Performance of a completely autotrophic nitrogen removal over nitrite process for treating wastewater with different substrates at ambient temperature

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Received 25 June 2012; revised 12 October 2012; accepted 17 October 2012

Abstract

The stability and parameters of a bio-ceramic filter for completely autotrophic nitrogen removal were investigated. The completely autotrophic nitrogen removal over nitrite (CANON) reactor was fed with different concentrations of ammonia (400, 300, and 200 mg N/L) but constant influent ammonia load. The results showed that the CANON system can achieve good treatment performance at ambient temperature (15–23°C). The average removal rate and removal loading of NH₄⁺-N and TN was 83.90%, 1.26 kg N/(m³·day), and 70.14%, 1.09 kg N/(m³·day), respectively. Among the influencing factors like pH, dissolved oxygen and alkalinity, it was indicated that the pH was the key parameter of the performance of the CANON system. Observing the variation of pH would contribute to better control of the CANON system in an intuitive and fast way. Denaturing gradient gel electrophoresis analysis of microorganisms further revealed that there were some significant changes in the community structure of ammonium oxidizing bacteria, which had low diversity in different stages, while the species of anaerobic ammonium oxidizing (anammox) bacteria were fewer and the community composition was relatively stable. These observations showed that anaerobic ammonia oxidation was more stable than the aerobic ammonia oxidation, which could explain that why the CANON system maintained a good removal efficiency under the changing substrate conditions.

Key words: completely autotrophic nitrogen removal over nitrite; bio-filter; anammox; pH; dissolved oxygen; alkalinity

DOI: 10.1016/S1001-0742(12)60094-1

Introduction

The complete autotrophic nitrogen removal over nitrite (CANON) process, which combines nitrification with anammox in a single reactor, can potentially remove ammonium by the cooperation between aerobic and anaerobic ammonium oxidizing bacteria (AOB) (Slièkers et al., 2003). It has been reported that the CANON process is the simplest nitrogen removal process so far due to its advantages of being completely autotrophic, without the need for carbon source addition, as well as having reduced energy requirements and lower biomass production compared to other processes. CANON could be very economical compared with the SHARON-Anammox (Jetten et al., 1997) two-step nitrogen removal process since it requires no nitrite addition and realizes nitrogen removal in a single reactor.

The research on the CANON process, at present, has been aimed mostly at wastewater with high temperature and high ammonium (Egli et al., 2003; Cho et al., 2011). Some research groups used this process to treat sludge digester liquids using different styles of reactor (Ahn and Choi, 2007; Yang et al., 2001; Vázquez-Padin et al., 2009). The CANON process may be a more economical and efficient option for high ammonia wastewater treatment, but the nitrogen loading and removal rate was lower than that of anammox. However, Chuang et al. (2007) obtained an ammonium removal rate of 1.46 kg N/(m³·day) in a closed down-flow hanging sponge reactor; moreover, the utilization of both sequencing batch reactor (SBR) and gas-lift for the CANON process contributed a very high N-conversion rate of 1.5 kg N/(m³·day) (Slièkers et al., 2003).

The performance of the CANON process depends on the microbial activity of AOB and anammox bacteria, and
the autotrophic organisms involved in the CANON process have different preferences for temperature. In general, the optimal temperature for expanding the differences of specific growth rates between AOB and nitrite oxidizing bacteria (NOB) is recommended to be above 25°C and the optimum temperature for the growth of anammox bacteria was 30–40°C (Strous et al., 1999). For those reasons, many studies were conducted at high temperature. In recent studies, some operations of the CANON process at ambient temperature (20°C) and low ammonium loading were also realized. Cema et al. (2007) successfully operated the anammox rotating biological contactor at 20°C, and Vázquez-Padín et al. (2011) achieved a mean removed nitrogen loading of 0.2 kg N/(m³·day) using the granular sludge reactor with the CANON process at 15°C.

In addition, our laboratory has made some attempts at the treatment of domestic sewage in SBR (Wang et al., 2010), while a CANON sponge reactor finally failed to realize stable performance at low ammonium and high temperature (35 ± 1°C).

