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Nitrogen removal from coal gasification wastewater by activated carbon technologies combined with short-cut nitrogen removal process

Qian Zhao, Hongjun Han*, Baolin Hou, Haifeng Zhuang, Shengyong Jia, Fang Fang

State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China. E-mail: zhaqian027@sina.com

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Abstract: A system combining granular activated carbon and powdered activated carbon technologies along with shortcut biological nitrogen removal (GAC-PACT-SBNR) was developed to enhance total nitrogen (TN) removal for anaerobically treated coal gasification wastewater with less need for external carbon resources. The TN removal efficiency in SBNR was significantly improved by introducing the effluent from the GAC process into SBNR during the anoxic stage, with removal percentage increasing from 43.8%-49.6% to 68.8%-75.8%. However, the TN removal rate decreased with the progressive deterioration of GAC adsorption. After adding activated sludge to the GAC compartment, the granular carbon had a longer service-life and the demand for external carbon resources became lower. Eventually, the TN removal rate in SBNR was almost constant at approx. 43.3%, as compared to approx. 20.0% before seeding with sludge. In addition, the production of some alkalinity during the denitrification resulted in a net savings in alkalinity requirements for the nitrification reaction and refractory COD degradation by autotrophic bacteria in SBNR under oxic conditions. PACT showed excellent resilience to increasing organic loadings. The microbial community analysis revealed that the PACT had a greater variety of bacterial taxons and the dominant species associated with the three compartments were in good agreement with the removal of typical pollutants. The study demonstrated that pre-adsorption by the GAC-sludge process could be a technically and economically feasible method to enhance TN removal in CGW.

Key words: coal gasification wastewater; short-cut nitrogen removal; granular activated carbon; PCR-DGGE; denitrification enhancement

Introduction

The coal gasification wastewater (CGW) is similar to coking wastewater, but more recalcitrant due to its more complex composition and toxic components (Zhu et al., 2009; Wang et al., 2012). In a coal gasification plant located in the north of China (Yang et al.,

*Corresponding author. E-mail: han13946003379@163.com
2006), the chemical oxygen demand (COD) and total phenols (TP) of the crude gasification wastewater was as high as 20,000 mg/L and 5,000 mg/L, respectively. Nowadays, guided by the National Development and Reform Commission, coal chemistry plants in China must meet the standard of zero liquid discharge (Tong et al., 2010). Therefore, a series of physiochemical and biological processes are usually employed to treat the harsh CGW, including ammonia stripping, solvent extraction, anaerobic process, aerobic system and follow-up membrane and crystallization technologies.

Aside from the refractory COD, the removal of nitrogen in CGW is also very difficult. One reason for the low efficiency of total nitrogen (TN) removal has been attributed to the harmfulness and toxicity of CGW. Typical nitrification inhibitors, such as phenol, polynuclear aromatic hydrocarbons and nitrogen heterocyclic compounds resulted in insufficient nitrification (Amor et al., 2005; Li et al., 2011). Anaerobic and aerobic reactors (Wang et al., 2011; Wang et al., 2012) have been successfully applied prior to the biological nitrogen removal processes to remove refractory organics by increasing sludge concentration, extending hydraulic residence time (HRT) and dosing with media (Lai et al., 2008) or powdered activated carbon (Widjaja et al., 2004). Another reason lies in the inadequate carbon resources for denitrification. The COD/N value is only an apparent overall indicator; the stoichiometric parameter, i.e. the fraction of organic carbon available for denitrification, is in fact a more accurate measure of the denitrification potential of wastewater. Although CGW contains a high concentration of COD, the low BOD₅/COD indicates a low amount of readily-available electron donors for the denitrifying bacteria. Shortage of carbon resources for biological denitrification is a common phenomenon in wastewater treatment plants. To solve this problem, pre-denitrification is widely employed to directly use carbon sources in the influent for denitrification (Ma et al., 2009). Supplying an external carbon source is another common and necessary alternative. Simple organics, such as sodium acetate, ethanol, and especially methanol, due to their availability as products or semi-finished products in the coal chemical field, are suitable alternatives to improve denitrification of CGW (Li et al., 2011; Oskar et al., 2007; Peng et al., 2007). However, this has the disadvantage of elevating the operation cost. To solve this engineering challenge, the shortcut biological nitrogen removal reactor (SBNR), a process in which ammonia is partially oxidized to nitrite and then directly reduced to nitrogen, was found to be a suitable alternative due to its lower oxygen and carbon resource demand (Hellinga et al., 1998; Hwang et al., 2006). This process is often selected to remove total nitrogen from the anaerobic effluent (Cui et al., 2011; de Graaff 2010).
In our previous study, powdered activated carbon technology (PACT) was shown to be a desirable treatment between the anaerobic process and SBNR, owing to its dual role in COD removal and in reducing the inhibitory effect on SBNR (Zhao et al., 2013). Powdered activated carbon (PAC) significantly improved the biodegradability of CGW. It partly or completely adsorbed refractory compounds rather than simple organic compounds produced in the anaerobic pathway, such as a butanoic acid and pentanoic acid (Zhao et al., 2013). These easily-degradable substances were primarily consumed by the microbial organisms in the PACT tank with dissolved oxygen as the electron acceptor. In fact, these simple organics could serve as the carbon source to improve the denitrification in SBNR with nitrite as the electron acceptor. Therefore, in this study, a combined granular activated carbon-powdered activated carbon technologyShortcut biological nitrogen removal system (GAC-PACT-SBNR), in which effluent of the GAC process was introduced into the SBNR during the denitrification period, was developed to enhance the TN removal for anaerobically treated CGW. PAC rather than GAC was employed in the PACT tank because some GACs cannot be mixed completely and therefore would accumulate in the corner or below the aerator. Therefore, different activated carbons were used in different compartments. This study aims to investigate the feasibility of the combined system by analyzing its performance, microbial phase and operation expenditure.

