Phenol biodegradation and simultaneous nitrogen removal using a carbon fiber felt biofilm reactor

Yingwen Chen, Mingqing Liu, Fuyuan Xu, Shemin Zhu and Shubao Shen

ABSTRACT
Phenol biodegradation and its effect on the biological nitrogen removal were studied in a biofilm reactor (15 L) packed with carbon fiber felt carriers. Meanwhile, the effects of the effluent internal recirculation ratios (0, 100% and 200%) and the air flow rates (0.42, 0.83, 1.46, 2.08 and 3.33 L/min) on the performance of system were tested. The system exhibited an excellent capacity for simultaneous phenol biodegradation and biological nitrogen removal without effluent internal recirculation when the influent phenol concentration was as high as 1,000 mg/L (organic loading rate of 9.54 kg COD/(m³ d)) and the ammonia loading rates of 0.20, 0.32 and 0.40 kg NH₄⁺-N/(m³ d) respectively. Nitrification process was inhibited at the influent phenol concentration of 1,200–1,300 mg/L with average ammonia removal efficiency of 26.9%. The nitrifiers activity could be recovered in the perfect performance of system for phenol biodegradation. However, denitrification was not affected by the process of phenol biodegradation. In the air flow rates of 1.46–2.08 L/min, the system manifested stable operation for phenol elimination and nitrogen removal. Dissolved oxygen (DO) distributions in carbon fiber felt biofilm descended gradually from the external to the center of the carrier in all air flow rates.

Key words | carbon fiber felt, dissolved oxygen (DO), phenol, simultaneous nitrification and denitrification (SND)

INTRODUCTION
Phenol as a typical pollutant is present in many kinds of industrial wastewaters. It is toxic, carcinogenic, mutagenic and teratogenic at high concentrations (Autenrieth et al. 1991) and is an inhibitor of normal biological processes to wastewater treatment. Heipieper et al. (1991) reported that the influence of phenol on Escherichia coli growth and the phenol inhibitory concentrations to bacteria was above 0.05 g/L.

Numerous studies have been reported in literatures on the elimination of phenol from wastewater by biological treatment processes (González et al. 2001; Hosseini & Borghei 2004; Sarfaraz et al. 2004; Shetty et al. 2007; Viggiani et al. 2006; Bajaj et al. 2008). Viggiani et al. (2006) studied on phenol bioconversion by Pseudomonas stutzeri OXI using either free or immobilized cells, and the result indicated that bacterial growth was substrate-inhibited, with a limiting phenol concentration of about 600 mg/L. Hosseini & Borghei (2004) used a moving bed biofilm reactor (MBBR) on laboratory scale to treat phenolic wastewater. MBBR was very stable against the hydraulic and toxic shocks and could handle phenolic wastes with concentration of 220 mg/L without any inhibition.

In some cases, ammonia may be present as well in the phenol containing wastewater, such as coking wastewater, phenolic resin wastewater and aniline producing wastewater. The biological process for nitrogen removal from wastewater is constituted of two steps of nitrification and denitrification. The nitrification process is generally the rate-limiting step owing to the poor growth characteristics of nitrifiers. Moreover, the nitrification process may be
inhibited by some chemical substances such as phenol (Wood et al. 1981; Kim et al. 2008). The inhibitory effects of ammonia, thiocyanate, free cyanide, ferric cyanide, phenol and p-cresol on nitrification in an activated sludge system were examined and phenol significantly inhibited nitrification above 200 mg/L (Kim et al. 2008).

In fact, nitrogen removal can be coupled with phenol elimination, which was used as a carbon source for carrying out heterotrophic denitrification (Yamagishi et al. 2001). This combination technology made the application of biological treatment processes of industrial wastewater containing ammonia and phenol doubly interesting. Eiroa et al. (2008) studied the effect of phenol biodegradation on the nitrogen removal in resin wastewater. TKN removal efficiencies of 85.8 and 87.1% were reached respectively, corresponding to different influent average nitrogen loading of 0.05 and 0.08 kg TKN/m³d at fixed influent phenol concentration of 250–4,000 mg/L and organic loading of 0.11 kg TOC/m³d. Amor et al. (2005) also observed inhibition effect of phenol on nitrification bacteria. When the influent organic loading varied in the range of 30–2,700 mg COD/(L d), high ammonia removal efficiency of 99.8% and phenol removal efficiency of 99.9% were simultaneously obtained.

