Simultaneous nitrification and de-nitrification in MBR

B. Wang, S. He, L. Wang and L. Shuo
Water Pollution Control Research Center, Harbin Institute of Technology (HIT), 202 Haihe Road, Harbin, China 150090

Abstract Experiments have been carried out to get an understanding of the effect of DO, C/N ratio and pH on the performance of a bench scale membrane bioreactor (MBR) in simultaneous nitrification and de-nitrification. It was found that under the conditions of MLSS in the range of 8000–9000 mg/L and temperature of water in the MBR of 24 °C, influent COD and NH3-N in the range of 523–700 mg/L and 17.24–24 mg/L respectively, the removals of COD, NH3-N and TN were 98%, 99% and 60%; 96.5%, 98% and 75%; 96%, 95% and 92%; 90%, 70% and 60% respectively at DO of 6, 3, 1 and 0.5 mg/L. It was also found that the changes in C/N ratio and pH in a certain range have a slight effect on COD removal but have significant influence on the removal of NH3-N and TN. The results showed that only under the conditions that each ecological factor was maintained relatively steadily, simultaneous nitrification and de-nitrification proceeded smoothly. It was found that when C/N ratio was 30, the influent pH 7.2, the temperature of water in MBR 24 °C and DO 1 mg/L, as optimum conditions, the removals of COD, NH3-N and TN were 96%, 95% and 92% respectively. In addition, mechanism research on simultaneous nitrification and de-nitrification in MBR has been conducted as well.

Keywords MBR; wastewater treatment; dissolved oxygen (DO); C/N ratio; ecological factors; simultaneous nitrification and de-nitrification

Introduction
The adverse environmental impacts associated with ammonia nitrogen include promotion of eutrophication, toxicity to aquatic organisms and depletion of dissolved oxygen in receiving waters due to bacterial oxidation of ammonia to nitrate (Klees and Silverstein, 1992). In consideration of these adverse impacts, it is in urgent need to restrict the ammonia nitrogen discharges. In the European directive on urban wastewater treatment (Cooper et al., 1994), the maximum allowable total nitrogen concentration including ammonia, nitrite and nitrate and organic nitrogen is 20 mg N/L (daily average). A further reduction to 10 mg/l is possible (Lefevre et al., 1993). The biological elimination of nitrogen in wastewater treatment plants is carried out by nitrification and de-nitrification. The two processes have been thought to be two separate reactions by different groups of microorganisms in activated sludge or biofilm. Aerobic autotrophic nitrifiers oxidize ammonia to nitrite and then nitrate. Under anoxic conditions, nitrite and then nitrate reduced to nitrogen gas by heterotrophic denitrifying bacteria. As autotrophic ammonia-oxidizing bacteria are generally characterized by low growth rates and yields, the nitrification is generally a rate-limiting step in a biological nitrogen removal process. Therefore, a major difficulty in biological nitrogen removal is to maintain adequate levels of nitrifiers in the aeration vessel.

Because of different environmental conditions of nitrifiers and de-nitrifiers, total nitrogen removal in wastewater treatment plants is most commonly achieved in a two-stage system. However, recent studies have revealed that these two important steps can take place simultaneously in the same reactor (Münch et al., 1996; Helmer and Kunst, 1998; Hao and Martinez, 1998; Menoud et al., 1999). This process is called simultaneous nitrification and de-nitrification.
Simultaneous nitrification and de-nitrification is gaining increasing interest because of its significant advantages compared to the conventional processes of separated nitrification and de-nitrification. If the efficiency of simultaneous nitrification and de-nitrification remains comparable with that in separate systems, the need for a separate de-nitrification tank can be eliminated or at least the size of the tank can be reduced. This could help to simplify the overall design dramatically.

In recent years, MBR has been proposed as an alternative to the conventional activated sludge process. The advantage of MBR is mainly due to the fact that it can maintain high MLVSS in the reactor. It has gained increasing use in wastewater treatment because of its several advantages (Silva et al., 1998).
1. The retention time of the biomass can be controlled as long as desired, which will create favorable conditions for normal growth of some species of bacteria with low growth rates, such as nitrifiers.
2. Possible implementation of simultaneous nitrification and de-nitrification because of its highly concentrated MLVSS, which make it easy to form an aerobic zone and an anoxic zone in the same reactor.
3. Better and more reliable effluent quality compared to that of the conventional process and no need for post-treatment.
4. Easy automatic control and compactness of the whole system.

