100-mesh sieve before analysis.

The 100 g samples were extracted using a 1 mol/L KCl solution (solid-to-water ratio of 1:10, W/V) for 1 hr in a horizontal shaker at room temperature. The suspensions were centrifuged at 5000 r/min for 15 min at 4°C and filtered through 0.22 μm Millipore filters (mixed cellulose ester membrane) to remove suspended solids and residual bacteria. The filtrates were stored at 4°C before analysis or fractionation.

#### 1.3. Resin separation protocol

DAX-8 resin was used to separate the sediment into hydrophobic and hydrophilic DON fractions. Before use, the resin was cleaned as described by Thurman and Malcolm (1981) and Liu et al. (2012). The cleaned DAX-8 resin (30 ml) was packed in a glass column, and 500 mL of the filtered sample was pumped through the column at a flow rate of 1.5 mL/min. The column effluent was acidified to pH 2.0 using 6 mol/L HCl and pumped through the column again at a flow rate of 1.5 mL/min. Next, the column was cleaned using 0.1 mol/L HCl and deionized water at a flow rate of 1.5 mL/min. All effluent was collected and passed through a cleaned cation exchange resin to remove
any NH₄⁺ in solution. The final effluent was considered the hydrophilic DON fraction. Next, the adsorbed fractions on the DAX-8 resin were eluted in the reverse direction with 0.1 mol/L NaOH and deionized water at a flow rate of 1.5 mL/min to obtain the hydrophobic DON fraction. The raw water from the extracted N4 and N14 samples was passed through cleaned cation exchange resin to remove NH₄⁺.

1.4. Laboratory incubation system

By considering the variations in DON uptake by different bacteria, the untreated sample and hydrophobic and hydrophilic fractions were inoculated with three bacterial communities derived from sediments, activated sludge and compost products. The bacterial solution was prepared using the following steps. Samples (33.3 mg) were extracted in deionized water (solid-to-water ratio of 1:3, W/V) for 1 hr in a horizontal shaker at room temperature. The mixtures were centrifuged at 5000 r/min for 15 min at 20°C, and the supernatants were sequentially filtered through 0.45 and 0.22 μm Millipore filters (mixed cellulose ester membrane). Particles collected on the 0.22 μm filters were resuspended in 100 mL of the 0.22 μm-filtered effluent as the bacterial inoculum.

Before microbial inoculation, the DON samples (200 mL) were adjusted to pH 7.0 by dropwise addition of 1 mol/L HCl and NaOH solution and packed into 250-mL Erlenmeyer flasks for sterilization. Next, 2 mL of each bacterial inoculum was added to each 200 mL sample. Each experimental group was incubated in a constant temperature incubator at 25°C.

1.5. Chemical analysis

The concentration of DON was calculated as the difference between the TDN and the sum of the inorganic nitrogen species (i.e., NO₃⁻ and NH₄⁺) (Huo et al., 2014a). NO₃⁻ and NH₄⁺ were measured using an ultraviolet spectrophotometric method and the Nessler colorimetric method, respectively (Huo et al., 2014a), and TN was determined using the persulfate digestion-ultraviolet spectrophotometric method (Huo et al., 2014b). The bacterial abundance was continuously monitored using the dilution-plate method, and the amino acid concentration was measured using the ninhydrin color method (Chutipongtanate et al., 2012).

Fluorescence spectra were obtained from the untreated, hydrophobic and hydrophilic samples by using a F7000 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence excitation-emission matrix (EEM) spectra were collected from subsequent scanning emissions at 5 nm increments between 300 and 500 nm by varying the excitation wavelength by 5 nm increments from 200 to 400 nm EEM fluorescence, and parallel factor analysis (PARAFAC) was used to characterize the DON fractions, as described previously by Stedmon and Bro (2008).