To keep a stable nitrification process, biofilm processes are widely adopted to improve the nitrification performance (Hem et al. 1994; Lazarova et al. 1997; Park et al. 2002; Rodgers & Zhan 2003). Meanwhile, simultaneous nitrification and denitrification (SND) occur in biofilm system usually using influent organic matter as carbon source for denitrification (Watanabe et al. 1992; Helmer & Kunst 1998; Puznava et al. 2000; Walters et al. 2009). But the studies on simultaneous nitrogen and phenol removal in biofilm system are seldom reported. Watanabe et al. (1995) developed a combined partially (aerobic) and fully (anaerobic) submerged RBC (CPFSR) reactor to achieve simultaneous nitrification and denitrification in the single reactor and phenol could be used as carbon sources for denitrification.

In this study, a lab-scale special biofilm reactor with carbon fiber felt carriers was carried out to achieve simultaneous phenol biodegradation and nitrogen removal. The aim of this experiment focused on phenol biodegradation characteristics and its effects on SND in the biofilm reactor. The influences of effluent recirculation ratio and air flow rate on the biofilm system operation were also investigated. By this way we hope to develop an efficient and economic technology to treat wastewater containing ammonia and phenol.

**MATERIALS AND METHODS**

**Set up of the lab-scale reactor**

A lab-scale plexiglass rectangular biofilm reactor was used in this study with an effective reaction volume of 15 L. The reactor was packed with special coiling carriers (carrier size: φ50 × 150 mm (Figure 1a,b) prepared by carbon fiber felt with plastic nets as skeleton. Physical properties of carbon fiber felt was shown as following: black color, monofilament diameter of 10–20 μm, bulk density of 0.04–0.06 g/cm³, and the total specific pore volume of 0.8 – 1.2 mL/g. Carbon fiber felt was provided by Nantong Senyou Carbon Fiber Co., Ltd, China. The total weight of packed carbon fiber felt was 120 g. The initial activated sludge was taken from Nanjing Jiangxinzhou Wastewater Treatment Plant with sludge concentration of 3 g/L and SV30% of 22%, dust-color. The schematic diagram of the reactor is shown as Figure 2.

Synthetic wastewater was pumped into the bottom of biofilm reactor using a peristaltic pump and flowed through the perforated board to contact with the carbon fiber felt carriers. Part of the effluent collected in an effluent tank was recirculated using another peristaltic pump and mixed with the influent in the water distribution region of the biofilm reactor. Compressed air flow was injected into the bottom of the biofilm reactor, controlled through an
air flow-meter. Excess sludge was discharged at regular intervals to keep biodegradation efficient and effluent SS concentration stable.

**Synthetic wastewater**

Ammonium sulphate and monopotassium phosphate were supplied as nitrogen and phosphorus sources. Ammonia concentrations in synthetic wastewater were fixed at 50, 80 and 100 mg/L respectively in the experimental process. Corresponding quantities of monopotassium phosphate with N/P ratio of 5/1 were added into the bulk solution. The initial pH was adjusted to 7.0–7.3, and NaHCO₃ was used as pH buffer. The nutrient solution was consisted of: CaCl₂·2H₂O 0.1 g, MgSO₄·7H₂O 0.1 g and 2.0 mL trace metal solution per liter of distilled water. Trace element solution was composed of (g/L): EDTA 5, ZnSO₄·7H₂O 0.43, MnCl₂·4H₂O 0.99, FeSO₄·7H₂O 5, NH₄MnO₄ 0.22, CuSO₄·5H₂O 0.25, CoCl₂·6H₂O 0.24 and NiNO₃ 0.19. Sodium acetate was added as the carbon source and the ratio of COD/N was taken as 6:1 in the SND cultivation period. Phenol was served as a substitute for sodium acetate at concentration from 0 to 1,300 mg/L. All reagents were analytic grade.

**Experimental procedure**

The experiments were operated at 20–25°C. The reactor was started-up with 10 L initial inoculated activated sludge and 1.60 L/min air flow rate. Synthetic wastewater was pumped continuously into the reactor with influent ammonia loading rate of 0.4 kg NH₄⁺-N/(m³ d). Biofilm formation on the carbon fiber felt carriers was confirmed by monitoring the physical characteristic of carrier surface and suspended solid concentration in reactor. When the nitrifiers were enriched on biofilm and the stable ammonia removal efficiency was achieved, sodium acetate was fed into synthetic wastewater as carbon source with a COD/N ratio of 6/1 to trigger SND on biofilm. Different effluent internal recirculation ratios (0, 100 and 200%) were tested to determine their effect on nitrogen removal. The test was conducted with effluent internal recirculation ratio of 200%, then with 100% and then with 0%. The running intervals for every recirculation ratios were 6 days.