From previous studies (Münch et al., 1996; Pochana and Keller, 1999; Yoo et al., 1999; Zhao et al., 1999), it was found that there are a lot of factors which influence simultaneous nitrification and de-nitrification, such as structure, size, density and concentration of sludge flocs, DO, F/M ratio, C/N ratio and pH, etc. The aim of the research is to illustrate the effect of ecological factors such as DO, C/N and pH on the performance of BMR in simultaneous nitrification and de-nitrification. In the test, a sludge concentration of 8000–9000 mg/L was maintained in the MBR through periodic sludge discharge, and the results revealed that simultaneous nitrification and de-nitrification can proceed smoothly when DO, C/N and pH were kept in a certain range.

Materials and method
A hollow fiber polysulfone ultrafiltration (UF) membrane module with 1.5 m² of total surface area, 0.05 μm of pore size and 0.35 m of length) was used as the test membrane. The volume of the reactor was 18 L. The temperature was maintained at 24 °C with a heater. A water float cock was used to control the water level of the reactor to keep the balance of influent and membrane permeate (effluent). The air was fed into the reactor with a micro-bubble aerator, and air flow was adjusted by an air flow-meter. A total of three runs were carried out, in which the HRT in the test MBR was kept at 5 h, but the values of DO, C/N ratio and pH were different. Three systems were run in parallel. A schematic diagram of the process configuration is presented in Figure 1.

A synthetic domestic wastewater made by mixing tapwater with certain quantities of starch, sugar, NH₄CL, Na₂HPO₄, NaH₂PO₄ and sodium bicarbonate was used in this test study, with an average COD 600 mg/L, NH₃-N 20 mg/L and TP was in the range of 2.5–5.1 mg/L.

The three reactors were run for over 1 month under different conditions to reach steady state. Then the test continued for a total of two months, and the effect of the ecological factors of DO, C/N ratio and pH on the performance of nitrification and de-nitrification in MBR was analyzed.

All analytical assessments were carried out according to the Chinese National Standard Guidelines or operation processes. [China NEPA, 1989]
Results and discussion

Effect of DO on the removal of COD

In different test phases, DO of MBR were kept at 6 mg/L, 3 mg/L, 1 mg/L and 0.5 mg/L respectively, and the relative results are shown in Figure 2. Initially, DO was maintained at 6 mg/L, and COD removal was averaged at 98%, then DO was reduced to 3 mg/L and the mean COD removal was 96%. When DO was kept over 3 mg/L, organic substance could be biodegraded significantly in the reactor. COD of the filtrate in the reactor (the mixed liquid samples collected were filtered by qualitative filter papers) and UF permeate were very low in both the samples. In the following phase, DO was dropped to 1 mg/L, and COD of filtrate had a slight rise, but the membrane permeate still kept a low value. This means that the UF membrane had excellent performance for COD removal. Finally, DO was reduced to 0.5 mg/L. A sharp increase in COD was observed both in filtrate and UF permeate, COD removal efficiency reduced to 91% and had a big fluctuation.

Effect of DO on NH3-N removal

Figure 3 shows NH3-N removal efficiencies at various DO levels. The removal of NH3-N was almost independent of DO when DO was over 3 mg/L. Under those conditions, NH3-N in the filtrate and membrane permeate were both below 1 mg/L, and NH3-N removal efficiencies were up to 98%–99%. In the following phase at DO of 1 mg/L in the MBR, NH3-N had a slight increase to about 2 mg/L in the filtrate, but the UF membrane still kept NH3-N to a low level and the removal efficiency reached 95%. Considering the small molecular weight of NH3-N, the UF membrane could not retain it through size-exclusion, and the NH3-N removal by membrane may be ascribed to the activated sludge.
attached to the outside of the hollow-fiber membrane. When DO was reduced to 0.5 mg/L in the reactor, NH$_3$-N removal was affected significantly, and removal efficiency was in the range of 40%–50%. The result is in line with many previous findings that DO influences the ammonia oxidation process significantly.