1. Materials and methods

1.1 Process description

A schematic illustration of the combined system is shown in Fig. 1, where the dimensions of GAC, PACT and SBNR compartments are 6 cm × 12 cm × 15 cm, 12 cm × 12 cm × 14 cm, and 12 cm × 12 cm × 14 cm with corresponding working volumes 1.08 L, 2.02 L, and 2.03 L, respectively, and the working volumes for both settling tank 1 and settling tank 2 were 0.52 L.

The GAC process and PACT reactor were set up in an integrated reactor. Both had continuously stirred tanks. 150 mg GAC was dosed in the GAC compartment, resulting in a filling ratio of 25%. With a gentle stirring speed of 120 r/min, the GAC could be stable in the first compartment rather than being drained away with effluent. PAC dosage of 1 g/L, HRT of 24 hr, DO of 4-6 mg/L and SRT of 20 days were used for PACT, and HRT of 24 hr, DO of 1.5-2.5 mg/L, SRT of 15 days were employed for SBNR to ensure accumulation of ammonium-oxidizing bacteria (AOB) and suppression of nitrite oxidation bacteria (NOB). The GAC and PAC used in this study were the commercial carbon materials most commonly-used for wastewater treatment in China. The characteristics are shown in Table 1.
The sludge in settling tank 1 and 2 flowed back to the PACT and SBNR compartments respectively. PACT was operated at room temperature while SBNR was maintained at 32 ± 1 °C with an electrothermal heating wire running around the SBNR zone. The SBNR was operated with oxic and anoxic reaction conditions in the time ratio 3 hr: 1 hr. Gas chromatography-mass spectrometry (GC-MS) was utilized for the three samples above to evaluate the PAC adsorptive preference.

1.2 Experimental design

The performance of the combined system was evaluated in three phases: phase I, GAC-PACT-SBNR process ran until till the granular carbon showed little adsorption ability; phase II, after the granules was renewed, the SBNR was fed the effluent from GAC during the denitrification period, i.e. the anoxic reaction; phase III repeated phase II after the GAC was renewed and seeded with 1.0 g waste sludge from PACT. COD, NH$_3$-N, TN and TP were determined daily for the inlet and outlet of every compartment during phase I and III. COD and TP were assayed every 2 days during phase II. In addition, the BOD$_5$ was tested every 5 days. On the 17th day of phase III, the microorganism profile of each compartment was determined by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

1.3 Feed and seed

Seed sludge for PACT and SBNR was respectively obtained from the full-scale aerobic tank 1 (after the anaerobic process) and aerobic tank 2 (after aerobic tank 1) treating Lurgi CGW at China Coal Longhua Harbin Coal Chemical Industry Co., Ltd. Before running the combined system, the sludge had been cultured in the separated PACT and SBNR tanks for 7 months. The sludge in PACT was grey-black while the sludge in SBNR was light maroon with good settling properties. MLSS was controlled at 3568-4210 mg/L. The VSS/SS ratio of the seed sludge was around 0.70. The seed sludge for the GAC process was obtained from the PACT process.