When the biofilm was in stable operation, the objectives of the study were divided into two aspects: (1) to investigate the simultaneous phenol biodegradation and biological nitrogen removal on carbon fiber felt biofilm. (2) to study the effect of the air flow rate on the operation of system and DO distribution characteristics of carbon fiber felt biofilm. In the first experiment process, the applied influent phenol concentration was increased from 0 to 1,300 mg/L, and ammonia concentrations were controlled at 50, 80 and 100 mg/L, with corresponding ammonia loading rates of 0.20, 0.32 and 0.40 kg NH₄⁺-N/(m³ d) respectively. The influent flow rate was kept constant with HRT of 6 h and air flow rate was 1.60 L/min. The influent phenol concentration was increased gradually because of the inhibition to normal biological process. For the second test, a total of five air flow rates (0.42, 0.83, 1.46, 2.08 and 3.33 L/min) were applied to investigate the effect on the biofilm operation with influent ammonia loading of 0.40 kg NH₄⁺-N/(m³ d) and phenol concentration of 800 mg/L. The running intervals for every air flow rates were 10 days. Additionally, three DO detecting points standing for center, interlayer and external of carbon fiber felt carrier (Figure 3) were selected to study DO distribution characteristics of biofilm in different air flow rates. DO probe was stretched into 1/2 depth of coiling carrier.

**Analytic methods and instruments**

Phenol was analyzed by HPLC (DIONEX, P680) using a C18 column. The mobile phase was methanol/water
(1:1, v/v) and the UV detector was used at 270 nm; nitrate (NO$_3^-$-N) and nitrite (NO$_2^-$-N) were analyzed by ion chromatograph (DIONEX, DX-120). Ammonia, COD, VSS and pH were evaluated according to Standard Methods (APHA 2005). Dissolved oxygen (DO) was measured using JENCO 9250M Dissolved Oxygen Meter. All data were averaged for three times.

RESULTS AND DISCUSSION

System start-up and effluent recirculation ratio test

After seven days operation, a slick carrier surface was observed and the suspended solid concentration in the reactor decreased to 43 mg/L. In thirty days of operation, 95% ammonia removal efficiency of biofilm was achieved. In the experiment process, activated sludge concentration on biofilm of 0.43 g VSS/g on average was controlled by eventual sludge withdrawal. Suspended sludge concentration in effluent was less than 20 mg/L. The excess sludge was discharged from biofilm with dry biomass of 2.73 g/d averagely. The effect of effluent internal recirculation ratios on the TN removal in carbon fiber felt biofilm system was investigated and the results are shown in Table 1. In the experiment process, the influent COD supplied from acetate and ammonia concentration were kept at 600 mg/L and 100 mg/L (ammonia loading rate of 0.4 kg NH$_4^+$/N/(m$^3$ d)). Effluent ammonia concentrations were 3.89, 3.95 and 4.12 mg/L and the corresponding TN removal efficiencies were as high as 93.5, 93.7 and 92.9% respectively at effluent internal recirculation ratios of 0, 100, and 200%. Meanwhile, no obvious differences of effluent COD were observed. The operation performance of biofilm for nitrogen and COD removal was not affected by the internal recirculation. The main reason was that the suitable anoxic microenvironment in the biofilm was benefit for denitrification. The pH in the biofilm reactor varied between 7.3 and 7.8.

Phenol biodegradation and effect on biological nitrogen removal

Phenol biodegradation

As mentioned in the experimental procedure, the influent phenol concentration was increased from 0 to 1,300 mg/L. The results of phenol biodegradation in carbon fiber felt biofilm reactor are illustrated in Figure 4a,b. In 1–37 days operation, the influent phenol concentration was increased to 100 mg/L slowly with sodium acetate concentration of 510 mg/L. The phenol removal efficiencies fluctuated from 80.5 to 100%, meanwhile the COD removal efficiencies changed from 70.5 to 95.9% correspondingly. Sodium acetate concentration in the influent decreased to

