Simultaneous nitrification and denitrification (SND) bioaugmentation with *Pseudomonas* sp. HJ3 inoculated for enhancing phenol and nitrogen removal in coal gasification wastewater

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**ABSTRACT**

A simultaneous nitrification and denitrification (SND) bioaugmentation system with *Pseudomonas* sp. HJ3 inoculated was established to explore the potential of simultaneous phenol and nitrogen removal in coal gasification wastewater (CGW). When the concentration of influent chemical oxygen demand (COD) and total phenols (TPh) was 1,765.94 ± 27.43 mg/L and 289.55 ± 10.32 mg/L, the average removal efficiency of COD and TPh at the stable operating stage reached 64.07% ± 0.76% and 74.91% ± 0.33%, respectively. Meanwhile, the average removal efficiency of NH₄⁻N and total nitrogen (TN) reached 67.96% ± 0.17% and 57.95% ± 0.12%, respectively. The maximum SND efficiency reached 83.51%. Furthermore, SND bioaugmentation performed with good nitrification tolerance of phenol shock load and significantly reduced toxic inhibition of organisms. Additionally, the microbial community analysis indicated that *Pseudomonas* sp. HJ3 was the predominant bacterium in the SND bioaugmentation system. Moreover, the indigenous nitrogen removal bacteria such as *Thauera*, *Acidovorax* and *Stenotrophomonas* were enriched, which further enhanced the nitrogen removal in the SND bioaugmentation system. The results demonstrated the promising application of SND bioaugmentation for enhancing simultaneous phenol and nitrogen removal in CGW treatment.

**Key words** | bioaugmentation, biotoxicity, coal gasification wastewater, phenol degradation, *Pseudomonas* sp. HJ3, simultaneous nitrification and denitrification

**INTRODUCTION**

Coal gasification wastewater (CGW) is a typical refractory industrial wastewater with high concentration of phenolic compounds, heterocyclic and polycyclic aromatic hydrocarbons, long chain alkanes and ammonia (Jia et al. 2016). Among these compounds, phenolic compounds are the main organic pollutants, accounting for about 30%–60% of the chemical oxygen demand (COD) content (Wang et al. 2014). In addition to phenolic compounds, ammonia is also one of the worst contaminants, resulting in a negative effect on CGW treatment (Zhao et al. 2015). Ammonia is usually treated by the conventional anoxic–oxic (A/O) nitrogen removal process, which requires nitrifying bacteria and denitrifying bacteria working in separate strictly aerobic and anaerobic conditions, thus increasing process complexity and energy consumption (Liu et al. 2015). What is more, the nitrification process is excessively inhibited by a variety of toxic refractory compounds (Kulkarni 2012; Zhao et al. 2014). Additionally, inadequate biodegradable carbon sources for denitrifying bacteria also lead to a low total nitrogen (TN) removal efficiency (Li et al. 2011). Therefore, enhanced nitrogen removal is of great importance for CGW treatment, as well as the elimination of toxic compounds to inhibit nitrification and denitrification.

Fortunately, simultaneous nitrification and denitrification (SND) is highlighted to be an attractive nitrogen removal strategy in industrial wastewater treatment (Herrero & Stuckey 2015). Recently, some bacteria have been reported to be capable of heterotrophic nitrification and aerobic denitrification, which can convert ammonia aerobically into nitrogen gas using organic substrates as carbon...
source and energy (Yang et al. 2018), such as Pseudomonas stutzeri (Zhu et al. 2014), Paracoccus versutus (Zhang et al. 2015), Alcaligenes sp. TB (Chen et al. 2016), Zobellella taiwanensis (Lei et al. 2016) and so on. These bacteria can simultaneously achieve nitrification and denitrification in a micro-oxygen reactor. Lu et al. (2017) isolated a heterotrophic nitrifying–denitrifying bacterium Serratia sp. LJ-1 that performs simultaneous ammonia and nitrate removal in aerobic conditions. Moreover, Serratia sp. LJ-1 presents much higher tolerance to phenol toxicity than that of the autotrophic nitrifying bacteria. Additionally, some heterotrophic nitrifying–denitrifying bacteria have 40% reduction of carbon source demand and 63% improvement of biological nitrogen removal efficiency (Yoo et al. 1999). Based on the above advantages, it is feasible to build a bioaugmentation system using high-efficiency SND bacteria for enhancing organics and nitrogen removal in a variety of complex industrial wastewaters. Compared with traditional biological nitrogen removal processes, the SND bioaugmentation system has many advantages: (i) nitrification and denitrification can take place simultaneously in the aeration tank, so it is unnecessary to construct additional advanced treatment systems for organics removal (Yang et al. 2018); (ii) the resistance capability to fluctuations can be greatly improved because the maximum growth rates of SND bacteria are higher than those of autotrophic nitrifying bacteria (Chen et al. 2015). Presently, efficient bioaugmentation processes have been achieved by immobilizing specific functional microorganisms on various types of support material, such as polyurethane foam cubes (Du et al. 2017a), mycelial pellets (Ma et al. 2015a) or microbial cellulose (Yu et al. 2011), thereby achieving an excellent removal ability for organic contaminants and nitrogen in situ. However, limited studies have explored the potential capability of SND bioaugmentation for simultaneous phenol and nitrogen removal in CGW treatment (Zhao et al. 2014; Ma et al. 2017).