2. Results and discussion

2.1. Separation of DON using DAX-8 and cation exchange resins

The DON at the N4 and N14 stations accounted for 50.7% and 52% of the TDN, respectively. Fractionating the DON in the extracted sediments into hydrophobic and hydrophilic fractions was accomplished using adsorption to and filtration through a DAX-8 resin. The cation exchange resin was applied to reduce the NH₄⁺ content. The total DON concentrations measured in the hydrophobic and hydrophilic fractions were similar to those measured in the untreated samples (Table 1), and the DON recovery rates in the N4 and N14 samples after resin separation were 96.17% ± 1.58% and 98.14% ± 0%, respectively, of those in the untreated samples. The hydrophilic DON fraction accounted for an average of 71.88% ± 2.85% of the TDN. The hydrophobic DON fraction had a higher C:N ratio than the hydrophilic DON fraction, indicating the former's complex molecular structure. No significant DON concentration variations were observed in the N4 and N14 samples.
Table 1 – Dissolved organic nitrogen (DON) concentrations of after resin separation.

<table>
<thead>
<tr>
<th>DON (mg/kg)</th>
<th>Recovery (%)</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>N4 untreated sample</td>
<td>68.34 ± 2.75</td>
<td>96.17 ± 1.58</td>
</tr>
<tr>
<td>N4 hydrophilic fraction</td>
<td>47.12 ± 5.66</td>
<td>1.87 ± 0.06</td>
</tr>
<tr>
<td>N4 hydrophobic fraction</td>
<td>18.60 ± 0.48</td>
<td>3.38 ± 0.09</td>
</tr>
<tr>
<td>N14 untreated sample</td>
<td>76.98 ± 3.07</td>
<td>98.14 ± 0</td>
</tr>
<tr>
<td>N14 hydrophilic fraction</td>
<td>57.58 ± 2.87</td>
<td>1.59 ± 0.01</td>
</tr>
<tr>
<td>N14 hydrophobic fraction</td>
<td>17.96 ± 0.15</td>
<td>3.20 ± 0.01</td>
</tr>
</tbody>
</table>

2.2. Characterization of nitrogen species concentration during incubation

The three bacterial communities exhibited a similar trend, with significantly decreasing DON concentrations in the first 5 days, slowly decreasing DON concentrations until day 15 or 20, and slightly increasing DON concentrations in the following days (Fig. 2). A similar DON trend has been reported in river and municipal wastewater effluent (Seitzinger and Sanders, 1997; Liu et al., 2014). It was assumed that the resin separation, pH adjustment, and elution procedures did not alter the DON or introduce substances that could inhibit bacterial growth. In this study, approximately 69% to 79% of the DON decreased by day 5. Many DON molecules rapidly adsorb onto cell wall surfaces during the initial inoculation period, and this fraction would be removed when passing the suspension through a 0.22 μm membrane filter and could not be determined. Meanwhile, the addition of labile DON to bacterial inocula resulted in a DON increase on day 0 (Liu et al., 2012). Next, the consumption of DON by bacteria was responsible for the subsequent slow decrease in DON concentrations from day 5 to day 15 or 20 (Wadhawan et al., 2014). During the decline phase, DON was released from the cells and could be determined. This release of DON resulted in increased DON on days 20 or 25. Approximately 59% to 96% of the DON in the untreated samples and hydrophilic and hydrophobic fractions was bioavailable for the three bacterial communities. DON accounted for 79%–91% of the TDN in the initial bacterial inoculums (i.e., 0 day), and TDN followed a trend that was similar to that of DON (Appendix A Fig. S1). This finding indicates that DON was the main nitrogen source for bacterial growth. NH4-N and NO2-N did not affect the behavior of DON due to their low percentages in the TDN and their stable variations during the bacterial growth phase (Appendix A Figs. S2 and S3).