Parameters like dissolved oxygen (DO) concentration, temperature and pH have great impacts on the performance of the system, due to the diversity of microbial species and their complex inter-relationship in the CANON system. As reported by the researchers, the optimum temperature for the CANON reactor is 30–35°C, and the pH value is 7.5–8.0 (Veyss et al., 2010). However, the conclusions about the optimal DO concentration and aeration are diverse due to different experimental conditions and researchers. Besides control parameters, a good balance is important as well between AOB and anammox bacteria in a CANON system. Changes in external factors like the ammonium substrate may disturb the microenvironment. It is therefore necessary to investigate the stability and the key parameters of the system under such disturbances for future application in practical engineering.

This research focused on the operating performance of a CANON bio-filter under variable substrate concentrations and analyzed the parameters and the succession of the microbial populations at ambient temperature (15–23°C).

1 Materials and methods

1.1 Reactor systems

An up-flow bio-filter (height 700 mm, diameter 150 mm) was used with working volume of 1.8 L and total volume of 8.15 L, as shown in Fig. 1. The vessel was manufactured from a Perspex column and was filled with volcanic activity bio-ceramic (packing height 550 mm, diameter 6–8 mm). An aeration device was attached on the bottom of the vessel while the pH/DO data acquisition system was on the top. The values of the parameters like temperature, DO concentration and pH were monitored and measured continuously on-line.

1.2 Long term operation

The CANON aerated bio-filter was continuously fed with a synthetic wastewater containing a defined amount of (NH$_4$)$_2$SO$_4$ (N source), NaHCO$_3$ (C source and buffer), and KH$_2$PO$_4$, without organic carbon. During the research, the DO was not stripped from the tap water; meanwhile, the oxygen from the air dissolved into the water continuously, which resulted in the existence of a certain concentration of NO$_2^-\text{-N}$. The amount of NO$_3^-\text{-N}$ in the influent came mainly from the tap water.

The reactor was successfully started-up with a feed of wastewater with high ammonia (400–500 mg N/L), and after long-term stable operation, the influent ammonia concentrations of the reactor gradually decreased from 400 to 300, and then to 200 mg N/L. This reactor operated at ambient temperature (15–23°C), maintaining a constant influent nitrogen rate (about 1.50 kg N/(m³·day)) by a decrease in hydraulic retention time (HRT). From 154th day on, under the condition that the influent ammonia concentration decreased to 100 mg N/L and even lower, the research entered a new stage that will not be mentioned in this article. In this study, in order to achieve a relatively steady state, the reactor operated for at least 3 weeks with one nitrogen concentration in the influent. pH, DO concentration, aeration and temperature were monitored, and ammonium, nitrite, and nitrate concentration of the influent and effluent were analyzed once a day. The removal efficiency and loading of ammonia nitrogen and total nitrogen (TN) were determined. The transformation of the microorganism strains was investigated by a DGGE experiment. The specific influent quality and operational conditions for the reactor are shown in Table 1.

1.3 Analytical methods

1.3.1 Chemical analysis

The total inorganic nitrogen was defined as the sum of the concentrations of NH$_4^+\text{-N}$, NO$_2^-\text{-N}$ and NO$_3^-\text{-N}$, while
the concentrations of these three substances in the influent and effluent were measured every day by the method of Nessler’s reagent photometry, N-(1-naphthyl)-ethylene diamine spectrophotometry and ultraviolet spectrophotometry macro-parameter separately. In addition, parameters such as DO, pH, water temperature and alkalinity were tested at the same time. For these measurements, we use a WTW inoLabStirOx multi-function on-line instrument for the detection of the DO concentration, pH and temperature of the effluent and also chose a portable pH meter to check the pH of the influent and effluent. The alkalinity was gauged by the means of potentiometric titration.