**Effect of DO on TN removal**

According to Figure 4, it is evident that DO has a significant effect on TN removal. In the first test phase of DO 6 mg/L, the removal of TN was only 60%. DO can penetrate into sludge flocs at the high DO, and hence it is hard to form an anoxic zone in sludge flocs. As a result, the majority of ammonia could be oxidized to nitrite and nitrate, but the de-nitrification process was inhibited, thus resulting in high concentration of TN both in the filtrate and membrane permeate. Based on the hypothesis of micro-environment, as a highly concentrated sludge could be maintained in the MBR, the anoxic zone may be formed in a sludge floc at an even higher DO concentration, so partial de-nitrification could take place in the MBR. In the next phase, when DO was reduced to 3 mg/L, the removal of TN increased because of the formation of some anoxic zones in sludge flocs. The TN in the UF permeate was about 7 mg/L and removal reached 75%. When DO was further reduced to 1 mg/L, an excellent membrane permeate with TN of 3 mg/L was obtained, and the corresponding TN removal efficiency reached 92%. At DO of 0.5 mg/L, nitrification was inhibited because of inadequate supply of DO for complete nitrification, which resulted in a high level TN in the effluent.

**Effect of C/N ratio on the performance of simultaneous nitrification and de-nitrification in MBR at 1 mg/L of DO**

According to the above findings, simultaneous nitrification and de-nitrification could take place most efficiently when DO was maintained at 1 mg/L. At this test stage, the emphasis was put on the effect of C/N ratio on the performance of MBR in simultaneous nitrification and de-nitrification. The results are presented in Table 1.
Based on these results shown in Table 1, it is evident that the NH$_3$-N in UF permeate increases with the decrease of C/N ratio. The change of C/N ratio was carried out either by increasing NH$_3$-N or decreasing COD of the influent. As the increase of NH$_3$-N load brought a shock to the biological system, and nitrification proceeded incompletely, there was a rise of NH$_3$-N in permeate. The increase of TN in the permeate was caused by the following two reasons: firstly, the increase of NH$_3$-N in the permeate, and secondly, the decrease of COD in the influent was insufficient for biological de-nitrification. If the sludge acclimatization was conducted at a certain C/N ratio to reach steady conditions, NH$_3$-N could be oxidized completely. However, this test was run at the C/N of 30 for a long time, and the system showed a good capacity for simultaneous nitrification and de-nitrification. It also showed the performance of MBR in simultaneous nitrification and de-nitrification may be affected by change in C/N ratio. Because the experimental result was obtained at the C/N ratio of 30, the change of C/N only showed the effect of shock load on the performance of the MBR in simultaneous nitrification and de-nitrification, but it is not able to determine a fixed minimum C/N ratio for completely simultaneous nitrification and de-nitrification.

Effect of pH on the performance of simultaneous nitrification and de-nitrification in MBR at 1 mg/L of DO

According to the results given in Table 2, the COD removals are comparable when pH of influent is in the range of 4.8–9.7, but pH has a significant influence on the removal of NH$_3$-N and TN. Initially, the average pH value of the influent was 4.7–4.8, the removals of NH$_3$-N and TN were 43–75% and 36.5–54.2% respectively. When pH was adjusted around 7.1–7.3, the removals of NH$_3$-N and TN increased to about 99.7–100% and 89.7–90.4% respectively. When pH was increased to 9.7, the removals of NH$_3$-N and TN were sharply decreased to about 75% and 62% respectively. The nitrification process is inhibited badly when pH is lower than 5. In the continuous flow reactor of the test, because of the dilution of mixed liquid, the pH values of 5 and 9.6 of influent were adjusted to 6.3 and 8.9, but both of them are not in the range of optimum pH for nitrification and de-nitrification. Based on the experimental results, it can be concluded that pH is