In this study, an SND bioaugmentation system with heterotrophic nitrifying–aerobic denitrifying bacterium Pseudomonas sp. HJ3 inoculated was established to evaluate the potential capability of simultaneous phenol and nitrogen removal in CGW. Meanwhile, phenol tolerance and biotoxicity reduction of the SND bioaugmentation system were assessed. Moreover, the interaction between Pseudomonas sp. HJ3 and the indigenous microbial community was analyzed. Based on above results, the study aims to provide useful guidance for practical application of bioaugmentation technology for enhancing phenol and nitrogen removal in CGW treatment.

MATERIALS AND METHODS

Wastewater and inoculum

The wastewater in this study was obtained from a full-scale CGW treatment plant located in Inner Mongolia, China. This wastewater had been pretreated by phenol and ammonia recovery processes. In addition, trace elements were added into the wastewater to provide a balanced feed for microbial growth (Wang et al. 2011). The main characteristics of the wastewater were as follows: COD of 1,600–1,800 mg/L, total phenol (TPh) of 250–300 mg/L, NH₄-N of 170–200 mg/L, TN of 200–240 mg/L, pH of 7.0–7.2.

The activated sludge was obtained from an aerobic tank located at a full-scale CGW treatment plant in Inner Mongolia, China. The ratio of mixed liquor volatile suspended solids (MLVSS) and mixed liquor suspended solids (MLSS) was around 0.72. Pseudomonas sp. HJ3 strain was isolated and characterized with heterotrophic nitrification and aerobic denitrification efficiency in a laboratory study. The cell morphology and a neighbor-joining tree of strain HJ3 are shown in Figure S1 (Supplement). The 16S rDNA nucleotide sequence of strain HJ3 has been submitted to GenBank nucleotide sequence databases with the accession number KY441413. Pseudomonas sp. HJ3 strain was inoculated in 200 mL Luria Bertani (LB) medium and then incubated for seven days in a rotary shaker at 120 rpm and 30 °C for further experimental study.

The polyurethane filler was prepared with a density of 0.008–0.015 g/cm³, surface area of 3,000 cm²/g, pore size of 2.0–2.5 mm and porosity >96%.

SND performance assessment of Pseudomonas sp. HJ3

The Luria Bertani medium for consortium enrichment contained 10.00 g/L of tryptone, 3.00 g/L of beef extract, 10.00 g/L of NaCl, at pH 7.2. The heterotrophic nitrification medium (HNM) for the heterotrophic nitrification study contained 7.90 g/L of Na₂HPO₄, 1.50 g/L of KH₂PO₄, 0.10 g/L of MgSO₄·7H₂O, 0.20 g/L of NH₄Cl, 0.20 g/L of phenol as sole carbon source, trace element solution 2.00 mL, at pH 7.2. The denitrification medium (DM1/DM2) for the aerobic denitrification study contained 7.90 g/L of Na₂HPO₄, 1.50 g/L of KH₂PO₄, 0.10 g/L of MgSO₄·7H₂O, 0.36 g/L of KNO₃ (0.27 g/L of KNO₂), 0.20 g/L of phenol as sole carbon source, trace element solution 2.00 mL, at pH 7.2. All solid mediums were prepared with addition of 1.5% agar. All the above mediums were sterilized for 20 min at 0.11 MPa, 121 °C.
The SND bioaugmentation reactor setup and operation

