Effects of the combination of aeration and biofilm technology on transformation of nitrogen in black-odor river

Mei Pan, Jun Zhao, Shucong Zhen, Sheng Heng and Jie Wu

ABSTRACT

Excess nitrogen in urban river networks leading to eutrophication has become one of the most urgent environmental problems. Combinations of different aeration and biofilm techniques was designed to remove nitrogen from rivers. In laboratory water tank simulation experiments, we assessed the removal efficiency of nitrogen in both the overlying water and sediments by using the combination of the aeration and biofilm techniques, and then analyzed the transformation of nitrogen during the experiments. Aeration (especially sediment aeration) combined with the biofilms techniques was proved efficient in removing nitrogen from polluted rivers. Results indicated that the combination of sediment aeration and biofilms, with the highest nitrogen removal rate from the overlying water and sediments, was the most effective combined process, which especially inhibited the potential release of nitrogen from sediments by reducing the enzyme activity. It was found that the content of dissolved oxygen in water could be restored on the basis of the application of aeration techniques ahead, and the biofilm technique would be effective in purifying water in black-odor rivers.

Key words | aeration, biofilm, black-odor rivers, combination techniques, nitrogen, self purification

INTRODUCTION

With the rapid development of urbanization, large numbers of urban rivers have become contaminated and turned to black-odor rivers. The features of the black-odor rivers include peculiar smell, decreasing number of aquatic organisms, and serious deterioration of structure and function of the river ecological system (He et al. 2013). Also, the eutrophication of black-odor rivers is a serious threat to the health of urban residents and ecological security. And the excess nitrogen plays a critical role in promoting river eutrophication. Since some of the internal nitrogen in the sediments has become the main source of N for rivers or lakes, which further sharpens eutrophication, how to effectively control the internal nitrogen has become a vital research topic. Assimilation, nitrification and denitrification are the predominant biological processes that the dissolved inorganic nitrogen compounds undergo in rivers or lakes (Palmer-Felgate et al. 2010).

In recent years, intermittent aeration operation modes have been widely studied in the world. It is commonly believed that nitrogen removal is efficient under the proper operating conditions. However, aeration technique can just improve the water quality temporarily, while the enhancement of the self-purification capability depends on the restoration of the overall ecosystem. Ecological restoration has become the main technology for controlling eutrophication on account of its advantages of low operating cost, flexible operation and positive effect on the environment. Especially, the biofilm techniques have been gradually applied to the field of water body bioremediation, with their rich microbial community structure (Sabater et al. 2002). Extracellular polymeric substances (EPS) are biopolymers of microbial origin in which biofilm microorganisms are embedded. They form matrices that are highly hydrated, helping to maintain a variety of microorganisms to fix on the biofilm for a long time, and to form a microbial symbiosis with diversified functions and collaborative divisions, including bacteria, fungi and algae, as well as other protozoa and metazoa (Jones et al. 2006; Flemming et al. 2007).
et al. (2012) confirmed the importance of biofilm in the transformation process of N. Arnon et al. (2007b) proved that the hydrodynamic force influenced the mass transport process of dissolved oxygen (DO) and N throughout the biofilm directly, and thereby affected its denitrogenation efficiency; meanwhile, their study also highlighted the impact of DO on the biofilm community structure. Existing research suggested that the formation of the biofilm community structure was influenced by the external environment directly or indirectly (Nogueira et al. 2002; Sabater et al. 2007), which could further affect the function and subsequent efficiency of biofilms to retain nutrients and pollutants. As a result, biological community structure is difficult to restore through bioremediation due to the inhibition created by the adverse external environment. First of all, it is conducive to the restoration of the ecological system to improve the overall water environment.