Table 1 | Effluent recirculation ratio tests for nitrogen removal

<table>
<thead>
<tr>
<th>Recirculation ratio (%)</th>
<th>Recirculation ratio (100%)</th>
<th>Recirculation ratio (200%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent COD (mg/L)</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Influent ammonia (mg/L)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Effluent COD (mg/L)</td>
<td>23.1 ± 1.8</td>
<td>20.5 ± 1.7</td>
</tr>
<tr>
<td>Effluent ammonia (mg/L)</td>
<td>3.89 ± 0.53</td>
<td>3.95 ± 0.60</td>
</tr>
<tr>
<td>Effluent nitrate (mg/L)</td>
<td>2.38 ± 0.42</td>
<td>2.10 ± 0.37</td>
</tr>
<tr>
<td>Effluent nitrite (mg/L)</td>
<td>0.23 ± 0.19</td>
<td>0.27 ± 0.20</td>
</tr>
<tr>
<td>TN removal efficiency (%)</td>
<td>93.5 ± 1.2</td>
<td>93.7 ± 0.9</td>
</tr>
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0 mg/L in the 44th day, while influent phenol concentration was increased to 1,000 mg/L with organic loading rates changing from 1.47 to 9.54 kg COD/(m³ d) correspondingly. It was noted that the performance of reactor for phenol and COD elimination was very good with average phenol and COD removal efficiencies of 97.9 and 94.7% in the period of 44–146 days. However, when the influent phenol concentration was increased as high as 1,200 mg/L, biological activity was inhibited severely, and phenol removal efficiency declined to 13.3% at the influent phenol concentration of 1,300 mg/L. Even though the system was operated at a lower influent phenol concentration of 800 mg/L for six days, no obvious improvement of biological activity was observed. In this experiment, a feasible method was established to improve the system performance by reduction of influent phenol concentration to 200 mg/L and addition of sodium acetate to wastewater of 255 mg/L. The phenol biodegradation efficiency was increased to 97.9% despite the influent phenol concentration being as high as 800 mg/L in the day of 205. But Bajaj et al. (2008) found that acetate which was a common intermediate product of phenol degradation retarded the phenol degradation. Additionally, as shown in Figure 4a,b, the trend of phenol biodegradation in the system was in accordance with that of COD elimination, which indicated few intermediate products were accumulated in the phenol biodegradation process. Bajaj et al. (2008) also studied on phenol biodegradation by an aerobic fixed bed reactor and there was no phenol intermediate present in the reactor.

Effect of phenol removal on nitrification process

In the operation period, the effect of phenol on nitrification process and the effluent ammonia concentration in different ammonia loading rates (0.20, 0.32 and 0.40 kg NH₄⁺-N/(m³ d)) are illustrated in Figure 5a. It was observed that ammonia removal efficiency in the system fluctuated with the increase of influent phenol concentrations, which indicated that phenol could inhibit the nitrifier activity temporarily. However, the inhibition to nitrification disappeared when the performance for phenol elimination (shown as Figure 4a) was very good. For example, in 39–44 days, when the phenol elimination efficiency changed from 88.2 to 100%, the removal efficiency of ammonia increased from 77.4 to 94.2% at the same time with influent phenol concentration of 200 mg/L and ammonia loading rate of 0.20 kg NH₄⁺-N/(m³ d). The nitrification performance was also influenced by the ammonia loading rates. When the influent phenol concentration was at 800 mg/L, with phenol elimination efficiency of 95.1–97.8%, the average ammonia removal efficiencies were 77.1% and 84.8% in the ammonia loading rates of 0.40 and 0.20 kg NH₄⁺-N/(m³ d) respectively. With further increase of the influent phenol concentration to 1,200–1,300 mg/L, the system performance for nitrification deteriorated with average ammonia removal efficiency of 26.9%. In this period, the phenol biodegradation efficiency was only 13.3% as shown in Figure 4a. In the following operation from 171 to 205 days, the nitrification activity of the biofilm was recovered and the ammonia removal efficiency was 74.5% averagely.
Probably it was resulted from the nitrification inhibition by high phenol concentration in the reactor. The research results obtained by Shinozaki et al. (1997) suggested that nitrifying activity could be sustained in an activated sludge process supplied with wastewater containing substances inhibitory to nitrification if the inhibitory substance was immediately removed from the wastewater to a non-inhibitory level. In summary, simultaneous phenol biodegradation and nitrification could be achieved by the carbon fiber felt biofilm reactor with the influent phenol concentration of 1,000 mg/L and ammonia loading rates of 0.40 kg NH₄⁺-N/(m³ d).