1.3.2 DNA extraction and PCR amplification

The cells were ruptured with a combination of enzymatic and chemical methods, followed by phenol/chloroform extraction, as well as ethanol precipitation. The extraction products were further purified with an agarose gel DNA purification kit (Tiangen, Beijing, China). A primer set amoA-1F (5′-GGGTTTCTTCACTGTGTTGT-3′) and amoA-2R (5′-CCCCTCGGGAAGCCTTCTTC-3′) (Ling and Ming, 2004) was used to selectively amplify the amoA gene. A GC clamp (CGC CCG CCG CCC CCC CCC CCC CCC G) was added to the forward primer AmoX368F at the 5′ was designed for subsequent DGGE. The composition of the 25 μL PCR reaction mixture was the same as the first phase, changing primers to AmoX368F and AmoX820. The PCR reaction conditions were as follows: initial denaturation at 94°C for 5 min and 30 cycles consisting of denaturation at 94°C for 30 sec, primer annealing at 53°C for 45 sec, and extension at 72°C for 60 sec; the final elongation step was extended to 10 min at 72°C. The products obtained were used as a template for a second amplification where the anammox bacteria specific primers AmoX368F (5′-CCTTTTCGGCCATGCGAA-3′) and AmoX820 (5′-AAAAACCTTTACTTATTGGCGCC-3′) were used. A GC clamp (5′-CGC CCG CCG CCC CCG CCC CCC CCC CCC CCC G-3′) added to the forward primer AmoX368F at the 5′ was designed for subsequent DGGE. The composition of the 25 μL PCR reaction mixture was the same as the first phase, changing primers to AmoX368F and AmoX820. The PCR reaction conditions were as follows: initial denaturation at 94°C for 5 min and 30 cycles consisting of denaturation at 94°C for 60 sec, primer annealing at 62°C for 60 sec, and extension at 72°C for 90 sec; the final elongation step was extended to 10 min at 72°C. The PCR products of the proper size were confirmed by electrophoresis through a 1.5% (W/V) agarose gel.

1.3.3 DGGE analysis of amoA gene and 16S rDNA gene

DGGE electrophoresis was performed with a D-CODE Universal Mutation Detection System (Bio-Rad Laboratories) according to the manufacturer’s instruction. The PCR products were applied on a DGGE gel of 8% polyacrylamide with a linear denaturing gradient ranging from 30% to 60%. Electrophoresis was run at a constant voltage of 120 V for 6 hr. Subsequently, the silver staining method was employed after electrophoresis and the gel images were obtained by using the Gel Doc 2000 system (Bio-Rad) (Bassam et al., 1991) for further analysis.

2 Results and discussion

2.1 Nitrogen removal

According to the NH4+-N concentration of the influent, the operational process was divided into three stages. As shown in Table 1, the NH4+-N concentration decreased from 400 mg N/L to 300 mg N/L and finally to 200 mg N/L. A small scale fluctuation in the feed ammonia concentration was caused by the artificial disturbance. Changes of the influent and effluent nitrogen elements in the three stages and the corresponding nitrogen removal can be seen in Fig. 2.

In Stage I (1–60 days), the reactor operated at 16–

### Table 1 Influent quality and operational conditions for the reactor

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time (day)</th>
<th>Temperature (°C)</th>
<th>NH4+-N (mg N/L)</th>
<th>NO2--N (mg N/L)</th>
<th>NO3--N (mg N/L)</th>
<th>pH</th>
<th>Alkalinity (mg CaO/L)</th>
<th>Aeration (L/min)</th>
<th>Nitrogen removal load (kg N/m³-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1–60</td>
<td>20</td>
<td>369.7–441.1</td>
<td>0–10</td>
<td>0–10</td>
<td>8.0–8.2</td>
<td>900</td>
<td>4.5–5.0</td>
<td>1.00</td>
</tr>
<tr>
<td>II</td>
<td>61–87</td>
<td>20</td>
<td>244.8–361.3</td>
<td>0–20</td>
<td>0–20</td>
<td>8.0–8.2</td>
<td>620</td>
<td>4.5–5.0</td>
<td>1.24</td>
</tr>
<tr>
<td>III</td>
<td>88–153</td>
<td>20</td>
<td>164.7–218.0</td>
<td>0–10</td>
<td>0–10</td>
<td>8.0–8.2</td>
<td>530</td>
<td>4.0–4.5</td>
<td>1.41</td>
</tr>
</tbody>
</table>

*Average value of each stage.*
17°C and HRT 1.2–1.6 hr with its influent ammonia concentration around 400 mg N/L, which was the same as that in the start-up period. From the 1st to the 32nd day, the reactor maintained a relatively stable performance: the average removal rate and removal loading of NH₄⁺-N and TN were 85.25%, 1.17 kg N/(m³·day) and 71.11% and 1.00 kg N/(m³·day), respectively. During the following 7 days, the reactor was paused due to a fault in the power system. As soon as it re-operated, the reactor performance recovered rapidly, and within less than two weeks the ammonia removal rate increased from 76% to 86%; a similar increasing trend was showed in the TN removal rate, which climbed from 60% to 70% at the same time. It was concluded that the system presented a steady treatment performance when it was under reversible influences, which was significant to other relevant studies.