<table>
<thead>
<tr>
<th>PH</th>
<th>COD</th>
<th>NH$_3$-N</th>
<th>TN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inf. (mg/L)</td>
<td>Eff. (mg/L)</td>
<td>Re. (%)</td>
</tr>
<tr>
<td>4.8</td>
<td>639.9</td>
<td>58.61</td>
<td>90.84</td>
</tr>
<tr>
<td>4.76</td>
<td>628.6</td>
<td>23.5</td>
<td>96.26</td>
</tr>
<tr>
<td>7.1</td>
<td>624</td>
<td>12.54</td>
<td>97.99</td>
</tr>
<tr>
<td>7.3</td>
<td>631</td>
<td>15.6</td>
<td>97.53</td>
</tr>
<tr>
<td>9.6</td>
<td>621.64</td>
<td>46.8</td>
<td>92.87</td>
</tr>
<tr>
<td>9.7</td>
<td>630.2</td>
<td>50.64</td>
<td>91.97</td>
</tr>
</tbody>
</table>
an important factor affecting simultaneous nitrification and de-nitrification, and the optimal pH range of 7.0–7.5 was determined in this study for most efficient removals of COD, NH$_3$-N and TN by nitrification and de-nitrification in MBR.

### Mechanism research on simultaneous nitrification and de-nitrification in MBR

There exists a large quantity of species of microorganisms in sludge flocs; the relationships between different groups are complex. Each group of bacteria has its optimum living environment. For all species in the activated sludge, some ecological factors favor the growth of certain groups but may be harmful to the growth of some other ones. Therefore, a suitable ecological environment should be created for nitrifiers and de-nitrifiers in biological nitrogen removal systems.

As shown in Figure 5, an anoxic zone and an aerobic zone is required in sludge flocs to form the essential environments for simultaneous nitrification and de-nitrification. To obtain a steady state, all ecological factors such as pH, temperature, DO, F/M and C/N influencing simultaneous nitrification and de-nitrification are required to keep stable. The optimum pH values for nitrification and de-nitrification are in the range of 8.0–8.4 and 6.5–7.5 respectively, and the optimum pH for simultaneous nitrification and de-nitrification is about 7.5, and the experimental results have substantiated the deduction. The optimum temperature for nitrification and de-nitrification are in the range of 20°C–30°C and 20°C–40°C respectively. When the temperature was below 15°C, the multiplication rate and metabolism rate of de-nitrifiers became slow, and de-nitrification rate reduced too. It has been confirmed from this test that DO should not be below 1 mg/L for nitrification, otherwise the process will be inhabited. For the de-nitrification process, DO should be kept below 0.5 mg/L. Much higher DO is unsuitable for simultaneous nitrification and de-nitrification, for DO will penetrate into the bio-flocs and the anoxic zone is then difficult to form.

The size and density of sludge flocs also affected the diffusion of DO, a large floc caused a long diffusion distance within it, thus needing much more DO and was more likely to form an anoxic zone in the sludge floc. The denser the floc, larger the quantity of microbes contained in its unit volume, and hence the more oxygen consumed. As a result, the oxygen was more difficult to diffuse into a larger and denser sludge floc and to form an anoxic zone within it.

The supply of organic substance as a carbon source is another important factor for simultaneous nitrification and de-nitrification. There exists a substrate overlap between

![Figure 5 Pattern of DO and substrate concentration in sludge](http://iwaponline.com/wst/article-pdf/52/10-11/435/433623/435.pdf)
heterotrophic aerobic bacteria in the aerobic zone and de-nitrifiers in the anoxic zone; they will compete for the same food source. The outer spaces of sludge flocs are occupied by heterotrophic aerobic bacteria, so it is more easy for them to get food from bulking solution than de-nitrifiers in the inner anoxic zone. During the process of organic substance diffusion from bulking solution into aerobic zone and further into anoxic zone, a large amount of organics will be utilized by heterotrophic aerobic bacteria and the remaining organics cannot meet the requirement for de-nitrification. In order to solve this problem, the following measures should be adopted: firstly, the quantity of supplied organics should be adequate to maintain a higher F/M value, otherwise, the low F/M value led to much consumption of organic substance and insufficient supply of carbon for de-nitrification. However, when the organic substance supplied was too much and the DO was limited, the bacterial activity of the floc in the aerobic zone was not too high, which meant more organics were not degraded and could diffuse into the anoxic zone of the floc. Secondly, DO should not be too high, otherwise a high DO will strengthen the penetration of oxygen, and it will become difficult to form an anoxic zone in the floc, also it will strengthen the activity of heterotrophic aerobic bacteria in the aerobic zone, and increase the rate of oxidation. Even though there is an anoxic zone in the sludge flocs, the de-nitrification will be reduced because of no or lack of carbon. Thirdly, NO$_2^-$ may directly intrude into anoxic zone and be denitrified instead of being oxidized by nitrobacteria into nitrate, thus reducing the demands of organics and oxygen. When 1 g NO$_2^-$ or NO$_3^-$ is reduced into N$_2$, the demand methanol is 1.53 g or 2.47 g respectively. Fourthly, the dead microorganisms in for sludge floc will release organics that will serve as the carbon source for denitrification.

