Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: Operating performance and bacterial community

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HIGHLIGHTS

• A 3-D biofilm-electrode reactor was used for wastewater denitrification.
• A nitrate removal of 98.3% was obtained with C/N ratio of 3.0 and HRT of 7 h.
• Both heterotrophic and autotrophic bacteria were responsible for nitrate removal.
• A phylogenetic tree of gene sequences in biofilm was established.
• The biofilm was abundant with Thauera-like and Enterobacter-like bacteria.

ABSTRACT

A three-dimensional biofilm-electrode reactor (3D-BER) was applied for nitrate removal from simulated municipal wastewater treatment plant (WWTP) effluent. It was found that when the influent C/N ratio ranged from 1.0 to 2.0, both heterotrophic and autotrophic denitrifying microorganisms played important roles in nitrate removal. The extension of hydraulic retention time (HRT) could enhance nitrate removal, but too long HRT was not necessary. A phylogenetic tree of gene sequences in biofilm was established, and the biofilm was abundant with Thauera-like and Enterobacter-like bacteria. The results illustrated that 3D-BER is a feasible and effective technology for the denitrification of WWTP effluent with poor organic carbon source. A nitrate removal of 98.3% was obtained with C/N ratio of 3.0 and HRT of 7 h. About 85.0–90.0% of nitrate removal was found at a C/N ratio of 1.5 and HRT of 10 h due to cooperative heterotrophic and autotrophic denitrification.

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1. Introduction

The shortage of water resources has become an exacerbating issue around the world as a result of accelerated industrialization and urban growth as well as changed climatic conditions. Among various measures of addressing this challenging problem, wastewater reclamation has received tremendous attention (Yi et al., 2011). The reclaimed water from municipal wastewater treatment plant (WWTP) can serve for many purposes, such as urban scenic and landscape greening, urban lake and river water replenishment, car washing, construction site water use, urban road sprinkling, industrial circulating cooling water use, irrigation water use, and the recharge of groundwater (Hao et al., 2013). As a result, the reclaimed water represents an indispensable part of water resources. For example, the utilization of reclaimed water in Beijing in Northern China (i.e. a region with serious water shortage problem) reached 0.65 and 0.68 billion m$^3$ in 2009 and 2011, accounting for about 18% and 20% of its total annual water consumption volume, respectively (Wei et al., 2011). In general, the quality of reclaimed water is affected by the available treatment processes whereas many pollutants could not be thoroughly removed. In particular, WWTP effluent may still contain a relatively high concentration of inorganic nitrogen such as nitrate and nitrite. For example, a total nitrogen (TN) concentration (mostly nitrate) of 15–40 mg/L in effluent was observed in many WWTPs in Beijing (Li et al., 2011). The discharge of nitrogen components into the environment through wastewater reclamation can lead to a number of public health and environmental concerns. These include the eutrophication of lakes and rivers, excessive vegetative growth and reduced fruit set of crops, decreased groundwater quality, and
other hazards (e.g., disease of methemoglobinemia in the fetus) for human and animal health (Chafari et al., 2008). As a result, the effective denitrification of WWTP effluent is of fundamental importance.

Biological denitrification has received the greatest interests among various processes since the bacteria can decompose nitrate into harmless nitrogen gas with the presence of an electron donor (Karanasios et al., 2010). However, WWTP effluent usually contains low concentration of chemical oxygen demand (COD) with little organic carbon source or internal electron donor left for bacteria to utilize, and it is thus necessary to use an external source as electron donor for denitrification. This problem can be addressed by using a biofilm-electrode reactor (BER) which produces hydrogen gas (H₂) through water electrolysis, while the denitrifying microorganisms immobilized on the cathode surface directly contact the hydrogen gas and use it as an electron donor (Zhao et al., 2012). If the anode of BER is made of graphite, CO₂ can also be generated to serve as an inorganic carbon source for microorganisms and a pH buffer in the denitrification system (Mousavi et al., 2012). Many studies of using BERs have been reported. For example, Park et al. (2005) applied a BER with a dimensionally stable anode (DSA) and a graphite felt cathode to treat an aqueous solution containing high concentrations of nitrate, and they observed a maximum nitrate removal efficiency of 98% at an applied current of 200 mA, corresponding to a nitrate removal of 0.17 mg NO₃⁻ N/(cm² of biofilm surface area day). Li et al. (2010) used Fe as a cathode and Ti/ IrO₂-Pt as an anode in an undivided cell to treat nitrate contaminated wastewater, and they found that the nitrate concentration decreased from 100.0 to 7.2 mg/L within 180 min with the presence of NaCl at 0.50 g/L, without the formation of ammonia and nitrite by-products. Feng et al. (2013) and Huang et al. (2013) used rectangular graphite electrodes in a cylindrical reactor to investigate the effects of different carbon sources and C/N ratio on the denitrification efficiency, and they found that starch could be utilized by microbes to generate electrons, while the increase of C/N ratio from 2.0 to 3.5 resulted in an increase of nitrate removal from 0.69 ± 0.02 to 1.09 ± 0.16 mg/L/h.