In Stage II (61–87 days), the feed ammonia concentration decreased to around 300 mg N/L. In order to maintain the influent ammonia load, we increased the influent discharge so as to reduce the HRT to 0.8–1.2 hr. The temperature in this stage, which changed from 15 to 18°C, fluctuated over a wider range than that in Stage I, so that it became an influencing parameter of the reactor in addition to the substrate concentration.

It can be observed from Fig. 2a and b that when other operation conditions were kept unchanged, the removal load significantly reduced. Analysis of this phenomenon suggested that the ammonia concentration in the initial part of Stage II encountered a sudden drawdown, causing a direct impact on AOB, which use NH₄⁺-N as substrate in the nitrification process. After that, adjusting the aeration led to the adaptation of AOB to the ammonia concentration of the system. The activity of AOB was thus recovered so that the removal loading increased rapidly and gradually became stable. In order to investigate whether or not the reactor could exhibit rapid adaptability to the variation of the influent substrate, the ammonia concentration was reverted back to the higher level of 325–361 mg N/L (day 71) after stably operating from the 64th to the 70th day. During this phase, the nitrogen removal performance of the reactor fluctuated and the TN removal rate fell to 58% at first, but then they recovered gradually and finally went back to the same removal efficiency as that in Stage I. In Stage II, the system achieved stability with an average ammonia removal efficiency of up to 82% with capacity of 1.44 kg N/(m³·day), and the TN removal was 69.1% at a rate of 1.23 kg N/(m³·day). This sufficiently demonstrated that the reactor had good capability under the higher influent ammonia concentration.

In Stage III (88–153 days), the NH₄⁺-N concentration fell further to 200 mg N/L and the HRT to 0.6–0.8 hr. This was in summer when the temperature rose from 15 to 23°C, and the average temperature was 21°C. The operation performance of the reactor, as shown in Fig. 2a and b, was similar to that in early Stage II, that is, the removal load experienced a sudden drop concomitantly with the
NIH + 1.5O2 → NO3− + 2H+ + H2O (1)

NH4+ + 1.32NO3− + 0.066HCO3− + 0.13H+ → 1.02N2 + 0.26NO3− + 0.066CH2O0.8N0.15 + 2.03H2O (2)

NO3− + 0.5O2 → NO3− (3)

When the ammonium concentration declined, which caused the subsequent reduction of NH4+ electron donors, the originally vulnerable bacteria NOB then competed for the electron acceptors of O2 and NO3− with the AOB and anammox bacteria. After the ammonium nitrogen was completely oxidized, the oxygen still remained and diffused to the inner biofilm. As a result, the bioactivity of the anammox bacteria was restrained, and the nitrite accumulated temporarily, indicating that decreased anammox and increased nitratation were responsible for a slight increase of the nitrite concentration in the system.

At this point, there would be no suitable microenvironment for NOB to survive when controlling the airflow to reduce the DO and increasing the water flow to ensure constant ammonia loading. Through adjusting the aeration, the activity of anammox bacteria would gradually recover, while the TN removal efficiency returned to a better level by degrees. Afterward, the reactor maintained a stable operation for several days, showing its stability and capacity against strong shock load.