Effect of phenol removal on the SND process

Experiments with Sodium acetate as carbon source for denitrification have been carried out in the carbon fiber felt biofilm reactor. The result of denitrification with phenol as sole carbon source is illustrated in Figure 5b. It was observed that nitrite and nitrate concentrations in effluent were below 1.0 mg/L all the time with the influent phenol concentration under 1,000 mg/L, while the performance of TN removal efficiency was not kept in a steady state. Referring to the results shown in Figure 5a, the fluctuation of TN removal efficiency was caused by the changing of ammonia concentration in effluent. The nitrate and nitrite could be eliminated through denitrification using phenol as electron donor. When the influent phenol concentration was 1,000 mg/L, an average TN removal efficiency of 76.5% was achieved. Even though the influent phenol concentrations were as high as 1,200–1,300 mg/L, nitrate and nitrite concentrations in effluent were not more than 5.0 mg/L. But the average TN removal efficiency was only 23.9% in 148–164 days owing to the biological inhibition to nitrifiers by high phenol concentrations in reactor. Eiroa et al. (2005) studied that denitrification percentage decreased because of the inhibition caused by phenol when influent phenol concentration was higher than 1,010 mg/L. In the study of Sarfaraza et al. (2004), when phenol loading was beyond 6.4 kg COD/(m³ d), the denitrification using granular sludge was inhibited with a sharp decrease in phenol removal efficiencies and an increase in nitrite concentration in the effluent. In this study, the carbon fiber felt biofilm reactor performed very well for the nitrogen removal with the influent phenol concentration of 1,000 mg/L (9.54 kg COD/(m³ d)).

![Figure 5](https://iwaponline.com/wst/article-pdf/62/5/1052/446541/1052.pdf)  
Figure 5 | (a) Effect of phenol biodegradation on nitrification process. (b) Effect of phenol biodegradation on SND process.
Effect of air flow rate on SND, phenol elimination and DO distribution characteristics

Effect of air flow rate on SND and phenol elimination

The running performances of biofilm reactor for phenol, ammonia and TN removal in five different air flow rates are shown in Figure 6 and Table 2. It was obvious that the performance of system got better with the increase of air flow rate. When the air flow rate was increased to 2.08 L/min, the phenol, ammonia and TN removal efficiencies on average of 99.5, 93.6 and 90.0% were achieved respectively. The fact that the relative lower removal efficiencies of phenol, ammonia and TN were obtained in the air flow rates of 0.42 L/min and 0.83 L/min indicated that oxygen supplied was insufficient for the biodegradation. There were some similarities between TN and ammonia removal efficiencies when the air flow rate was below 2.08 L/min. However TN removal efficiency decreased severely to 72.1% averagely in the air flow rate of 3.33 L/min, although the performance of system for phenol and ammonia elimination was very good. The possible reason was due to the destruction of anoxic microenvironment and the reduction of phenol which would easily lead to bad denitrification at higher air flow rate.

DO distribution characteristics

DO distribution characteristics of detected points 1, 2, 3 on biofilm in different air flow rates are shown in Figure 6 and Table 2. The DO in points 1, 2, 3 increased gradually when the air flow rate changed from 0.42 L/min to 3.33 L/min. Substantial increases of detected DO in three points occurred with the air flow rate higher than 2.08 L/min, which illustrated that the oxygen injected for biological process was sufficient. Especially for point 3, the DO increased on average from 1.78 mg/L to 3.21 mg/L in the air flow rates of 0.42 L/min and 2.08 L/min respectively. The curve features of DO in point 1 and 2 were similar owing to the buffering of biofilm. There were distinct differences among the DO distribution of three points in the different air flow rates. DO in point 3 were always higher than those of point 1 and 2, and in the air flow rates of 2.08 L/min and 3.33 L/min, DO in point 2 were a little higher than those of point 1. It was concluded that DO distributions on carbon fiber felt biofilm descended gradually from the external to the center of carriers, which was attributed to the special coiling structure of carriers. In the air flow rate of 2.08 L/min, 0.89, 1.07, 3.21 mg/L of DO on average in points 1, 2 and 3 were achieved respectively, with the stable phenol, ammonia and TN removal efficiencies illustrated in the Table 2.

CONCLUSIONS

Phenol biodegradation and simultaneous biological nitrogen removal using a biofilm reactor packed with carbon fiber felt carriers were investigated in this study. Performance of the system was very good with influent phenol concentration of 1,000 mg/L (organic loading rate of 9.54 kg COD/(m3 d)) and ammonia loading rates of 0.20, 0.32 and 0.40 kg NH3-N/(m3 d) respectively. When the influent phenol concentrations were increased to 1,200–1,300 mg/L, the biological activity for phenol elimination declined severely with phenol removal efficiency of only 13.3%, and nitrification was also inhibited with average ammonia removal efficiency of 26.9%. Sodium acetate contributed to the biological activity recovery of
biofilm system. However, no effect was observed on denitrification in the process of phenol biodegradation. The system manifested a stable operation for phenol elimination and nitrogen removal in the air flow rates of 1.46–2.08 L/min. DO distributed in carbon fiber felt biofilm descended gradually from the external of carrier to the center.

REFERENCES