Remarkable differences were observed in the DON reduction rates for the different DON fractions. The rates of DON reduction in the growth phase were 0.65–1.27 μg/L (L · day) for the untreated samples, 0.86–1.25 μg/L (L · day) for the hydrophilic samples, and 0.29–0.38 μg/L (L · day) for the hydrophobic samples. The order of DON bioavailability was ranked as follows: hydrophilic DON > untreated DON > hydrophobic DON. Hydrophilic DON was associated with lower MWs and lower C:N ratios (e.g., amino acids, urea, proteins, and nucleic acids), whereas hydrophobic DON was composed of a biodegradation-resistant pool with a higher MW, higher C:N ratio and complex structure (Reemtsma et al., 2008; Baker and Curry, 2004). Urea is a major component of hydrophilic DON that can be used by many bacteria possessing urease. In Lake Shankou, urea is another important labile DON component in sediments. As mentioned above, the sloping cultivated land is an important type of land around the N4 and N14 stations (Fig. 1). Excess urea in farmlands was washed into the lake and deposited into the sediments. After a small decrease on day 5 (Fig. 2), the concentration of hydrophobic DON remained stable. These results indicate that most of the hydrophobic DON was not bioavailable. The small decrease in hydrophobic DON was attributed to the degradation of a small amount of low-MW DON that was loosely held by or adsorbed to the humic core structure (Bermann and Bronk, 2003; Stepanauskas et al., 1999; Watanabe et al., 2014). The C:N ratio reportedly indicates the source of organic matter (Lee et al., 2006). The C:N ratio of the hydrophobic fractions averaged 3.38 and 3.20, which was higher than that of the hydrophilic fractions (1.87 and 1.59). The high C:N ratio of the hydrophobic fractions creates a higher N demand for bacterial decomposition (Liu et al., 2012), affirming the conclusion that the hydrophilic DON in sediments was more bioavailable than the hydrophobic DON.

There was a clear regional difference in DON bioavailability in the sediments. As shown in Fig. 3, the average bioavailable DON ratios of hydrophilic DON and hydrophobic DON were 93
and 80%, respectively, in the N4 samples and 82% and 71%, respectively, in the N14 samples. These results indicate that the DON in the sediments at N4 was more bioavailable than that at N14. N4 was located in the lake branch of the Changshuihe River, where agricultural activities, urban runoff and untreated wastewater contribute significantly to the DON in sediments. N14 was located in the lake branch of the Nanbeihe River, where agricultural and forest runoff was the main source of DON for the sediments.

The soil DON contents changed with the surrounding ecological environment, primarily depending on the soil and land use types. In the town of Changshuihe, untreated wastewater results in the discharge of large amounts of N into the lake branch of Changshuihe. DON in domestic sewage is easily degraded because of its simple chemical structure. Influent DON from urban wastewater reportedly averages 6.13 mg/L as N, and the <1 kDa fraction contributes an average of 40%–57% of the DON (Huo et al., 2013). In forest soils, hydrophobic N accounts for ca. 50% of DON and includes proteins, polyphenol mixtures and humic substances containing amino groups (Gao et al., 2011). Some hydrophilic DON components in forest soils can be degraded and deposited into rivers with sediments from runoff (Yu et al., 2002). However, most polyphenol N has a high MW (>100 kDa) and is not easily degraded in sediments.

Sloping farmland around Lake Shankou was created by forest clear-felling and burning before Lake Shankou was built. During replanting, the residual tree trunks, fallen leaves and other organic matter was washed into the valley by the rising water level and rainfall. Field et al. (2003) reported that nearly 56% of suspended charcoal is carried into the sediment during the first storm after forest clearing. Long-term application of chemical fertilizers can result in higher C and N mineralization rates and can increase microbial biomass.

![Fig. 3 – Bioavailable DON percentage after 25 d incubation process. DON: dissolved organic nitrogen.](image)