This study proposed a combined technique by integrating aeration and biofilm to avoid the shortcoming of a single restoration technique. The sediment and water aeration were simulated in laboratory experiments simultaneously. After the water quality had been improved to a certain extent, carbon fiber grasses served as substratum for biofilm growth in the specified tanks. Then, the effect of the proposed combined techniques on the changes of the enzymatic activity in sediments and the release as well as transformation of internal N in the overlying water is described, with the purpose of providing a theoretical basis for the feasibility of ecological bioremediation.

MATERIALS AND METHODS

Sampling

Surface sediment samples (0–5 cm) were collected using a UWITEC sediment corer from a section of a typical urban river (32°00′N and 118°49′E). Then the samples were kept in ice boxes, and sufficient amount of water was taken from the corresponding position. After being homogenized, the sediment samples were divided into three parts. The first part was dried at room temperature and the rocks among sediments were removed; then the sample was kept in sealed bags after filtering, for detection of physical and chemical properties. The second part was used for the detection of the urease activity (UA) and the dehydrogenase activity (DHA) of sediments. The remaining part was used to simulate the water restoration experiment in an indoor environment. The water samples were filtered with 0.45 μm glass fiber filters and tested for basic physical and chemical properties; a portion of water samples was added into the simulation water tank; the remaining samples were kept at low temperature (4°C) for subsequent laboratory experiments. Every sample plot had three replicates.

Laboratory experiment

Customized PVC water tanks were used to simulate the river channel restoration experiment. The experimental apparatus was composed of six independent water tanks, each with the dimension of 40 cm × 50 cm × 50 cm (Figure 1). Homogeneous sediment samples were separately injected into the six tanks to a height of 4 cm from the bottom, and then the filtered river water was slowly added to a height of 40 cm. For W2-1 and W2-2, two air pumps were used for aerating to overlying water respectively. And the air pumps in W3-1 and W3-2 were used for aerating to sediments. W1-1 served as the controlling container that was static, meaning that no restoration technology was done to the sediments or water. Aeration was performed intermittently by using perforated pipes with the pore diameter of 3 mm. The process of aeration lasted for 2 h every day with the airflow of 100 L/h. And the same amount of river water was added immediately to the original level after sampling each time. On the 18th day (as aeration lasted for 18 days in this experiment), the carbon fiber grasses started to serve as substratum for biofilm growth in W1-2, W2-2 and W3-2 tanks. All of the sediment aeration and water aeration was terminated on the 48th day of the experiment. During the whole process of experiments,
samples in the six tanks were taken to monitor the concerned indicators on the 8th, 18th, 25th, 38th, 48th, 68th and 80th day, respectively. Meanwhile, the transformation and purification efficiency of N in the water body were examined to provide a reference for the technology optimization under different experimental conditions.

Analytical methods

The total phosphorus (TP) of the overlying water and sediment was detected by the ammonium molybdate spectrophotometric method. Ammonia nitrogen (NH$_4^+$-N) was determined by Nessler’s reagent spectrophotometry. The total nitrogen (TN) and nitrate nitrogen (NO$_3^-$-N) were detected by UV spectrophotometry. And the chemical oxygen demand (COD) was detected by the potassium permanganate oxidation method (State Environmental Protection Administration of China 2002). The loss on ignition method was adopted to determine the contents of organic matter (OM) in sediments. The UA and DHA of sediments were assayed by indophenol colorimetric method and triphenyl tetrazolium chloride spectrophotometry (Bao 2000). The DO and pH were measured with a portable dissolved oxygen analyzer (Hach, HQ30d). All the data were expressed as the mean values of three replicates.

Microscope observation

Biofilms were separately scraped from carbon fiber grasses in W1-2, W2-2, and W3-2 with three replicates. Biofilms and overlying water were observed by using a light optical microscope at 400× magnification (Nikon, YS100).