The combined system was fed with synthetic wastewater containing crude phenol to simulate real CGW. The crude phenol obtained from the same company was a typical product of coal chemistry plants. It was actually a condensed mixture of phenols, aromatic hydrocarbons, alkanes, etc, with extremely high COD and low biodegradability. The proportion of crude phenol in the synthetic wastewater was 2% (V/V). The synthetic wastewater also contained acetate, phenol, NH$_4$Cl and macro-nutrients. The main characteristics are as following: COD 1328.8-1807.3 mg/L, BOD$_5$ 387.2-514.3 mg/L, NH$_3$-N 130.4-173.3 mg/L, TP 410.8-617.9 mg/L, bicarbonate alkalinity 10.0-15.1 mmol/L, pH 7.2-7.5,
MgSO$_4$$\cdot$7H$_2$O 50 mg/L, K$_2$HPO$_4$ 20 mg/L, CaCl$_2$$\cdot$2H$_2$O 20 mg/L, FeSO$_4$$\cdot$7H$_2$O 15 mg/L, KH$_2$PO$_4$ 10 mg/L and NaHCO$_3$ 25 mg/L.

1.4 PCR-DGGE

PCR-DGGE of the bacterial community was performed on 16S rRNA gene fragments. The DNA of the samples was extracted with a DNA extraction kit (purchased from Haigene Co. Ltd., Harbin, China). PCR was performed on the DNA extracts on a S1000™ Thermal Cycler (Bio-Rad Laboratories, Richmond, CA, USA) with primers against the V3-V5 region of the 16S rRNA gene of the bacterial groups. The nucleotide sequences of the primers were as follows: 338f-GC (5′-GC-Clamp [CGC CCG CCG GCG GCG GGC GGG GCG GGG GCA CGG GGGC]-ACT CCT ACG GGA GGC AGC AG-3′) and 518f (5′-ATT ACC GCG GCT GCT GG-3′) (Moura et al., 2007; Wang et al., 2007). The PCR amplification mixture consisted of 5 μL 10×Ex taq, 4 μL dNTP mixture (2.5 mmol/L each), 0.2 μmol/L of each primer, 1 μL of template DNA; the solution was made up to a volume of 50 μL with sterile water. The PCR amplification was performed at 98°C for 5 min, followed by 35 cycles of 94 °C for 45 sec, 56 °C for 45 sec, and 72 °C for 45 sec, and final single extension at 72 °C for 10 min. The DGGE analysis was conducted according to Muyzer et al. (1993) using a DCode system (Bio-Rad Laboratories, Hercules, CA, USA). 8% polyacrylamide gels having gradients of 40%-60% denaturant were used to run the gel electrophoresis. The 100% denaturing solution strength was defined as 7 mol/L urea and 40% formamide. The gel was run using TAE buffer for 12 hr at 60°C with 75 V and visualized by silver staining (Assam et al., 1991). The cluster and densitometric analysis of DGGE bands were performed using the GelCompar II software. The Shannon-Weaver biodiversity index was then determined based on analysis of the photograph using the BioRad Quantity One software. The Shannon-Weaver diversity index ($H$) was calculated using Eq. (1) (Zhou et al., 2010):

$$H = -\sum (pi) \log pi$$

(1)

where, $pi$ is the proportion of the $i$th phylotype.

The selected bands were eluted and a re-PCR was performed to sequence the amplicons. The sequencing was performed in the ABI 3730x1 DNA Analyzer with services provided by the State Key Laboratory of Urban Water Resource and Environment in Harbin Institute of Technology. To determine the phylogenetic affiliation, a similarity search was performed using the BLAST program. The nucleotide sequences were aligned with the CLUSTAL Xprogram and the phylogenetic trees were constructed with the Mega 4.0 program.
1.5 Analytical methods

COD, BOD₅, MLVSS, TP, ammonia and TN were determined in accordance with standard methods (AHPA et al. 1998). DO and pH values were determined daily with a pH meter and a DO meter (HACH 30d). It is noteworthy that value of MLVSS of PACT includes the mass of the powdered activated carbon.