The SND bioaugmentation reactor made of a polyvinyl chloride (PVC) column with a working volume of 2.5 L was established. R1 was inoculated with 50% (V0/V) of pre-cultured Pseudomonas sp. HJ3 suspension and activated sludge with MLSS of 3,000 mg/L. Polyurethane fillers were added into R1 for steady biofilm formation, which was also conducive to immobilizing the Pseudomonas sp. HJ3 strain in the reactor. Meanwhile, R2 was seeded with the same proportion of polyurethane fillers and activated sludge without Pseudomonas sp. HJ3 inoculated as a control under the identical operating conditions. The bacterial suspension, activated sludge and polyurethane fillers in the two reactors were suspended and mixed by a mixing device (JJ-1A, Skyray Instrument Co., Ltd, China) and a micro-pore aeration device (YXLY, LANXESS Co., Ltd, Germany). Dissolved oxygen (DO) concentration range was controlled at 0.5–1.0 mg/L using the micro-pore aeration device. Sludge residence time (SRT) was controlled at 25 d. The two reactors were operated with two cycles a day, of which one cycle of 12 h consisted of 10 min for feeding, 630 min for aeration, 60 min for settling, and 20 min for withdrawal. Considering that high-strength CGW might lead to a destabilization of the SND bioaugmentation system, stepwise increase of influent COD load was adopted at the initial operating stage. When influent COD and NH4+-N reached about 1,800 mg/L and 200 mg/L, respectively, the concentration of COD and NH4+-N in the effluent decreased significantly and a biofilm was formed on the surface of the fillers, indicating that the reactor had achieved stable operation. The temperature was maintained at 25–27 °C. All samples were periodically taken and centrifuged at 8,000 rpm to measure the concentration of COD, TPh, NH4+-N, NO3-N, NO2-N and TN. All of the above data were measured in triplicate. The SND efficiency was calculated as follows:

$$\eta_{SND} = \left(1 - \frac{\text{NO}_x^- \text{produced}}{\text{NH}_4^+ \text{removal}}\right) \times 100\%$$

where NOx produced is production of NO2-N and NO3-N, and NH4+ removal is decrease of NH4+-N (Third et al. 2003).

Phenol shock load study

At the stable operating stage, R1 and R2 were operated at the influent phenol concentration of about 500 mg/L. This loading was continued for four days before switching back to normal loading. Then the influent phenol concentration was further increased to about 1,000 mg/L for four days. TPh and NH4+-N concentrations were analyzed during the shock load stage.

Biototoxicity assessment

The raw CGW was assessed at five dilution ratios (5%, 10%, 20%, 30%, 40%), and the treated effluent of R1 and R2 was assessed at five dilution ratios (10%, 25%, 50%, 75%, 100%). A measure of 0.2 mL of pre-cultured Tetrahymena thermophila (T. thermophila) cells (cell density of about 1 x 10^5 cells/mL) were put into the 96-well microplate, then 0.8 mL of a diluted water sample or negative control (culture medium) was added. The T. thermophila was originally obtained from the Chinese Academy of Sciences (Beijing, China). Each diluted water sample and negative control was conducted in three replicates. After 24 h exposure, the cell density of T. thermophila was determined at 490 nm using a microplate luminometer (ELx800, BioTek, USA). The EC50 value was calculated by logistic fit using Origin 8.5 software (Origin Lab, USA). The toxic unit (TU) was calculated as the reciprocal of EC50 to reflect the toxicity level.

Microbial community analysis

The biofilm samples were collected from R1 and R2 at the stable operating stage (65 d) to analyze the microbial community via high-throughput sequencing. Total DNA was extracted using a DNA extraction kit (E.Z.N.A. Mag-Bind Soil DNA Kit) according to the manufacturer’s instructions. The extracted DNA was polymerase chain reaction (PCR)-amplified using the V3–V4 region of 16S rDNA gene universal primers with 341F (CCTACGGGAGGCAGCAG) and 805R (GACTACHVGGGTATCTAATCC). The PCR procedure was conducted as described by Jia et al. (2015). The obtained PCR products were sequenced using MiSeq Illumina by Sangon Biotech Co., Ltd (Shanghai, China). The resulting sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold.
Analytical methods

The concentrations of COD, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and TN were determined according to Standard Methods (APHA 1998). TPh concentration was determined using the Folin phenol spectrophotometry method. DO concentration was measured with an oxygen meter (LDO10103, HACH, USA) and pH value was determined with a pH meter (pHS-3C, Leici, China).