RESULTS AND DISCUSSION

Characteristics of the overlying water and sediments in urban rivers

The water in the urban river showed a dark green color. The results showed that the contents of DO, TP, TN, NH$_4^+$-N, NO$_3^-$-N and COD of the overlying water are 1.80, 0.79, 14.84, 3.32, 0.68 and 55.86 mg/L respectively. The water quality of the sampled urban river was inferior to level ‘V’ according to the Chinese national standards (China EPA 2002). The sediments were oily-black and muddy, containing domestic garbage and non-decomposed plant residues. The contents of TN, TP and OM of sediments were high (2.55 mg/g, 0.75 mg/g, 3.32%), and the NH$_4^+$-N and NO$_3^-$-N of sediments were 0.10 mg/g and 0.011 mg/g, respectively, suggesting that N mainly existed in the form of organic N in sediments, which accounted for about 90% of TN. The high DHA and UA (38.02 mg/(g·h) and 0.28 mg/(g·h)) of the sediments indicated that organic N was prone to mineralization. Some existing studies suggested that the enzymatic activity can be used as an indicator for the dynamics of nutrients in the water ecosystem (Hill et al. 2006, 2010). The contents of TN and NH$_4^+$-N in the overlying water were relatively high. However, due to the low contents of DO in the overlying water, the mineralization and decomposition rate of OM were relatively low, resulting in high proportion of NH$_4^+$-N and low proportion of NO$_3^-$-N, which also suggested that ammonification was quite strong, and the microbial growth as well as reproduction processes were inhibited. In addition, the microorganisms in the overlying water were mainly facultative bacteria such as ammonifying bacteria and anaerobic bacteria, which meant that the water environment was not suitable for the growth of nitrifying bacteria (Ribot et al. 2012).

The effect of artificial aeration on N transformation and water quality of urban rivers

The laboratory simulation experiment explored and compared the effects of water aeration (W2-1) and sediment aeration (W3-1) on N transformation of the water body. Figure 2 showed that the contents of DO in water tanks were in the following sequence: W3-1 < W1-1 < W2-1 during the first 18 days of aeration, suggesting that the acceleration of hydrodynamic force intensified the mass transport and the reaction between DO and organic N at the early stage of aeration, in
the sediment aeration tank. As a result, the mineralization and decomposition of organic N were enhanced, leading to massive consumption of DO (Arnon et al. 2007b; Ruan et al. 2009). The DO content of the sediment aeration tank was even lower than that of the static water body, which reflected that the polluted water consumed a large amount of DO and accumulated a lot of OM. After 18 days of aeration, the DO content in W3-1 was increasing continuously, suggesting that the mineralization rate of OM decreased, and the OM was transformed to inorganic matters, leading to the decreasing consumption rate of DO simultaneously. Both water aeration and sediment aeration were terminated at the 48th day. Thereafter, the DO content of W3-1 also decreased, but the decline range was much lower than that of the water aeration tank (W2-1). Through the entire aeration process, the DO content of W2-1 exhibited a rising trend, but the trend flattened out a bit after 25 days. Meanwhile, upon the termination of aeration, the DO content of W2-1 declined rapidly, suggesting that the OM in sediments cannot react with DO thoroughly under the water aeration condition, as a consequence of which DO can only penetrate through the surface sediments (Arnon et al. 2007a). Thus, this approach can only improve the water quality temporarily. Even though aeration was terminated, the internal N in sediments still released pollutants and consumed DO continuously. What is more, sediment aeration consumed a large amount of DO in the initial stage, but the supply of DO would gradually exceed consumption under the continuous aeration condition. Under oxygen depletion conditions, the redox-sensitive NO₃⁻/C₃O⁻ fraction was released from anaerobic sediments. Hence, it can be considered as a potential source of nitrogen for denitrification (He et al. 2015). Furthermore, since the degradable OM in sediments had been mineralized greatly upon the termination of aeration, the consumption of DO would drop (Figure 2). In addition, during the entire experiment process, the DO in W1-1 exhibited a declining trend, suggesting that the OM in sediments consumed DO continuously. But the overlying water in W1-1 has a lower oxygen uptake rate than that in W2-1 and W3-1, due to the fact that the overlying water was in static state.