2.2 Key operational parameters of the reactor

2.2.1 Effect of pH

The pH was affected by many factors in the CANON system: the process by which part of the ammonia nitrogen was converted to nitrite by AOB would consume alkalinity (Eq. (1)); subsequently, the residual alkalinity along with the generated nitrite would be converted to dinitrogen gas by the action of anaerobic ammonium oxidizers, which led to alkalinity production; furthermore, microbial metabolism would engender CO2 and lead to a reduction of the pH value; the stripping effect of CO2 would raise the pH value. The ultimate outcome obtained by these multiple factors was the reduction of pH during the reaction. The overall reaction could be described by Eq. (4) (Sliekers et al., 2002).

\[
\text{NH}_4^+ + 0.85\text{O}_2 \rightarrow 0.11\text{NO}_3^- + 0.44\text{N}_2 + 0.14\text{H}^+ + 1.43\text{H}_2\text{O}
\]

The influent pH in this experiment was adjusted to 8.0–8.4 by the addition of NaHCO3. The ΔpH (∆pH = pHfinal − pHinitial) varied with the changes of TN removal rate and the fluctuations of the curves were similar (Fig. 3).

In order to investigate the relationship between the two parameters mentioned above, the Statistical Program for Social Sciences software (SPSS 16.0), which is one of the most influential international major statistical software...
programs, was used for correlative analysis. Among the
three common correlation coefficient calculation methods
(Pearson, Spearman and Kendall), Pearson correlation was
chosen to analyze the relationships among the 122 pairs
of data. In statistics, the Pearson correlation coefficient
is used to measure the relationship between the two variables
X and Y (linear correlation) values in the range [–1,
+1], and the larger the absolute value of the correlation
coefficient, the closer the correlation coefficient is to 1 or
–1.

As the output results show in Table 2, it was determined
that ΔpH and the TN removal rate correlated with each
other significantly at the 0.01 level (bilateral) with Pearson
correlation coefficient of 0.480. Furthermore, the ΔpH in
this test fluctuated in a range of 0.2–0.8. After arranging
the entire set of data, we found that when ΔpH was
greater than 0.65, the corresponding average ammonia
removal rate and TN removal rate were greater than 85%
and 65% respectively. However, when ΔpH was less than
0.35, the associated treatment performance seemed worse
and more volatile. Overall, during the running process
of the CANON system, the performance of the reactor
could be judged by observing the changes of pH directly.
This means that a greater ΔpH could represent a better
performance for the reactor. Consequently, pH could be the
key operational parameter of the reactor.

2.2.2 Effect of alkalinity
The ammonia oxidation process consumes alkalinity. It
was proposed that alkalinity was correlated to the influent
nitrogen concentration and could influence the formation
of nitrite, especially with a high influent volumetric load-
ing rate. The activity of AOB would be limited and fail
in the competition for oxygen with NOB, resulting in the
production of nitrate nitrogen when alkalinity was in short
supply (Gaul et al., 2005). Moreover, there were also some
other processes consuming a small portion of the alkalinity
besides the aerobic ammonium oxidation reaction, such as
the metabolic activity of the microorganisms themselves,
the anaerobic ammonium oxidation reaction, the stripping
of CO2, and so on. Therefore, sufficient alkalinity was
needed to guarantee the good performance of the CANON
system.

Alkalinity in the test was adjusted by addition of
NaHCO3. The influent and effluent values of alkalinity
and Δalkalinity from 60th to 153rd day are shown in
Fig. 4. Similar to ΔpH, Δalkalinity could also reflect
the removal efficiency. In a given stage, the more ammonium
was oxidized, the more alkalinity was consumed, and the
fluctuation trend of the change of alkalinity was similar
to that of the TN removal rate curve. It could be concluded
that these two quantities had a significant correlation on the
0.01 level (bilateral) after finishing the correlation analysis
of 82 pairs of data involving Δalkalinity and TN removal
rate with SPSS software (Table 3).

As well as ΔpH, the change of alkalinity could also
indicate the performance of the reactor with a relatively
high Pearson correlation coefficient, but the complexity
of the determination of alkalinity and associated lag in
adjusting it was inevitable. Once the performance of the
reactor worsened, it would be difficult to observe and take

<table>
<thead>
<tr>
<th>Table 3 Correlation between Δalkalinity and TN removal effect</th>
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<tbody>
<tr>
<td>ΔAlkalinity</td>
</tr>
<tr>
<td>Pearson correlation</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>TN removal effect</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td>n</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
measures to deal with changes in alkalinity in time. However, with decreasing NH₄⁺-N concentration, Δalkalinity also showed a tendency to drop. Therefore, the removal efficiency of the reactor could not be judged by observing the change of the alkalinity quantitatively.