RESULTS AND DISCUSSION

The SND properties of *Pseudomonas* sp. HJ3 strain

As plotted in Figure 1(a), phenol and ammonia were degraded significantly by *Pseudomonas* sp. HJ3 at 48 h. Phenol removal efficiency reached 90.12% ± 0.14%. Meanwhile 90.26% ± 0.09% of NH$_4^+$-N was removed with a maximum removal rate of about 2.10 mg/L/h, which was lower than that of *Pseudomonas stutzeri* YG-24 (8.75 mg/L/h) and *Zobellella taiwanensis* DN-7 (17.3 mg/L/h) (Li *et al.* 2015; Lei *et al.* 2016). Because the carbon source used in these studies is citrate and succinate, they are more easily utilized by cells than phenol, which can accelerate cell growth rate and result in a higher NH$_4^+$-N removal rate. Furthermore, the nitrate (DM1) and nitrite (DM2) were respectively used as sole nitrogen source to evaluate the aerobic denitrification ability of strain HJ3. As shown in Figure 1(b) and 1(c), phenol as denitrification carbon source was significantly degraded in the aerobic denitrification process, which performed with a similar trend to the heterotrophic nitrification process. As shown in Figure 1(b), NO$_3^-$-N removal efficiency was about 73.38% ± 0.11%. The corresponding maximum NO$_3^-$-N removal rate reached about 1.51 mg/L/h, which was similar with that of *Acinetobacter junii* YB (Yang *et al.* 2015). Meanwhile, nitrite was slightly accumulated at 9–21 h, and then decreased gradually to 0.47 ± 0.09 mg/L at 30 h. When nitrite was sole nitrogen source, the NO$_2^-$-N removal efficiency was 50.26% ± 0.18% (Figure 1(c)) and the corresponding maximum removal rate was about 0.98 mg/L/h. Meanwhile, no obvious accumulation
of nitrate was detected. The above results illustrate that *Pseudomonas* sp. HJ3 could utilize phenol as the carbon source for the heterotrophic nitrification and aerobic denitrification processes.

**Phenol and nitrogen removal performances in SND bioaugmentation system**

As shown in Figure 2, R1 and R2 had similar behavior with regards to COD, TPh, NH$_4^+$-N and TN removals at the influent COD concentration of 545.81 ± 17.12 mg/L. The average removal efficiencies of COD, TPh, NH$_4^+$-N and TN in R1 (0–20 d) were 58.20% ± 1.03%, 64.86% ± 0.54%, 53.40% ± 0.27% and 44.11% ± 0.38%, respectively. This indicated that *Pseudomonas* sp. HJ3 was not effective because the biofilm attached to the filler was not mature during the start-up stage. With COD concentration increasing to 1,120.33 ± 24.65 mg/L, the concentrations of effluent COD, TPh, NH$_4^+$-N and TN gradually decreased in R1 (21–40 d), and the corresponding average removal efficiency reached 60.54% ± 0.32%, 70.79% ± 0.54%, 54.10% ± 0.13% and 46.79% ± 0.61%, respectively. In addition, COD and TPh average removal efficiency in R2 was maintained at 52.51% ± 0.15% and 58.58% ± 0.76%, respectively. However, NH$_4^+$-N and TN average removal efficiencies were only 42.92% ± 0.24% and 5.64% ± 0.45%, respectively. It has been reported that the activities of nitrifying and denitrifying bacteria are inhibited due to the toxicity of high-concentration refractory compounds (Joo et al. 2006; Kim et al. 2008). When influent COD and TPh concentrations increased to 1,765.94 ± 27.43 mg/L and 289.55 ± 10.32 mg/L, COD and TPh average removal efficiency in R1 in the stable operating stage (55–70 d) reached 64.07% ± 0.76% and 74.91% ± 0.33%, respectively. Meanwhile, the average removal efficiency of NH$_4^+$-N in R1 reached 67.96% ± 0.17% at the stable operating stage, which was increased by about 44.09% over that of R2. This suggested that R1 with *Pseudomonas* sp. HJ3 bioaugmentation could enhance phenol and other