The changing trends of NH₄⁺-N, NO₃⁻-N and TN in the overlying water are shown in Figure 3, Figure 4 and Figure 5 respectively. Under either aeration or static condition, the content of NH₄⁺-N exhibited a rising trend during the first 18 days. During the initial aeration stage (8 days), the contents of NH₄⁺-N in the overlying water of W2-1 and W3-1 increased sharply, which was the result of the ammonification of organic N in the sediments (Ma et al. 2003). The rising rate of NH₄⁺-N in W3-1 was very rapid; a reasonable explanation was that organic N reacted with DO thoroughly, and then released a large amount of NH₄⁺-N from sediments under the stimulation of high-activity enzymes (Li et al. 2015). In addition, the release rate of NH₄⁺-N of sediments in W2-1 was slightly faster than that in W1-1. However, the NH₄⁺-N contents of overlying water in W3-1 and W2-1 began to decline after the first 18 days. The reason could be as follows: (1) DO began to rise on the 18th day, indicating that the mineralization rate of the sediments was reduced (Racchetti et al. 2011); (2) the nitrifying bacteria grew, and a part of the NH₄⁺-N was transformed into NO₃⁻-N attributable to the increase of DO in aeration tanks (Ruan et al. 2009). Figure 4 shows that the contents of NO₃⁻-N of the overlying water both in W2-1 and W3-1 exhibited a rising trend during the aeration period (first 48 days). For W3-1, the DO content and nitrification rate were
still lower than those in W2-1 during the first 18 days, but the release of organic N led to high contents of NH$_4^+$-N in the overlying water; thus, the rising rate of the total amount of NO$_3^-$-N was higher than that in W2-1 (Nogueira et al. 2002). However, the slope of the rising trend during the first 18 days was always less than that during the 18–48th days, which was because the supply rate of DO became higher 18 days was always less than that during the 18–48th days, which was because the supply rate of DO became higher during the first 18 days, which might be because the mineralization rate was far higher than the nitrification rate during the initial aeration stage. However, due to the organic N being released from sediments into the overlying water, the mineralization rate was much higher than the N transformation rate in overlying water. Thus, the content of TN in W3-1 was still the highest before the 25th day (Figure 5).

The aeration was terminated on the 48th day of the experiment. The content of NH$_4^+$-N in W3-1 exhibited a rapid after the first 18 days (Burford & Lorenzen 2004). As shown in Figures 3 and 4, the growth rate of NH$_4^+$-N was higher than that of NO$_3^-$-N during the first 18 days, which might be because the mineralization rate was far higher than the nitrification rate during the initial aeration stage. However, due to the organic N being released from sediments into the overlying water, the mineralization rate was much higher than the N transformation rate in overlying water. Thus, the content of TN in W3-1 was still the highest before the 25th day (Figure 5).

In general, the changing trends of DO, NH$_4^+$-N, NO$_3^-$-N, and TN in W1-1 confirmed that organic N in sediments consumed a part of DO under the anaerobic condition and became the source of N pollution in the water body, which was consistent with the decrease of TN in W1-1 shown in Table 1. From Table 1, we could also infer that the long-time sediment aeration had a more positive effect on reducing nitrogen loading in overlying water and sediments compared to the water aeration. Particularly, sediment aeration can effectively reduce N contents, as well as UA and DHA in sediments, which lowered the contents of internal N and the potential risk of N release significantly (Burford & Lorenzen 2004). Circulation of water under aerobic and anoxic conditions through adopting either the water aeration or the sediment aeration as driving force could enhance the effect of N removal. As shown in Table 1, the increase of enzymatic activity and N contents in W1-1 further proved that high DHA and UA could easily induce the mineralization of OM and organic N (Hill et al. 2010; Li et al. 2015).