2 Results and discussion

2.1 Performance of the combined system in phase I

Figure 2 shows the performance of the combined system in phase I. Initially, GAC exhibited excellent adsorption ability and achieved high COD and TP removal efficiency up to 57.9% and 92.3%, respectively. Accordingly, PACT was loaded with a relatively harmless and higher BOD₅/COD influent. Thus it was reasonable to obtain COD and TP removal efficiency of 75.7% and 98.8% in the PACT compartment. Around day 30, the active sites of GAC were exhausted and adsorption equilibrium was reached. Based on the amounts of COD loaded onto the granular carbon, the cumulative adsorption capacity of GAC was calculated as 0.5 g COD/g GAC. With progressive deterioration of GAC adsorption, the PACT compartment was substantially loaded with inlet to the combined system, resulting in the organic loading rate and TP loading rate increasing by more than a factor of two. However, the steady-state performance of PACT was marked by near constant effluent COD and TP values, with less than 5% fluctuation. Given that the MLVSS was 4620.2 mg/L, the average TP utilization rate during this period was calculated to be 67.0 ± 7.7 mg TP/ (g (VSS+AC)·d). Meanwhile, no ammonia removal was observed in the GAC process. A diminishing NH₃-N conversion rate was observed in the PACT, from 38.7% to 13.7%. In SBNR, up to 71.0%-92.7% NH₃-N was converted to NO₂-N while the TN removal efficiency was only 32.2%-49.6%, which suggested a severe suppression of denitrifying bacteria activity.

The MLSS in SBNR was 1540.5 mg/L less than that in PACT, indicating more biomass was produced in the PACT compartment. This could be attributed to the greater growth rate of heteroautotrophic bacteria in PACT than the nitrogen-related bacteria that predominated in SBNR. The microbial ecology within each compartment is elaborated in Section 2.4.

2.2 Comparison of nitrogen removal efficiency between phases I and II

In Phase II, GAC was renewed and the effluent from GAC was intermittently pumped into the SBNR reactor to favor the denitrifying bacteria and enhance the TN removal. Figure 3 shows the comparison of TN efficiency between phases I and II. The TN removal in SBNR displayed a strong dependency on the performance of GAC preceding it. During the first 16
days when GAC showed relatively high adsorption ability for TP and COD, up to 68.8%-75.8% of TN was removed in SBNR, compared to only 43.8%-49.6% during the same period in phase I.

**Table 2** shows the profile of COD and BOD in each compartment from day 1 to 16 in phase II. The COD and BOD concentration in the raw wastewater was 1563.5 mg/L and 498.9 mg/L, respectively. Hence the non-biodegradable COD was 1064.7 mg/L. After GAC adsorption, non-biodegradable COD and BOD were 474.7 mg/L and 398.7 mg/L. In contrast, the BOD in the PACT outlet was only 45.1 mg/L. Therefore, the TN removal could probably be explained by the nutrient substances in the stream introduced into the SBNR. In our previous study, GC-MS analysis revealed that the activated carbon preferentially absorbed aromatic compounds with complex molecule structure rather than simple organic acids, e.g. the intermediates produced from the anaerobic metabolism such as butanoic acid, pentanoic acid, propanoic acid (Zhao et al., 2013). These simple organics were quite beneficial for the biomass in SBNR. Not only can they provide an energy source for the denitrification reaction as the electron donor, but they might also play the role of a co-substrate to promote the degradation of the remaining recalcitrant substances. This fact was in good agreement with the excellent performance of SBNR, especially the remarkable increase in TN removal in phase II. Therefore, the GAC compartment of the reactor may act as a filter to remove all toxic and inhibitory materials in the feed, thus allowing the SBNR compartments to be loaded with a relatively harmless and readily-biodegradable influent. In this respect, the SBNR compartment would be more likely to support active populations of the relatively sensitive ammonia oxidizing bacteria and denitrifying bacteria. In addition, the production of some alkalinity during denitrification resulted in a net savings in alkalinity requirement for the nitrification reaction and refractory COD degradation by autotrophic bacteria in SBNR under anoxic conditions.

However, the limiting factor of activated carbon technology is the short service-life. With the progressive deterioration of GAC adsorption ability caused by chemical fouling, the TN removal efficiency in SBNR declined accordingly. On the 17th day of phase II, a sharp decrease in TN removal in SBNR was observed. Hence, the stream from GAC with COD > 920 mg/L and TP> 150 mg/L seriously suppressed the denitrifying bacteria activity (data not shown in **Fig. 3**). On the 30th day of phase II, the TN removal efficiency in SBNR decreased to the same level as that in phase II.