![Figure 2](https://iwaponline.com/wst/article-pdf/80/8/1512/641500/wst080081512.pdf)
organics degradation in CGW treatment. Moreover, the removal of ammonia by heterotrophic nitrifying-aerobic denitrifying bacteria and the utilization of the organic carbon source were coupled. It was reported that electrons generated by the degradation of phenolic compounds can be transferred to ammonia monooxygenase to simultaneously remove ammonia. It is worth noting that an obviously higher TN removal efficiency (57.95% ± 0.12%) was achieved in R1, while only 4.62% ± 0.11% of TN was removed in R2, indicating that in R2 without Pseudomonas sp. HJ3 inoculated it was difficult to achieve the denitrification process. In the bioaugmentation system, bacteria are attached to the fillers to form a stable biofilm, which supplies a long residence time to retain large amounts of the inoculated strain in the reactor (Du et al. 2017b). Moreover, fillers can provide a gradient change of DO concentration from the outside to inner spaces, which can provide an anoxic microenvironment for denitrification (Körner & Zumft 1989). Accordingly, the SND bioaugmentation system could significantly strengthen simultaneous phenol and nitrogen removal in CGW treatment.

**Nitrogen transformation and SND efficiency in SND bioaugmentation system**

In order to further explore the SND mechanism in the bioaugmentation system, nitrogen transformation and SND efficiency were investigated. As plotted in Figure 3, NH$_4^+$-N was significantly removed with a removal efficiency of 70.26% ± 0.13% in R1. Meanwhile, NO$_2^-$-N and NO$_3^-$-N as the nitrification intermediates increased, but gradually decreased after 8 h. The reason for the accumulation and consumption of nitrification products might be that the ammonia was sufficient at the bacterial rapid growth stage, so Pseudomonas sp. HJ3 performed with stronger heterotrophic nitrification than aerobic denitrification, which resulted in the accumulation of nitrification products. With the decrease of ammonia concentration, the aerobic denitrification was stronger than the heterotrophic nitrification, so that the nitrification products were reduced. Moreover, SND efficiency reached 83.51%, indicating that NH$_4^+$-N was effectively reduced to nitrogen gas in the SND bioaugmentation system. By contrast, the removal efficiency of NH$_4^+$-N in R2 was only 38.32% ± 0.09%, and 19.76 ± 3.96 mg/L of NO$_2^-$-N and 40.34 ± 4.31 mg/L of NO$_3^-$-N were accumulated, resulting in an SND efficiency of only 17.79%. Evidently, the lower SND efficiency indicated that nitrogen removal in R2 possibly depended on microbial assimilation or filler absorption rather than denitrification. Moreover, the large accumulation of nitrification products may aggravate the potential toxicity to surrounding bacteria (Bancroft et al. 1979; Hao et al. 2016).

**Nitrification tolerance characteristics under high phenol shock load**

CGW is subjected to a high variability of phenol concentration due to fluctuations in the pre-treatment, which is known to inhibit nitrification (Fang et al. 2013). To evaluate the nitrification tolerance of the SND bioaugmentation system to phenol toxicity, the characteristic of nitrification efficiency was analyzed under phenol shock loads. As shown in Figure 4, when influent phenol concentration was increased from 300 ± 12.25 mg/L to 500 ± 14.32 mg/L, the performance of R1 was obviously disturbed. The removal
efficiency of phenols decreased from 75.70% ± 1.03% to 58.08% ± 0.31%. However, the system rapidly recovered to the initial level as the influent phenol concentration decreased to about 320 ± 16.40 mg/L. Meanwhile, NH₄⁺-N removal efficiency was 58.41% ± 0.26% at a phenol concentration of 500 ± 14.37 mg/L, and gradually recovered to 74.21% ± 0.87% as the phenol concentration decreased to 320 ± 16.40 mg/L within 4 d. When influent phenol concentration was further increased to 1,000 ± 24.65 mg/L, the removal efficiency of NH₄⁺-N in R1 rapidly decreased to 47.91% ± 1.01% and gradually recovered to 60.51% ± 0.56% with decreasing phenol concentration. By contrast, remarkable reductions of NH₄⁺-N and phenol removal efficiency were observed in R2. When phenol concentration increased to 1,000 ± 24.65 mg/L, the removal efficiency of NH₄⁺-N significantly decreased and could not be recovered to the initial level. Obviously, R2 performed with higher sensitivity to phenol shocking. These results indicated that the SND bioaugmentation system performed with tremendous resistance and recovery capacity to phenol shocking, thereby resulting in an excellent performance for ammonia removal. In previous reports, the nitrification process by autotrophic nitrification bacteria could easily be inhibited at phenol concentrations as low as 10 mg/L (Pagga et al. 2006), while Pseudomonas sp. HJ3 was capable of heterotrophic nitrification and aerobic denitrification performed with higher phenol tolerance, which was favorable for the improvement of nitrification.