Table 2 – Bacteria cell density (×10^5) of N4 and N14 extracted samples during incubation process adding sediment bacteria, activated sludge bacteria or compost bacteria.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adding sediment bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4 untreated samples</td>
<td>15.0 ± 0.0</td>
<td>11.9 ± 0.7</td>
<td>58.4 ± 2.3</td>
<td>2930.0 ± 70.7</td>
<td>25800.0 ± 4242.6</td>
</tr>
<tr>
<td>N4 hydrophilic fraction</td>
<td>1.4 ± 0.0</td>
<td>28.4 ± 2.3</td>
<td>144.0 ± 33.9</td>
<td>3600.0 ± 282.8</td>
<td>28220.0 ± 28.3</td>
</tr>
<tr>
<td>N4 hydrophobic fraction</td>
<td>0.2 ± 0.0</td>
<td>28.0 ± 2.8</td>
<td>33.7 ± 5.2</td>
<td>3730.0 ± 183.8</td>
<td>30000.0 ± 2828.4</td>
</tr>
<tr>
<td>N14 untreated samples</td>
<td>2.5 ± 0.0</td>
<td>19.4 ± 3.1</td>
<td>130.8 ± 15.3</td>
<td>3580.0 ± 84.9</td>
<td>21000.0 ± 1414.2</td>
</tr>
<tr>
<td>N14 hydrophilic fraction</td>
<td>0.1 ± 0.0</td>
<td>20.4 ± 5.1</td>
<td>146.6 ± 19.0</td>
<td>3446.0 ± 121.6</td>
<td>27900.0 ± 1555.6</td>
</tr>
<tr>
<td>N14 hydrophobic fraction</td>
<td>0.2 ± 0.0</td>
<td>31.0 ± 4.2</td>
<td>99.0 ± 1.4</td>
<td>3699.0 ± 80.6</td>
<td>31600.0 ± 565.7</td>
</tr>
<tr>
<td>Adding activated sludge bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4 untreated samples</td>
<td>0.4 ± 0.0</td>
<td>28.5 ± 0.6</td>
<td>162.4 ± 3.4</td>
<td>3740.0 ± 84.9</td>
<td>22400.0 ± 1923.3</td>
</tr>
<tr>
<td>N4 hydrophilic fraction</td>
<td>0.3 ± 0.0</td>
<td>90.8 ± 1.7</td>
<td>104.0 ± 33.9</td>
<td>2580.0 ± 537.4</td>
<td>22860.0 ± 2121.3</td>
</tr>
<tr>
<td>N4 hydrophobic fraction</td>
<td>0.2 ± 0.0</td>
<td>42.0 ± 22.6</td>
<td>151.0 ± 12.7</td>
<td>3760.0 ± 56.6</td>
<td>28280.0 ± 961.7</td>
</tr>
<tr>
<td>N14 untreated samples</td>
<td>0.3 ± 0.0</td>
<td>98.0 ± 2.8</td>
<td>167.0 ± 1.4</td>
<td>3905.0 ± 21.2</td>
<td>30500.0 ± 989.9</td>
</tr>
<tr>
<td>N14 hydrophilic fraction</td>
<td>6.0 ± 0.0</td>
<td>92.4 ± 5.1</td>
<td>108.2 ± 16.7</td>
<td>3396.0 ± 192.3</td>
<td>25400.0 ± 1979.9</td>
</tr>
<tr>
<td>N14 hydrophobic fraction</td>
<td>0.2 ± 0.0</td>
<td>48.4 ± 14.7</td>
<td>171.0 ± 12.7</td>
<td>3125.0 ± 227.7</td>
<td>32400.0 ± 565.7</td>
</tr>
<tr>
<td>Adding compost bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4 untreated samples</td>
<td>7.4 ± 0.0</td>
<td>53.4 ± 37.6</td>
<td>139.2 ± 20.4</td>
<td>3400.0 ± 282.8</td>
<td>27360.0 ± 2828.4</td>
</tr>
<tr>
<td>N4 hydrophilic fraction</td>
<td>10.0 ± 0.0</td>
<td>53.6 ± 0.0</td>
<td>60.0 ± 8.5</td>
<td>2960.0 ± 56.6</td>
<td>36640.0 ± 565.7</td>
</tr>
<tr>
<td>N4 hydrophobic fraction</td>
<td>7.4 ± 0.0</td>
<td>8.1 ± 5.5</td>
<td>60.4 ± 0.6</td>
<td>3800.0 ± 141.6</td>
<td>29920.0 ± 2715.3</td>
</tr>
<tr>
<td>N14 untreated samples</td>
<td>2.0 ± 0.0</td>
<td>36.1 ± 0.7</td>
<td>160.2 ± 0.3</td>
<td>3400.0 ± 28.3</td>
<td>30140.0 ± 84.9</td>
</tr>
<tr>
<td>N14 hydrophilic fraction</td>
<td>4.9 ± 0.0</td>
<td>22.8 ± 1.7</td>
<td>89.4 ± 0.8</td>
<td>3798.0 ± 48.1</td>
<td>24200.0 ± 141.4</td>
</tr>
</tbody>
</table>
more biodegradation-resistant DON in sediments than N4. Due to their low MW and simple structure. Thus, N14 received Williams, 2005). Most of these components are bioavailable during the first 5 days that was followed by a rapid increase in density. There was a temporary adaptation by the bacteria during the incubation period (Table 2). The bacterial abundance of the three bacterial communities did not significantly differ. The release of amino acids was reported during substrate metabolism for biomass growth (Laspidou and Rittmann, 2002), some of which are soluble and are use-associated products, e.g., carbohydrates, proteins and nucleic acids. This fraction is considered as a component of DON and is responsible for increasing the DON concentration (Jarusutthirak and Amy, 2007).

