Simultaneous nutrients and carbon removal from low-strength domestic wastewater with an immobilised-microorganism biological aerated filter

Q. Chen, L. Qu, G. Tong and J. Ni

ABSTRACT

To improve the efficiency of low-strength domestic wastewater treatment, an immobilised-microorganism biological aerated filter (I-BAF) was established for simultaneous carbon, nitrogen and phosphorus removal. The I-BAF performance was systematically evaluated under continuous and intermittent aeration modes. At the optimal condition with an intermittent aeration control schedule of 2 h on/1 h off, the maximum removal rates of COD, NH$_4^+$-N, TN and P were 82.54%, 94.83%, 51.85% and 61.49%, respectively, and the corresponding averaged effluents could meet the first class standards of China. Further analysis of PCR-DGGE profile revealed that members of the gamma and alpha proteobacterium bacterial groups were probably responsible for the nitrogen and phosphorus removal. The I-BAF system showed excellent performance in carbon and nutrients removal, which provided a cost-effective solution for the treatment of low-strength domestic wastewater.

Key words | BAF, low-strength wastewater, PCR-DGGE, simultaneous nitrogen and phosphorus removal, SOUR

INTRODUCTION

The concentrations of chemical oxygen demand (COD), total suspended solids (TSS), ammonia nitrogen (NH$_4^+$-N) and total phosphorus (TP) are usually very low in the domestic wastewater from medium and small towns in China (Jin et al. 1998). For example, the reported annual average concentrations of COD, TSS, NH$_4^+$-N and TP are 80–150 mg/L, 20–80 mg/L, 12–16 mg/L and 1–3 mg/L, respectively (Yang, 2006). Unfortunately, activated sludge systems fail to treat such a low-strength wastewater due to their operational complexity, low amount of active biomass, high cost in the apparatus and frequent occurrence of the sludge bulking.

The biological aerated filter (BAF), as a novel wastewater treatment system, has been developed in Europe and then extensively applied over the world (Ryu et al. 2008). It is better than the activated sludge systems in terms of system stability and compactness, because high concentration of active biomass can be maintained and suspended solids in the influent can be captured physically. Moreover, by reason of the gradient of dissolved oxygen (DO) from the interior to the exterior of the carrier, it is effective in removing carbon and nitrogen concurrently (Lemoine et al. 2006; Rogalla et al. 2006). In particular, phosphorus removal can be achieved in BAF systems operated under alternated anaerobic/aerobic conditions (Shanableh et al. 1997; Zheng & Long, 2008). The phosphorus extraction depends on backwashes to discharge the phosphorus-rich attached biomass. However, little attention has been paid to the possibility of combining carbon, nitrogen and phosphorus removal using a single BAF.

This study would focus on achieving simultaneous removal of carbon, nitrogen and phosphorus from low-strength domestic wastewater with the help of a single immobilised-microorganism biological aerated filter (I-BAF). Particular attention would be paid to the operation modes of both continuous and intermittent aeration.
MATERIALS AND METHODS

Experimental apparatus and operation

The experiments were conducted in a lab-scale I-BAF reactor with an effective volume of 2 L and a packing ratio of 70% (Figure 1). Custom-made patented FPUFS carriers, 8 × 8 × 8 mm³ cubes, were used to immobilise microorganisms. Activated sludge taken from the Gaobeidian sewage treatment plant (Beijing, China) was seeded into the reactor and cultivated with the synthetic wastewater, containing (mg/L): CH₃COONa of 125, NH₄Cl of 57.3, KH₂PO₄ of 4.39, NaHCO₃ of 33.5, NaCl of 63.0, MgSO₄·7H₂O of 5.1, FeSO₄·7H₂O of 6.5. The averaged concentrations of COD, NH₄⁺-N, and PO₄³⁻-P were 100 ± 12.8, 12.8 ± 1.15, and 1.13 ± 0.20 mg/L, respectively.