**Biotoxicity reduction of CGW in SND bioaugmentation system**

The results of the acute toxicity of influent and treated effluents of R1 and R2 are depicted in Table 1. The regression

![Graphs showing phenol and NH₄⁺-N concentrations over time in R1 and R2.](image-url)
T. thermophila exposed to raw influent and treated effluents from R1 and R2

<table>
<thead>
<tr>
<th>Sample</th>
<th>24 h EC50 (%)</th>
<th>R²</th>
<th>TU</th>
<th>Toxicity class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>7.31</td>
<td>0.9978</td>
<td>13.68</td>
<td>Class IV high acute toxicity</td>
</tr>
<tr>
<td>Effluent</td>
<td>R1 61.44</td>
<td>0.9922</td>
<td>1.63</td>
<td>Class III acute toxicity</td>
</tr>
<tr>
<td></td>
<td>R2 30.09</td>
<td>0.9997</td>
<td>3.32</td>
<td>Class III acute toxicity</td>
</tr>
</tbody>
</table>

analysis showed the $R^2$ value for each sample was higher than 0.99, indicating that *T. thermophila* was a suitable model organism for biotoxicity assessment of CGW. As shown in Table 1, the 24 h EC50 value of the influent reached 7.31%. The acute TU value of the influent was 13.68, which could be classified as Class IV (high acute toxicity) according to the toxicity classification (Persoone et al. 2005). Apparently, CGW performs with strong acute toxicity to *T. thermophila* because of the high concentration of organic contaminants and ammonia that exist (Zheng et al. 2018). This indicates that the high toxicity of CGW has a negative effect on nitrogen removal. In contrast, the biotoxicity of CGW was significantly decreased through SND bioaugmentation treatment. As shown in Table 1, the 24 h EC50 value of the R1 effluent was 61.44%, indicating that this effluent performed with slight toxic inhibition of *T. thermophila*. The acute TU value of the R1 effluent was only 1.63, which was effectively reduced by 88.08% compared with that of raw CGW. This suggests that a reduction in toxicity could be achieved in the SND bioaugmentation system. However, the effluent from R2 performed with relatively higher toxic effect on *T. thermophila*, and the corresponding 24 h EC50 value and acute TU value were 30.09% and 3.32, respectively. This suggests that many toxic compounds were not degraded completely in R2, thus leading to a higher toxicity than the R1 effluent. Apparently, the SND bioaugmentation system significantly decreased the toxic inhibition of organisms owing to the efficient phenol and nitrogen removal in CGW.

Overall analysis of microbial community in SND bioaugmentation system

The diversity and richness of the microbial community

The species coverage and diversity of biofilm samples in R1 and R2 at the stable operating stage (65 d) were analyzed by high-throughput sequencing. As shown in Table 2, good coverage was found in R1 (0.9987) and R2 (0.9969). This indicates that the sequencing results reliably reflect the characteristics of the microbial communities. The ACE index and Chao1 index are commonly used to evaluate the richness of species in a microbial community. The two indexes of R1 are lower than that of R2, indicating that microbial richness decreased slightly after inoculation of *Pseudomonas* sp. HJ3. The Shannon diversity index not only presents the simple species richness, but also describes the abundance of each species distribution. The Shannon index of R1 is 5.96, which is also lower than that of R2. The possible reason for relatively lower richness and diversity of microbial community in the SND bioaugmentation system is that inoculated *Pseudomonas* sp. HJ3 was predominant, which resulted in the elimination of other bacteria in competition.