### 2.3 Bio-regeneration of GAC process

This part of the study aims to explore the possibility of extending GAC service-life via
addition of seed sludge to the GAC compartment, i.e. turning it into a GAC-sludge process. 

Figure 4 shows the contrast in GAC performance between phases II and III. In the GAC-sludge hybrid system, it took more days for the GAC to completely lose its adsorption capacity in phase III than that in phase II. Thus the adsorptive capacity of GAC rose more than the equilibrium adsorption in the presence of biomass that had already acclimated to CGW in PACT. It seemed that the GAC finally reached a balance between bioregeneration and adsorption after 45 days, with the COD and TP removal efficiencies staying at approx. 10% and 20%, respectively. Meanwhile, the TN removal efficiency in SBNR during the latter period of phase III (after day 45) was constant at approx. 43%, compared to 20% during the last 7 days in phase II. Although the denitrification was not highly improved in phase III, the replacement frequency for granular carbon decreased and the demand for external carbon resources became lower. Hence, it was feasible and practical to enhance denitrification with less carbon source by introducing the GAC outlet into SBNR and adding seed sludge to the GAC compartment.

After additional 1.0 g sludge was added to the GAC compartment, the GAC performance experienced slight fluctuation, and eventually the increase in performance from addition became insignificant. Therefore, increasing the amount of seed sludge added to GAC may not be effective in improving its performance. Eventually, the matrix reached the above-mentioned balance, with similar COD and TP removal efficiencies. Since no oxygen was supplied for the GAC process, the original aerobes died and the facultative aerobes dominated in the microorganism consortia, which was responsible for the bio-regeneration of spent granular carbon. This fact was verified by the SEM assay, which showed microorganisms residing in holes or gaps of GAC (SEM picture not shown). It was also consistent with the microbiology community characterization elaborated in Section 2.4.

The economic feasibility of GAC-PACT-SBNR was evaluated through a rough economical contrast with the case of supplying carbon resource. In a previous study, 75.54% of TN was removed with sodium acetate addition of 3164.55 g/m³ (Zhao et al., 2013). The sodium acetate was 1900 CNY/ton, so the cost of external carbon resource was 6.0 CNY/m³. The GAC replacement frequency in this study was approx. 1/20 day⁻¹ at inlet flow rate of 6.0 L/day. The GAC in China was approx. 4000 CNY/ton, so the cost of activated carbon consumption was 4.5 CNY/m³. The expense for the GAC would be even lower than this value because the spent adsorbent can be recycled after bio-regeneration. The energy consumption, i.e. electricity expenditure on pumps for the GAC stream in phase II, was negligible for a coal chemistry plant. The calculation demonstrated the pre-adsorption by GAC-sludge process was
an economically feasible alternative to enhance TN removal for CGW.