2.3. Characterization of the bacterial abundance during incubation

Bacterial growth followed a similar trend in the untreated samples and the hydrophobic and hydrophilic fractions during the incubation period (Table 2). The bacterial abundance of the three bacterial communities did not significantly differ. There was a temporary adaptation by the bacteria during the first 5 days that was followed by a rapid increase in bacterial abundance from day 5 to day 20, with a growth rate ranging from $1.40 \times 10^9$ cell/day to $2.44 \times 10^9$ cell/day and a subsequent decrease until day 25. This result is consistent with the variations in DON observed during the incubation periods.

During the first 5 days, the specific bacterial growth rates on hydrophilic DON ranged from 0.31 to 1.14, and the bacterial growth rates on hydrophobic DON ranged from 0.02 to 1.10, which indicated that bacteria can adapt to hydrophilic DON more quickly than hydrophobic DON because hydrophilic DON has a low MW and simple structure that is easily degraded. From days 5 to 20, the bacterial abundance increased rapidly on both hydrophilic and hydrophobic DON. This trend was different from the DON bioavailability trend because relatively stable DON concentrations were observed in the two fractions from days 5 to 20. In addition, these results indicate that not all bioavailable DON was used for bacterial growth. Extracellular polymeric substances are reportedly released during substrate metabolism for biomass growth (Laspidou and Rittmann, 2002), some of which are soluble and are use-associated products, e.g., carbohydrates, proteins and nucleic acids. This fraction is considered as a component of DON and is responsible for increasing the DON concentration.

2.4. Variations of amino acids and fluorescence characterization

Amino acids followed a similar trend, slowly decreasing during the first 5 days and increasing during the following days (Fig. 4). Approximately 27.05% to 73.84% of amino acids were reduced by day 5, which was attributed to the direct utilization of amino acids by bacteria (Veuger et al., 2004). As the bioavailable amino acid concentration decreased, the bacteria began to degrade dissolved proteins into peptides and DFAA (Hollibaugh and Azam, 1983; Berman and Bronk, 2003), which caused the amino acid concentrations to increase slowly. A sudden peak was observed on day 25 in the three bacterial communities. The release of amino acids due to cell lysis during the contract phase potentially caused the concentration to suddenly increase. The amino acid concentrations in the hydrophobic fractions increased, resulting in averages of 1.75 mg N/L for N4 and 1.83 mg N/L for N14. In the hydrophilic fractions, the amino acid concentration increased, resulting in averages of 1.73 mg N/L for N4 and 1.76 mg N/L for N14.

EEM fluorescence spectroscopy was used to characterize DON variations during the incubation period. The fluorescence excitation–emission maps of DON in three bacterial communities were consistent with each other. The EEM spectra of the untreated samples and the hydrophobic and hydrophilic fractions with added compost bacteria are shown in Fig. 5. Peaks corresponding to two types of protein-like substances and one FA-like substance were observed at excitation/emission (Ex/Em) values of 225–275/350 (component 1), 275/350 (component 3) and 250/400–410 (component 2). During the incubation period, the fluorescence intensity of component 1 decreased, and the fluorescence intensities of components 2 and 3 increased.