Bacterial community characteristics in SND augmentation system

In order to explore the diversification of the bacterial community, the microbial communities in an SND bioaugmentation system and a control without *Pseudomonas* sp. HJ3 inoculated were analyzed. As shown in Figure 5, Proteobacteria was the largest phylum in R1 and R2 with relative abundances of 70.53% and 75.41%, respectively. Bacteroidetes and Firmicutes were the secondary dominant phyla in R1 and R2 (data not shown). It has been reported that Proteobacteria and Bacteroidetes are closely correlated with nitrification and denitrification (Chen et al. 2018). Firmicutes are helpful for degradation of organic pollutants and tolerance to extreme conditions (Fang et al. 2017). Obviously, there was no significant difference ($P > 0.05$) at phylum level between R1 and R2.

However, the bacterial communities at the genus level in R1 and R2 were distinctively different. The detailed description of bacterial community composition at genus level is summarized in Figure 5. The relative abundance of *Pseudomonas* in R1 was 18.74%, which was obviously higher than that in R2, attributed to inoculation of the *Pseudomonas* sp. HJ3 strain suspension. As shown in Figure 6, although the relative abundance of *Pseudomonas* sp. HJ3 gradually decreased during the operating process, its relative abundance was maintained at 14.85% in R1 at 65 d, which
was significantly higher than that in R2. This indicates that *Pseudomonas* sp. HJ3 was the dominant strain in the SND bioaugmentation system, thereby facilitating the improvement of SND efficiency in the bioaugmentation system. Additionally, *Thauera* (12.47%), *Acidovorax* (13.85%) and *Stenotrophomonas* (7.96%) were the relatively higher abundance genera in R1 (Figure 5), which played a vital role in the nitrification and denitrification processes.

Based on previous findings, *Thauera* are the main functional genus responsible for nitrogen removal and organic-compound degradation (Du *et al.* 2017b). *Acidovorax* are the common bacteria in wastewater treatment systems, which have been reported to be a novel aerobic denitrifying bacteria genus (Mergaert *et al.* 2001; Shen *et al.* 2013). In addition, the relative abundance of *Zoogloea* in R1 was 4.57%, which was significantly higher than that in R2. It has been found that *Zoogloea* species are favorable to the forming and holding of a biofilm (Gao *et al.* 2014). In contrast, *Acinetobacter* (21.46%), *Comamonas* (9.21%) and *Allicytophilus* (5.70%) were dominant bacteria in R2, which are found to be capable of degrading phenolic compounds and polycyclic aromatic hydrocarbons in a wastewater treatment system (Ma *et al.* 2015b; Qu *et al.* 2015). However, indigenous nitrogen removal bacteria such as *Thauera* and *Acidovorax* accounted for only a small proportion in R2, which was a possible reason for the lower nitrogen removal efficiency in R2. It has been reported that a bioaugmentation system can change inoculated bacteria abundance and reform the indigenous microbial community structure (Zhao *et al.* 2016; Zhang *et al.* 2018). On the one hand, the enrichment of indigenous nitrogen removal bacteria might compete with *Pseudomonas* sp. HJ3, resulting
in a decrease in the abundance of *Pseudomonas* sp. HJ3. On the other hand, SND bioaugmentation with *Pseudomonas* sp. HJ3 inoculated would stimulate the growth of indigenous nitrogen removal bacteria, thereby offering a large biological nitrogen removal potential. Therefore, the interaction of *Pseudomonas* sp. HJ3 with indigenous nitrogen removal bacteria enhanced the nitrogen removal in the SND bioaugmentation system.

**CONCLUSION**

The SND bioaugmentation system with *Pseudomonas* sp. HJ3 inoculated achieved efficient simultaneous removal of phenols and nitrogen in CGW. The corresponding average removal efficiencies of COD, TPh, NH₄⁻N and TN at the stable operating stage reached 64.07% ± 0.76%, 74.91% ± 0.33%, 67.96% ± 0.17% and 57.95% ± 0.12%, when the concentration of influent COD, TPh and NH₄⁻N was 1,765.94 ± 27.43 mg/L, 289.55 ± 10.32 mg/L and 187.25 ± 14.07 mg/L, respectively. Meanwhile, the maximum SND efficiency reached 85.51%. Moreover, the SND bioaugmentation reactor exhibited a better capability to resist fluctuations in influent phenol loading and reduce the toxic inhibition of organisms. Additionally, SND bioaugmentation with *Pseudomonas* sp. HJ3 inoculated altered the microbial community structure and established a new bacterial community balance in situ for enhancing nitrogen removal.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2019.399.

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