Experimental schedule was demonstrated in Table 1. Excess sludge was removed from the bottom every month. All processes were operated at room temperature and the pH was maintained at 6.8–7.5.

Tracking study

To determine the activity of microorganisms in the intermittently aerated mode, tracking studies were conducted at 20 min intervals during alternate aeration/non-aeration cycles. The changes of nitrogen and phosphorus concentrations as a function of time were analysed.

Community analysis

Samples were collected on days 26, 48 and 80. The carriers were cut into small chips and submerged in the PBS buffer (pH 7.4), followed by vigorous vortexing. Genomic DNA was then extracted from the mixture with a 3 S-DNA isolation kit (Shenergy Biocolor, Shanghai). PCR-DGGE procedure and data analysis were performed as previously described in Wang et al. (2010).

Analytical methods

COD, ammonia and phosphate were analysed according to the standard methods. Nitrite was determined by N-(1-naphthyl)-ethylene diamine spectrophotometry and nitrate was determined by ultraviolet spectrophotometry. DO was monitored with a dissolved oxygen meter (YSI 550A, USA). The amount of TN was calculated by adding concentrations of ammonia, nitrate and nitrite.

The biomass concentration was measured as total solids on the carriers. Biofilm detachment from the carrier was done by submerging the carrier in 1M NaOH at 80°C for 30 min. Biofilm activity was determined by oxygen uptake rate (OUR) method, which was based on the successive addition of NaClO₃ and allylthiourea (ATU), selective inhibitors of nitrite oxidising bacteria (NOB) and ammonia oxidising bacteria (AOB), respectively. First, the total OUR was measured. When the DO concentration decreased about 3 mg/L, NaClO₃ (final concentration 20 mM) was added to the mixed sample and the OUR₁ was detected. The difference between the total OUR and the OUR₁ was considered as the oxygen uptake of NOB. Then, after the DO concentration decreased by another 2 mg/L, allylthiourea (ATU, final concentration 5 mg/L) was added to the mixed sample and the remaining OUR₂ was determined. The difference between the OUR₁ and the OUR₂ represented the oxygen uptake of AOB. The OUR₂ reflected the oxygen consumption of the heterotrophs (SurmaczGorska et al. 1996). The specific OUR of total biofilm, heterotrophic bacteria, AOB and NOB were represented as SOURₜ, SOURₜₕ, SOURₜₐ and SOURₜₙ, respectively.

Table 1 | Operational conditions of each mode

<table>
<thead>
<tr>
<th>Mode</th>
<th>Period (d)</th>
<th>HRT (h)</th>
<th>DO (mg/L)</th>
<th>Aeration mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–26</td>
<td>4</td>
<td>4–5</td>
<td>Continuous aeration</td>
</tr>
<tr>
<td>2</td>
<td>27–48</td>
<td>4</td>
<td>2–3</td>
<td>Continuous aeration</td>
</tr>
<tr>
<td>3</td>
<td>49–94</td>
<td>4</td>
<td>2 h aeration on (2–3)/1 h off (0–1)</td>
<td>Intermittent aeration</td>
</tr>
</tbody>
</table>

Figure 1 | Schematic of the I-BAF system (1) inflow (2) peristaltic pump (3) sample ports (4) inflator (5) air diffuser (6) excess sludge (7) timer control.
RESULTS AND DISCUSSION

Performance of the immobilised BAF system

Carbon removal

The stability of I-BAF with respect to COD removal efficiency was evidenced by the low effluent variations despite the different aeration control. At an inflow organic loading rate of 0.6 kg COD/m$^3$-d, the system achieved COD removals of 86.13%, 83.53% and 82.54% in Modes 1, 2 and 3, respectively (Figure 2(A)). This is consistent with other BAF studies at similar organic loading rate (Ling et al. 2006).