### The effect of biofilm technique on strengthening water purification based on aeration

In this study, as an excellent support material for biofilms (Matsumoto et al. 2012), carbon fiber grasses were used as the biofilm substratum in W1-2, W2-2 and W3-2

<table>
<thead>
<tr>
<th>Water tank</th>
<th>DHA (%)</th>
<th>UA (%)</th>
<th>TN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1-1</td>
<td>-5.52</td>
<td>-14.29</td>
<td>6.67</td>
</tr>
<tr>
<td>W2-1</td>
<td>36.86</td>
<td>25</td>
<td>24.71</td>
</tr>
<tr>
<td>W3-1</td>
<td>41.21</td>
<td>53.57</td>
<td>52.94</td>
</tr>
</tbody>
</table>
May be con
water and sediments in W2-2, since the mass transport be the constraint of mass transport between overlying sediments into the overlying water. Another reason might be the constraint of mass transport between overlying water and sediments in W2-2, which was mainly ascribed to the fluctuation of sediments accelerating the release of microorganisms from the sediments into the overlying water. Another reason might be the constraint of mass transport between overlying water and sediments in W2-2, since the mass transport may be confined to the surface sediments, and only a small part of the microorganisms in sediments were released to the overlying water (Arnon et al. 2007a). Similarly, these reasons could account for the amount of biofilms in W1-2 being relatively the smallest among the three tanks.

Table 2 shows that biofilms can effectively improve the water quality. Particularly, based on the comparisons of W1-2 (biofilm alone), W2-1 (water aeration alone) and W3-1 (sediment aeration alone), the purification efficiency of sediment aeration was the best. According to the pair comparisons of W1-1 versus W1-2, W2-1 versus W2-2 and W3-1 versus W3-2, it could be deduced that biofilms can further adsorb and transform pollutants in the water body and consolidate the purification performance based on the aeration, which brought about high removal efficiencies of NH$_4^+$-N and TN. Particularly, the morphology of biofilms had a direct influence on the mass transport of nutrients and DO in the biofilms (Rinaudi et al. 2006). Since the biofilm was in an aerobic environment, its surface was suitable for the survival of aerobic nitrifying bacteria, whereas the inside of the biofilm was in an anaerobic or facultative condition that was more suitable for denitrifying bacteria due to the constraint of DO mass transfer (Arnon et al. 2007a).

Under the sediment aeration condition (W3-2), a large amount of NH$_4^+$-N was released into the overlying water (Figure 3), and nitrification and denitrification were triggered by the biofilm at the same time, which can generate N$_2$ that escapes from the water body (Ribot et al. 2012; Ruan et al. 2009). In other words, the morphology of biofilms determined the community structure of biofilms, which would contribute to the growth of nitrifying and denitrifying bacteria and in turn affect the purification performance of the biofilm (Arnon et al. 2007a). Based on the approach of sediment aeration combined with biofilms, the removal rates of NH$_4^+$-N, TN, COD and TP in the overlying water of W3-2 reached up to 63.25, 69.14, 53.06 and 72.15%, respectively. Meanwhile, the content of DO increased from 1.8 to 3.8 mg/L. And the water quality was improved to level ‘III’ or ‘IV’ (China EPA 2002), indicating that overall purification effect was considerable and the application of biofilms combined with aeration could