2.2.3 Effect of DO

In the CANON system, the concentration of DO produces a direct effect on the population and activity of ammonia oxidizers, nitrite oxidizers and anaerobic AOB and also restricts the conversion among ammonia, nitrite, nitrate and TN. The DO concentration should not only ensure a high removal rate of ammonia, but also maintain the activity of anammox bacteria in the inner layer of the biofilm so as to prevent the accumulation of nitrate and the reduction of TN. It was reported that the ammonia nitrogen conversion rate would achieve 90% or more with a low concentration of nitrate and nitrite in the effluent at a DO concentration of 0.5–0.7 mg N/L (Liao et al., 2005); all the ammonia nitrogen would be converted into nitrite and nitrate when DO was controlled at 5 mg N/L (Helmer et al., 2001).

DO was controlled by adjusting the aeration, and the effluent DO concentration of the reactor is shown in Fig. 5. It can be seen that the average value of the effluent DO was 6.5 mg N/L, which was greater than that inside the reactor, and also greater than values mentioned above. Actually, there are two main reasons for the higher effluent DO level: one is the remaining DO which was not consumed by microorganisms and the DO from the atmosphere; the other is that volcanic activity bio-ceramic packing materials with high inner porosity were used in the reactor, and they required much more DO for aerobic bacteria in high hydraulic loading and thus produced a thick outer layer of biofilm which could provide oxygen to the anammox bacteria in the inner layer. Thus the reactor could have good performance under high DO concentration.

With the previous analyses, the correlation of 80 pairs of data involving effluent DO and TN removal effect was also analyzed using SPSS software (Table 4). The statistical results showed that the two variables were uncorrelated; in other words, the effluent DO value could not reflect the operating performance of the bio-filter system properly. Besides, there was a difference between the effluent DO value and the actual value inside the reactor. Therefore the effluent DO value could not be the key parameter of the reactor.

Although DO was not a determining parameter of the CANON system, the removal of TN mainly relied on the process of anaerobic ammonium oxidation. Keeping a proper DO concentration was necessary during the research: excessive DO concentration caused by excessive aeration was not beneficial for the growth of anaerobic ammonium oxidizing bacteria, and could not suppress NOB growth; while a too small amount of aeration would decrease the activity of AOB, which would affect anaerobic ammonium oxidation with the substrate of nitrite by insufficient aerobic ammonium oxidation and less production of nitrite. Therefore, a more appropriate amount of aeration not only supplied a good living environment for AOB in the outer layer of the biofilm, but also contributed to the activity of anammox bacteria, thus ensuring the removal of TN.

2.3 Influence of temperature

At present, the CANON system has generally been operated successfully at 30–35°C (Third et al., 2001; Furukawa et al., 2006). According to the research reports, it was beneficial to the maintenance of a dynamic balance between aerobic ammonium oxidation and anaerobic ammonium oxidation when the temperature was controlled at 30°C (Villegas et al., 2011). By conducting an experiment on the CANON process in a sequencing batch biofilm reactor, it could be concluded that a reactor which was operated at 26–35°C could ensure a better TN removal efficiency, while the performance of the anammox reaction would be influenced at a temperature of 20°C and finally be affected seriously when the temperature dropped to 15°C (Zhang et al., 2009).

The influence of temperature on the CANON process lies mainly in the difficulty of achieving a stable accumulation of NO₂⁻-N, which is the matrix of the anaerobic ammonium oxidation reaction (Zhang et al., 2009). The temperature was not controlled in this experiment and changed with the changing ambient temperature, as shown in Fig. 6. Compared to other CANON systems, the oper-
ating temperature of this experiment was lower. However, there was little or no influence on the TN removal efficiency caused by the changing temperature. Compared with Stage II and III, the temperature in Stage I was relatively steady, while the temperature in Stage II fluctuated significantly. As a limiting factor, temperature played a leading role in the metabolic activity of microorganisms in Stage II, which led to some negative effects on the treatment performance of the reactor. In late Stage II, although the temperature was decreasing, the TN removal efficiency showed a rising trend. This could be explained by the fact that the adaptability of the microorganisms and other impact factors became dominant factors which determined the performance of the reactor rather than temperature at this point. The temperature in Stage III presented a slow rising trend, but the TN removal fluctuated to some extent and even appeared to drop at a few discrete points, which indicated that temperature was not a dominant factor and had no effect on TN removal. In the late stable period, the temperature rose to more than 20°C: then the ammonia nitrogen conversion rate and nitrogen removal efficiency increased with rising temperature. Therefore, higher temperature made a significant contribution to the nitrogen removal performance of the reactor.