Nitrogen removal

As shown in Figure 2(B), satisfactory ammonia nitrogen removal rate of over 94% was achieved throughout the experiments. However, TN removal was greatly influenced by the operating DO concentration. In Mode 1, the removal rate of TN was only 15.22%. In Mode 2, denitrification was improved and TN removal rate achieved 48.31%. It was suggested that simultaneous nitrification and denitrification occurred when the DO was maintained between 2–3 mg/L. In Mode 3, when the intermittent aeration was carried out, TN removal rate reached 51.85%.

Moreover, the nitrogen removal was not greatly improved in Mode 3. Tracking study showed that nitrification occurred even during the non-aeration period, indicating aerobic zone might still exist in the reactor during a certain period, and denitrification in aeration conditions was very similar to that in non-aeration conditions (Figure 3). Thus, addition of an intermittent non-aeration period was not effective for enhancing nitrogen removal in the I-BAF.

Phosphorus removal

During Mode 1, significant phosphorus accumulation occurred (Figure 2(C)). The poly-$\beta$-hydroxy-alkanoates stored in the polyphosphate-accumulating organisms (PAOs) probably have worn off and counteracted phosphorus uptake under excessive aeration conditions. During Mode 2, the phosphorus removal rate reached 30.09%, typical for a conventional activated sludge system without chemical addition. Except for the anoxic zone in the inner carrier, the use of acetate as a carbon source probably promoted the release of phosphorus in the continuous aeration condition (Patel et al. 2006).

Interestingly, during Mode 3 of intermittent aeration, 61.49% removal efficiency was observed. Metabolic, especially assimilation, would play a less important role in the phosphorus reduction because the sludge yield was very low in the I-BAF with a long SRT of more than 30 days. Furthermore, significant phosphorus release was observed in the tracking study (Figure 3). It was reasonable to consider that the principle behind TP removal in biofilm systems was
similar to that in suspended systems (Chlou et al. 2000). In the I-BAF, the alternating aeration/non-aeration condition and the inner sludge recycles between anoxic and oxic zone in the carrier may induce the accumulation of PAOs. For long-term operation, complete phosphorus removal was only achieved by removing the P-rich biomass. In our study, the effluent P could be well controlled to a reasonable level at a long solid retention time (SRT) of 30 days. It was likely that the reactor was operated at low strength conditions, leading to lower growth rate of microorganisms. Moreover, PAOs are more predominant in systems at longer SRT, where a number of PAOs can be preserved (Lee et al. 2007).

Phosphorus and nitrogen removals were expressed relative to the biomass concentration. In this study, the values were 0.25 P-removed/gMLSS and 2.37 N-removed/gMLSS. Fan et al. (2009) applied a modified A2/O process to treat low-strength wastewater, and the values were 0.14–0.71 P-removed/gMLSS and 0.59–2.26 N-removed/gMLSS when the MLSS concentration was between 1,200 and 3,500 mg/L. Considering simultaneous phosphorus and nitrogen removal, the I-BAF system was more efficient. It also had cost-saving advantages over A2/O systems because only single reactor was required and there was no need for nitrate rich liquid and sludge recirculation. Based on the intermittent aeration control, 35% of power consumption could be saved every day compared to continuous aeration systems. Besides, the I-BAF system was more eminent in nutrients removal when compared with other representative intermittent-aeration systems (Shanableh et al. 1997; Chlou et al. 2001; Zheng & Long, 2008). Therefore, the proposed I-BAF system was high-efficiency, cost-saving and energy-efficient, which can be regarded as an alternative solution to simultaneous carbon and nutrients removal.

**Microorganisms in the reactor**

**SOUR**

As depicted in Figure 4, SOURt was found to be 8.16 mgO2/gMLSS-h in Mode 1. Under similar conditions, a value of 8.47 was reported by Fan et al. (2009). With the reduction of oxygen supply, SOURt decreased to 6.1 mgO2/gMLSS-h in Mode 3. The change of the heterotrophic bacteria activity was the same as that of the total biofilm. Because the oxygen diffusion limitation was small at high DO concentration, the bacteria reached higher activity as expressed by SOUR.