2.4 PCR-DGGE results

The microbial community analysis for different compartments is helpful in understanding the microbial reactions and their roles in pollutant removal. Figure 5 shows the DGGE profiles of the 16S rDNA gene fragments amplified from extracted DNA of the biomass obtained from PACT, SBNR, and GAC. Each band on the DGGE profile represents a specific species in the microbial community and the staining intensity of a band represents the relative abundance of the corresponding microbial species. Sludge samples of PACT showed the most complex profile with more visible bands, with Shannon-Weaver diversity indexes of 4.16, compared to 3.51 and 3.95 for GAC and SBNR, respectively. Thus PACT had a greater variety of bacterial taxons. Among the detectable bands in the DGGE profiles of the samples in the three compartments, bands 2, 3, 5, 13, 14 were common in the three samples. Bands 1 and 4 showed the highest intensity in the sample from the PACT compartment, and bands 6 and 10 appeared with highest intensity in the SBNR sample. Bands 7, 8, 9 and 15 showed high intensity in the samples from both PACT and SBNR. For the samples from GAC, the bands of highest intensity were bands 11 and 15.
Separated DGGE bands were excised from the gels and purified using the PCR Purification Kit. Then the excised bands were sequenced and each sequence was submitted to a BLAST search. The 16S rRNA gene sequences of the strains were deposited in the GenBank database under accession numbers of KF817580-KF817592. Results are shown in Table 3. The common bacterial species in the three compartments (bands 2, 3, 5, 13, 14) shared 97%-99% 16S rDNA sequence homology with Uncultured alphaproteobacterium, Clostridium sp. enrichment culture clone, Roseomonas sp. enrichment culture clone, Uncultured Firmicutes bacterium clone, and Bacillus sp. S110(3)-116S ribosomal RNA, respectively, indicating a relatively close phylogenetic relationship (Fig. 6). The unique bacterial species in PACT (bands 1 and 4) exhibited 98% and 91% similarity with the Uncultured Pseudomonas sp. clone and Uncultured Nitrosomonas sp. isolate DGGE gel band L20 (Fig. 6). Pseudomonas sp. has been proved to be the functional consortium for the degradation of aromatic compounds, such as p-cresol (Ho et al., 2010), phenol (Zhou et al., 2010; He et al., 2007) and quinoline (Carl and Fetzner, 2005; Sun et al., 2009). The detected species with high homology with Uncultured Nitrosomonas sp. indicated the role of nitrogen removal in PACT, even though it was much weaker than in the SBNR. In the SBNR, the bacterial species (band 6) showed 100% 16S rDNA sequence homology with the Nitrosomonas sp. Nm59, which was in good agreement with the nitrogen removal efficiency of SBNR. Nitrosomonas-related organisms as dominant population have previously been found in some anammox-like or CANON systems used for nitrogen removal (Liu et al., 2008, 2012). The dominant and unique bacteria species in GAC were of 97% and 98% 16S rDNA sequence homology with the Uncultured beta proteobacterium isolate DGGE gel band 45 and unidentified beta proteobacterium 16S rRNA gene, respectively (Fig. 6). The species might be affiliated with nitrosomonas or nitrospira members in the Betaproteobacteria class. DNA from dead bacteria was detected or it could be that the bacteria simply did not express their machinery for nitrogen removal. Another great likelihood was related with the facultative anaerobes and members of the Betaproteobacteria, which conferred the oxidation of aromatic compounds.

3 Conclusions

The GAC-PACT-SBNR combined system was developed to enhance the TN removal efficiency for anaerobically treated CGW. The TN removal in SBNR was significantly improved by introducing the effluent from GAC as carbon resource.
After the GAC was seeded with activated sludge, the granules had a longer service-life and the demand for external carbon resource in SBNR became lower. The PACT showed excellent resilience to the increasing organic loadings. Microbial community analysis showed that the dominant bacterial species in the three compartments were consistent with the removal of typical pollutants. The combined system could be an appropriate alternative to enhance TN removal for CGW.

Acknowledgements
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References


Table 1 Characteristics of GAC and PAC

<table>
<thead>
<tr>
<th>Items</th>
<th>GAC</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation method</td>
<td>Thermal</td>
<td>Thermal</td>
</tr>
<tr>
<td>Surface area (m²/g)</td>
<td>427.87</td>
<td>1237.99</td>
</tr>
<tr>
<td>Iodine number (mg/g)</td>
<td>&gt;600.00</td>
<td>&gt;700.00</td>
</tr>
<tr>
<td>Methylene blue adsorption (g/100 g)</td>
<td>20.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Total pore volume (cm³/g)</td>
<td>0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>Micro (Φ&lt; 2 nm)</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>Meso (Φ 1.7-30 nm)</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Macro (Φ&gt; 30 nm)</td>
<td>0.02</td>
<td>0.00</td>
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</table>

Table 2 Average COD and BOD of inlet and outlet of each unit on days 1-16, phase II

<table>
<thead>
<tr>
<th></th>
<th>Inlet</th>
<th>GAC outlet</th>
<th>PACT outlet</th>
<th>SBNR outlet</th>
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<tr>
<td>COD (mg/L)</td>
<td>1563.5</td>
<td>873.4</td>
<td>180.2</td>
<td>87.3</td>
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<tr>
<td>BOD₃ (mg/L)</td>
<td>498.9</td>
<td>398.7</td>
<td>45.1</td>
<td>5.5</td>
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</table>

Table 3 Characterization of isolated 16S rRNA gene fragments derived from PACT, SBNR, and GAC