As an abiotic factor, temperature, together with other ecological factors, had an effect on the microorganisms in the CANON system. At the same time, with the change of environment and microbial adaptability, the influence on the system due to any factor will also change in different periods. The reactor in this experiment operated at ambient temperature, which was 15–23°C throughout the study. The reactor produced a good adaptability to the temperature and the removal efficiency was also stable. Further microbiological studies need to be done on microorganisms that adapt to lower temperatures in the CANON system.

### 2.4 DGGE analysis

Biomass samples of different operating stages (day 30, 80 and 143) were taken for the DGGE analysis. The DGGE profile of the amoA gene and the PCR products of anammox’s 16S rDNA fragments are shown in Fig. 7.

Three samples were taken from the steady periods of Stage I, II, and III. There were differences in the number and intensity of the DGGE bands of AOB at different stages, which indicated that the community structure of AOB and the bacteria population of each community were diverse in different stages.

The amount of bands of Stage I was large, showing that with the influent ammonia concentration of 400 mg N/L, there existed abundant species of AOB; while with the reduction of the feed ammonia concentration, the bands of Stage II and Stage III became fewer and fewer, compared to Stage I. In addition, the community structure of AOB became relatively simple, which indicated that AOB that were suited to the high ammonia environment were eliminated as the ammonia concentration decreased, and the remaining bacteria were ones that could adapt to the environment of low ammonia concentration. The difference in concentration of influent ammonia nitrogen probably led to the reduction of the diversity of AOB, so that competition for ammonia nitrogen among the AOB communities emerged, which led to the elimination of parts of the AOB. The mutual bands belonging to the three stages represented those bacteria which could survive in all stages.

Compared with Fig. 6a, there were fewer bands in anammox bacteria DGGE profiles of 16S rDNA fragments in Fig. 6b; and with the decrease of the influent ammonia concentration, the profile seemed to have only small changes in the species abundance, which illustrated that in the inner biofilm of the CANON system, there were few kinds of anammox bacteria and a relatively stable community composition. The reduction of the feed ammonia concentration did not lead to a significant shift of the anammox species. The community composition of
anammox bacteria was more steady than that of AOB, which was due to the anammox bacteria having a lower growth rate and a longer growth period (μ = 0.0027 hr⁻¹, doubling time was 10.6 days). Consequently, it was hard to represent the diversity of the anammox bacteria through the shift with changing ammonia concentration. Anaerobic ammonia oxidation was more stable than the aerobic ammonia oxidation, which could explain why the CANON system exhibited good removal efficiency under the changing substrate conditions.

3 Conclusions

The upflow bio-filter CANON process had a strong capacity and stability for the treatment of wastewater with high ammonia (369.7–441.1 mg N/L), with TN removal efficiency and load reaching 71.1% and 1.00 kg N/(m²·day), respectively. When the influent substrate concentration was reduced, the performance of the operation could be stabilized by regulating the airflow and this illustrated that the CANON process could have good treatment performance in variable and lower substrate concentrations with the temperature varying between 15 and 23°C. Compared with alkalinity, pH could indicate the performance of the treatment for the reactor more straightforwardly, due to its sensitivity, accuracy, and convenience of measurement. DGGE profiles showed that there were some significant changes in the community structure of AOB, while the species of anammox bacteria were fewer and the community composition was relatively stable. That is to say, anaerobic ammonia oxidation was more stable than the aerobic ammonia oxidation reaction. For further development of the CANON process, research should be directed towards sustainable control of the process at lower substrate concentration, and deep exploration of the microorganisms involved will certainly help to improve the research.

Acknowledgments

This work was supported by the Trans-Century Training Program Foundation for Talents from the Ministry of Education of China (No. NCET-10-0008), the Open Project of State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (No. QAK201005), and the National Water Pollution Control and Management Technology Major Projects (No. 2012ZX07202-005).

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