To interpret the EEM spectra of DON, a PARAFAC model was applied to analyze the variations in the concentrations...
and fluorescence intensities of three components during the incubation periods. The PARAFAC model for DON with added compost bacteria distinguished two protein-like substances (component 1 and component 3) and one FA-like substance (component 2) for all samples, as shown in Appendix A Fig. S4. Component 1 contained two peaks (Ex/Em 275–285/226–251) that corresponded to tryptophan-like protein substances, which have been identified by Yamashita and Tanoue (2003).

Fig. 5 – Three-dimensional excitation emission matrix fluorescence spectroscopy of dissolved organic nitrogen with added compost bacteria during the incubation periods.
in ocean water. The fluorescence characteristics of component 2 have rarely been identified. Chen et al. (2003) observed similar fluorophores (Ex/Em 214–220/440–450) for standard FA from the Suwannee River. The fluorophore of component 3 was characterized by two peaks and exhibited peaks that were similar to those of tyrosine-like protein derived from waste sludge (Guo et al., 2014).

The changes in the fluorescence intensities of the three components in the untreated samples and hydrophobic and hydrophilic fractions with added compost bacteria are shown in Fig. 6. The contents of components 1 and 3 varied differently during the incubation periods. The relative fluorescent intensity of component 1 decreased to 40.2%–42.1% in the untreated samples to 45.4%–51.3% in the hydrophilic fractions and to 40.0%–44.4% in the hydrophobic fractions by day 25. The tryptophan-like proteins in the DON fractions were highly bioavailable, especially in the hydrophilic fractions. For protein-like component 3, the relative fluorescent intensity slowly increased during the first 20 days. In addition to the inoculation fluid, the biotic origin was responsible for the production of tryptophan- and tyrosine-like substances (Elliott et al., 2006). Tyrosine, with an aromatic protein-like structure, is useful for maintaining the stability of microbial communities (Zhu et al., 2012). The gradually accumulated tyrosine-like substances are primarily attributed to the release of soluble microbial products during bacterial metabolism (Laspidou and Rittmann, 2002; Jarusutthirak and Amy, 2007).

For FA-like component 2, the relative fluorescence intensity increased during the first 5 days and then decreased during the following days. During the incubation periods, DFAA and DCAA were preferentially used through direct uptake and hydrolysis or mineralization by bacteria (Pehlivanoglu and Sedlak, 2004). These processes contribute to the release of FA-like substances adsorbed to the humic core structure and increase its relative fluorescence intensity. When large amounts of low-MW DON were utilized, FA-like substances were decomposed and taken up by bacteria, which resulted in a relative decrease in fluorescence intensity.

3. Conclusions

DAX-8 resin coupled with cation exchange treatment was successfully employed to separate the components of DON and to reduce ammonia nitrogen in sediments from Lake Shankou (resulting in a DON recovery rate of 97.16%–98.14%). The hydrophilic fraction was the main form of DON at the N4 and N14 stations. Approximately 59% to 96% of the sediment-derived DON was available for uptake in the presence of a bacterial inoculum. Hydrophilic DON was more easily degraded than hydrophobic DON, with a DON reduction rate of 0.86–1.25 μg/(L·day) due to its low MW. The average degradation rates of hydrophilic and hydrophobic DON in the N4 samples were higher than those in the N14 samples. Bacteria can utilize free amino acids directly, and the decomposition of dissolved proteins can increase amino acid concentrations. The EEM-PARAFAC model identified three DON components, tryptophan-like protein substances (component 1), FA-like substances (component 2), and tyrosine-like protein substances (component 3). Tryptophan-like protein and FA-like substances were degraded during the incubation periods. Simultaneously, tyrosine-like proteins began accumulating during bacterial metabolism due to the release of SMPs.

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Appendix A. supplementary data

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