<table>
<thead>
<tr>
<th>Band</th>
<th>GenBank accession numbers</th>
<th>Closest identification (GenBank accession numbers)</th>
<th>Homology (%)</th>
<th>Taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ᵃ</td>
<td>KF817580</td>
<td>Uncultured <em>ammonia-oxidizing</em> bacterium clone CL1-5/E 16S ribosomal RNA gene, partial sequence (DQ068706)</td>
<td>98%</td>
<td><em>Ammonia-oxidizing bacterium</em></td>
</tr>
<tr>
<td>2ᵇᶜ</td>
<td>——</td>
<td>Uncultured <em>alphaproteobacterium</em> (AJ867903)</td>
<td>99%</td>
<td><em>Proteobacteria</em></td>
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<tr>
<td>3ᵇᶜ</td>
<td>KF817581</td>
<td><em>Clostridium</em> sp. enrichment culture clone (JF312734)</td>
<td>99%</td>
<td><em>Clostridium</em></td>
</tr>
<tr>
<td>4ᵃ</td>
<td>KF817582</td>
<td>Uncultured <em>Pseudomonas</em> sp. clone (EU706076)</td>
<td>91%</td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td>5ᵇᶜ</td>
<td>KF817583</td>
<td><em>Roseomonas</em> sp. enrichment culture clone (HQ436503)</td>
<td>97%</td>
<td><em>Acetobacteraceae</em></td>
</tr>
<tr>
<td>6ᵇ</td>
<td>KF817584</td>
<td><em>Nitrosomonas</em> sp. Nm59 (AY123811)</td>
<td>100%</td>
<td><em>Nitrosomonas</em></td>
</tr>
<tr>
<td>7ᵇᶜ</td>
<td>KF817585</td>
<td>Uncultured bacterium clone NF089 (JX391706)</td>
<td>99%</td>
<td><em>Bacteria</em></td>
</tr>
<tr>
<td>Sequence</td>
<td>Accession</td>
<td>Description</td>
<td>Identity</td>
<td>Phylum</td>
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<td>-----------</td>
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</tr>
<tr>
<td>8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>KF817586</td>
<td>Uncultured <em>Synergistaceae</em> bacterium clone (HQ290315)</td>
<td>95%</td>
<td>Deferribacter</td>
</tr>
<tr>
<td>9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>KF817587</td>
<td>Uncultured <em>Clostridium</em> sp. isolate DGGE gel band FC-7 16S ribosomal RNA gene, partial sequence (GQ985493)</td>
<td>99%</td>
<td>Clostridium</td>
</tr>
<tr>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>KF817588</td>
<td>Uncultured <em>Tissierella</em> sp. (AB331495)</td>
<td>96%</td>
<td>Micromonas</td>
</tr>
<tr>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>KF817589</td>
<td>Uncultured <em>beta proteobacterium</em> isolate DGGE gel band 45 (EF075376)</td>
<td>97%</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>12&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>——</td>
<td>Uncultured <em>Firmicutes</em> bacterium clone (HQ444234)</td>
<td>96%</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>13&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>KF817590</td>
<td><em>Bacillus</em> sp. S110(3)-116S ribosomal RNA (GU136567)</td>
<td>98%</td>
<td>Bacillus</td>
</tr>
<tr>
<td>14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>KF817591</td>
<td><em>Trichococcus flocculiformis</em> strain (JF505984)</td>
<td>99%</td>
<td>Trichococcus</td>
</tr>
<tr>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>KF817592</td>
<td>Unidentified <em>beta proteobacterium</em> 16S rRNA gene (AJ003767)</td>
<td>98%</td>
<td>Betaproteobacteria</td>
</tr>
</tbody>
</table>

<sup>a</sup> sample from the PACT compartment; <sup>b</sup> sample from the SBNR; <sup>c</sup> sample from the GAC process.

Sequences 2 and 12 were not submitted to the NCBI system.
**List of Figure Captions**

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**Fig. 2** COD, TP, NH3-N removal efficiencies of the combined system in phase I

**Fig. 3** Comparison of nitrogen removal by SBNR between phase I and II

**Fig. 4** Comparison of COD, TP and TN removal efficiencies between phase II and III

**Fig. 5** Profiles of PCR-DGGE analyses of the bacterial community structure in 3 compartments: PACT (A), GAC process (B) and SBNR (C)

**Fig. 6** Phylogenetic analysis of fifteen 16S rDNA bands obtained from combined system...

Phylogenetic tree determined by maximum likelihood analysis of the sequences of PCR-amplified V3-V5 region in 16S rDNA. GenBank accession numbers are shown in brackets.

a, sample from the PACT compartment; b, sample from the SBNR; c, sample from the GAC process.

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