In MBR, when each ecological factor is in a relatively stable condition, even if a certain factor has a slight fluctuation, highly concentrated sludge flocs have the ability to balance its disadvantageous effect on simultaneous nitrification and de-nitrification. For example, the activity of heterotrophic aerobic bacteria will strengthen with a slight increase of DO, which will make them consume more DO and organic substance as well as lessen the volume of the anoxic zone. In this case, the de-nitrification will be affected, but the system still has a certain capacity for simultaneous nitrification and de-nitrification. In addition, the change of F/M will induce the activity change of heterotrophic aerobic bacteria, Nitrosomonas, nitrobacteria and denitrifiers, then they will cooperate to implement simultaneous nitrification and de-nitrification. The MBR is also effective in maintaining optimum pH level without the addition of external acid or base source. During nitrification, oxidizing 1 g NH$_3$-N will consume 7.14 g (in CaCO$_3$) alkalinity, but reducing 1 g NO$_3^-$ will produce 3.5 g alkalinity as a compensation for alkalinity loss in de-nitrification.

There exist “food chains” in MBR. For instance, ammonia produced through ammonification by heterotrophic aerobic bacteria will serve as the substrate for Nitrosomonas and nitrobacteria, nitrite produced by Nitrosomonas will be consumed by nitrobacteria. In addition, both the nitrite and nitrate produced by either Nitrosomonas or nitrobacteria will be utilized by denitrifiers. It is necessary for a large community of microorganisms to coexist for simultaneous nitrification and denitrification in MBR.

**Conclusion**

The effect of DO, pH and C/N on simultaneous nitrification and de-nitrification in MBR was studied. It was found that under the conditions of MLSS in the range of 8000–9000 mg/L and the temperature of water in MBR of 24°C, the influent COD and NH$_3$-N in the range of 523–700 mg/L and 17.24–24 mg/L respectively, the removals of COD, NH$_3$-N and TN were 98%, 99% and 60%; 96.5%, 98% and 75%; 96%, 95% and 92%;
90%, 70% and 60% respectively at DO of 6, 3, 1 and 0.5 mg/L. When DO was higher than 1 mg/L the removals of COD and NH$_3$-N were adequate, and the removal of TN increased with the decrease of DO. However, at DO of 0.5 mg/L, the removals of COD, NH$_3$-N and TN showed a sharp drop.

In the tested MBR, at DO of 0.5 mg/L and C/N ratio in the range of 20–30, the removals of COD, NH$_3$-N and TN were high; when C/N ratio was 10, the removal of COD was still high, but that of NH$_3$-N and TN decreased. When the C/N ratio was 5, the removal of COD was not affected significantly, but that of NH$_3$-N and TN were reduced. Because the experiment was a result obtained at the C/N ratio of 30, the change of C/N ratio only showed the effect on the resistance to shock loading, but it is not able to determine a fixed minimum C/N ratio for complete nitrification and de-nitrification.

When the influent pH was in the range of 4.76–9.7, the variation of COD removal was insignificant, but the variation of NH$_3$-N and TN with pH was significant, which showed that pH is one of main ecological factors that affect simultaneous nitrification and de-nitrification in the MBR significantly.

The results showed that when C/N ratio was 30, the influent pH 7.2, the temperature of water in MBR 24$^\circ$C and DO 1 mg/L, as optimum conditions the removals of COD, NH$_3$-N and TN were very high, which resulted from a completely simultaneous nitrification and de-nitrification that took place in the tested MBR.

References