### Table 2 | The effect of different treatment techniques on purifying water

<table>
<thead>
<tr>
<th>Water tank</th>
<th>W1-1</th>
<th>W1-2</th>
<th>W2-1</th>
<th>W2-2</th>
<th>W3-1</th>
<th>W3-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$-N</td>
<td>C (mg/L)</td>
<td>3.69</td>
<td>2.02</td>
<td>2.45</td>
<td>1.34</td>
<td>1.68</td>
</tr>
<tr>
<td>R (%)</td>
<td>-11.14</td>
<td>39.16</td>
<td>26.20</td>
<td>59.64</td>
<td>49.40</td>
<td>63.25</td>
</tr>
<tr>
<td>NO$_3^-$-N</td>
<td>C (mg/L)</td>
<td>0.42</td>
<td>0.33</td>
<td>0.92</td>
<td>0.54</td>
<td>1.02</td>
</tr>
<tr>
<td>R (%)</td>
<td>38.24</td>
<td>51.47</td>
<td>35.29</td>
<td>20.59</td>
<td>50.00</td>
<td>33.82</td>
</tr>
<tr>
<td>TN</td>
<td>C (mg/L)</td>
<td>18.87</td>
<td>9.65</td>
<td>9.65</td>
<td>7.65</td>
<td>6.58</td>
</tr>
<tr>
<td>R (%)</td>
<td>-27.16</td>
<td>34.97</td>
<td>34.97</td>
<td>48.45</td>
<td>55.66</td>
<td>69.14</td>
</tr>
<tr>
<td>TP</td>
<td>C (mg/L)</td>
<td>0.92</td>
<td>0.73</td>
<td>0.55</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>R (%)</td>
<td>-16.46</td>
<td>7.59</td>
<td>30.31</td>
<td>55.70</td>
<td>50.63</td>
<td>72.15</td>
</tr>
<tr>
<td>COD</td>
<td>C (mg/L)</td>
<td>59.25</td>
<td>57.22</td>
<td>40.12</td>
<td>35.25</td>
<td>36.25</td>
</tr>
<tr>
<td>R (%)</td>
<td>-6.07</td>
<td>-2.43</td>
<td>28.18</td>
<td>36.90</td>
<td>35.11</td>
<td>53.06</td>
</tr>
<tr>
<td>DO</td>
<td>C (mg/L)</td>
<td>1.2</td>
<td>1.5</td>
<td>2.4</td>
<td>2.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

C: corresponding to concentration.
R: corresponding to removal rate.
achieve a better purification effect. According to comparative analysis (Table 3), DHA and UA of the sediments decreased significantly after the biofilm restoration, which probably resulted from the contents of OM and organic N in sediments being significantly reduced, as was TN. Moreover, the result also suggested that biofilms can promote the transformation of N in overlying water and sediments (Arnon et al. 2007a).

**CONCLUSIONS**

The results verified that aeration could improve the DO contents of polluted black-odor rivers to a certain extent, and had a significant effect on the transformation of all forms of N as well as the microbial activity. In particular, sediment aeration was better than water aeration in terms of restoring river water quality.

For the seriously polluted urban rivers, the reoxygenation capability is the prerequisite for the effective application of bioremediation. On the basis of aeration, the biofilm technique could significantly remove NH$_4^+$-N and TN from the water body. On the one hand, the biofilm system possessed the advantageous conditions for the biological denitrification in terms of time and space. On the other hand, nitrification and denitrification were triggered by the biofilms, which could accelerate N$_2$ generation and escape from the water body. However, research about the mechanism and function of biofilms is not yet mature. And more in-depth studies still need to be conducted with respect to the matter cycling inside biofilms, the community structure and function of biofilms, and the physical, chemical and biological factors that influence the biological community. Overall, the sediment aeration combined with biofilm technique, following reasonable procedures, could significantly improve the effect and stability of restoration of black-odor urban rivers with serious eutrophication, which would have a long-term significance for sustainable management of water environment.

**ACKNOWLEDGEMENTS**

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**Table 3** The effect of different treatment techniques on purifying sediments

<table>
<thead>
<tr>
<th>Water tank</th>
<th>Removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHA</td>
</tr>
<tr>
<td>W1-1</td>
<td>–5.52</td>
</tr>
<tr>
<td>W1-2</td>
<td>10.21</td>
</tr>
<tr>
<td>W2-1</td>
<td>36.86</td>
</tr>
<tr>
<td>W2-2</td>
<td>45.68</td>
</tr>
<tr>
<td>W3-1</td>
<td>41.21</td>
</tr>
<tr>
<td>W3-2</td>
<td>48.67</td>
</tr>
</tbody>
</table>

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