SOURa was 2.44, 2.39 and 2.34 mgO2/gMLSS-h in Modes 1, 2 and 3, respectively, implying that AOB activity was stable with the decrease of oxygen supply. Rongsayamanont et al. (2010) found that in the suspended cells, the SOURa were constant when DO > 2 mg/L; whereas in the entrapped cells, the SOURa changed from 4.0 to 2.0 mgO2/gMLSS-h when the DO concentration decreased from 5 to 2 mg/L. In the I-BAF system, it was more close to suspended cells because the AOB located on the surface of the porous carriers, where oxygen transfer limitation was not intensive.

SOURn of each Mode was 1.92, 1.29 and 0.68 mgO2/gMLSS-h, respectively. Rongsayamanont et al. (2010) pointed out that when the DO concentration decreased from 5 to 2 mg/L, the SOURn decreased from about 2.7 to 1.6 mgO2/gMLSS-h in the entrapped cells. These observations indicated that the level of oxygen supply had a strong effect on NOB activity. This is in accordance with previous reports (Pollice et al. 2002), which illustrated that a limited oxygen supply and alternating aeration were beneficial to controlling nitrification to nitrite and inhibiting the activity of NOB. It is
speculated that part of the nitrogen removal in Mode 3 may be via nitrite rather than nitrate.

**Community analysis**

PCR-DGGE profile indicated that the system was composed of a few dominant strains and each sample exhibited a distinct DGGE pattern (Figure 5). A change in aeration mode differentially selected for some different populations. Considering lane 1 as the benchmark, the respective dice index values of lanes 2 and 3 were 74.0% and 67.3%, implying that the diversity of microbial composition was more complex in the intermittent aeration condition.

In order to determine the dominant bacteria in the system, the major bands on the DGGE gel were excised, sequenced and compared to publicly available databases. Sequence analysis showed that bands 2-1, 3-1 and 2-2 corresponded phylogenetically to *Thiothrix* spp. (97%), *T. caldifontana* (93%) and *Roseospira* sp. (92%), respectively. But band 1-1 was not close to previously characterised bacteria. Besides, phylogenetic analysis was conducted to show the relationship among the excised DGGE bands. The results showed that bands 2-1 and 3-1 belonged to the *gamma proteobacterium* group, while band 2-2 belonged to the *alpha proteobacterium* group. Band 1-1 probably belonged to the high G + C gram-positive group of bacteria.

The dominant species in lanes 2 and 3 (*gamma* and *alpha proteobacterium*) may represent bacteria that played key roles in nitrogen and phosphorus removal, which is consistent with previous studies (Falkenfort et al. 2002; Ahmed et al. 2008). The operation of AOB are recognised to dominate in WWTPs, while *gamma proteobacteria* denitrifying bacteria are the dominant species in the anoxic packed bed. *Gamma proteobacteria* are also reported for the ability of phosphorus removal (Ahmed et al. 2008).

**CONCLUSIONS**

The performance of a proposed immobilised BAF system was evaluated for simultaneous removal of nutrients and carbon from low-strength domestic wastewater. Laboratory experiments were conducted under three different aeration modes.

The optimal operation condition was found at HRT = 4 h with an intermittent aeration time schedule of 2 h aeration on/1 h off. Under this condition, the highest removal efficiencies of carbon, nitrogen and phosphorus could be achieved, and the corresponding average effluent concentrations of COD, NH$_4$$^+$-N, TN and P were 16.83, 0.67, 6.39 and 0.39 mg/L, respectively. Moreover, analysis of DGGE profile revealed that more communities of microorganisms were involved in the intermittent aeration system than in the continuous mode. Further phylogenetic analysis showed that members of the *gamma* and *alpha proteobacterium* bacterial groups were responsible for the nitrogen and phosphorus removal.

The I-BAF system, characterised with its low power consumption and high removal capacity of carbon and nutrients, would be one of the promising alternatives as a reliable and cost-effective technology for low-strength domestic wastewater treatment in medium and small towns.

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