In general, the BER denitrification process can be affected by many operating parameters, such as temperature, nitrate concentration, carbon source, salinity, pH, hydraulic retention time (HRT), electrode materials, reactor configuration, and current intensity (Zhao et al., 2011). In conventional BER (with two electrodes), the cathode not only provides hydrogen for autotrophic denitrifying bacteria as their electron donor, but also is used as the carrier for microorganisms. As a result, its nitrate removal capacity can be restricted by the surface area of cathode. Moreover, the denitrification can be affected by limited inorganic carbon source in BER (Zhao et al., 2011). Consequently, the BER is usually associated with a relatively low denitrification rate and requires a long hydraulic retention time. To address this problem, a number of efficient and economical alternatives of BER configuration have been proposed. These include the combined electrochemical reactor and biological reactor, and multi-electrode reactor or three-dimensional biofilm-electrode reactor (3D-BER) (Mousavi et al., 2012). In 3D-BER, activated carbon (AC) or AC mixed with another substance is filled in the cathode part as a third bipolar electrode. This third electrode (i.e., AC filler) provides a high surface area for biomass growth and attachment as well as increases hydrogen gas yield. It also produces more CO₂ to provide a desirable anoxic condition where less organic carbon source is required to remove nitrate (Zhou et al., 2007). For example, Zhou et al. (2009) used PbO₂ as an anode and activated carbon fiber (ACF) as a cathode to treat nitrate-polluted groundwater, while the anode and cathode were set in the center and surrounding the inner wall of the reactor, respectively. The conventional BER (or 2D-BER) was converted to a 3D-BER when activated carbon (AC) was added and packed in the reactor, and they found that the 3D-BER’s nitrate removal was increased from 57.93% to 78.10% as compared to 2D-BER at a HRT of 4 h, while the nitrate removal was further increased to 99.80% at a HRT of 8 h (Zhou et al., 2009). The 3D-BER was also applied to oxidize pollutants from industrial solid waste landfill leachate (Rao et al., 2009), remove acid orange 7 in simulated wastewater (Zhao et al., 2010), and remove NOx from flue gas (Zhou et al., 2012).

The novel 3D-BER technology is still in its early development stage with only a few denitrification studies being reported in literatures, and even very few studies were found for nitrate removal from WWTP effluent. In addition, the denitrification performance of BER can be greatly affected by the involved microorganisms. However, previous studies on both conventional BER and 3D-BER technologies have mainly focused on reactor design (e.g., electrode materials and reactor configurations) and the effects of various operating factors. The information of involved denitrifying bacterial community as well as the interaction between bacteria community and reactor operating conditions are still unclear (Mousavi et al., 2012; Feng et al., 2013). In fact, the understanding of microbial structure and diversity during denitrification process is of great importance for optimizing BER performance. The development of new molecular biology techniques such as the 16S rRNA-based methods and other methods like scanning electron microscopy (SEM) can make it possible to characterize and evaluate the bacterial community in BER (Park et al., 2006; Cho et al., 2008). Such techniques have been applied to characterize the microbial communities in many biological treatment studies of wastewater (Osaka et al., 2008; Srinandan et al., 2012). As described above, the 3D-BER can be a promising choice for achieving a higher denitrification rate and a shorter HRT due to the reactor’s unique configurations. It is thus of importance to extend its application to WWTP effluent treatment. The objective of this study was then to investigate the performance of a 3D-BER for removing nitrate from simulated WWTP effluent under various C/N ratio and HRT conditions. The graphite cathode and anode were used, and the activated carbon (AC) was filled in the reactor to form a third electrode. The bacterial community in the biofilm developed on AC filler was characterized through the analysis of 16S rDNA. Such results can provide valuable information for designing more cost-effective denitrifying process for wastewater reclamation.

2. Methods
2.1. Experimental apparatus and simulated wastewater

Fig. 1 shows the schematic diagram of experimental setup and the 3D-BER configuration. The reactor made of Plexiglas was cylindrical with a diameter of 15 cm and a height of 56 cm. The cathode consisting of six graphite rods (diameter of 2 cm and height of 42 cm) was set surrounding the inner wall of the reactor, while the graphite rods were linked using insulated electrical copper wires. The anode was a graphite rod (diameter of 3 cm and height of 42 cm) and was set in the center of the reactor. The remaining space in the reactor was filled with granular activated carbon (AC) with particle size of 3–5 mm and a total volume of 6 L. The reactor had an effective volume of 3.4 L. Prior to use, the AC was washed with H₂SO₄ solution (0.02 M) and deionized water for several times and was then dried at 105 °C for 24 h (Zhou et al., 2009). The AC filler can serve not only as a biofilm carrier but also as the third electrode. A DC regulated power supply (model HSPY-60-2 provided by Beijing Hansheng Puyuan Technology Co., Ltd. in China, 0–60 V, 0–2 A) was used to provide constant current for water electrolysis.

2.2. Experimental conditions

The experimental conditions were as follows: the influent nitrate concentration was 0.50 mg/L, the influent pH was 7.0 ± 0.2, the hydraulic retention time (HRT) was 8 h, the temperature was 25 ± 2 °C, and the current density was 200 mA/cm². The influent flow was 45 mL/min. The reactor was operated for 24 h, and the effluent was continuously sampled and analyzed.

2.3. Analytical methods

The nitrate concentration was analyzed using ion chromatography. The pH was measured using a pH meter. The conductivity was measured using a conductivity meter. The voltages were measured using a digital multimeter. The current was measured using a current meter. The temperature was measured using a thermometer. The current density was calculated as the ratio of current to the effective area of the cathode. The HRT was calculated as the ratio of the reactor volume to the inflow rate. The cathode potential was measured as the difference between the cathode and anode potentials. The voltages were measured as the difference between the cathode and anode potentials.
was added into concentration of 334–373 mg/L. Such nutrient-containing
K2/C0 was measured using a COD analyzer (model 5B-6C provided
is the initial concentration of nitrate or total nitrogen (mg/
synthetic wastewater of KNO3 was added to a plastic tank (12 L). A
through adaptation, enrichment, immobilization and acclimatiza-
trate-reducing microorganisms. The immobilization of denitrifying
K NO3 was a simulated WWTP effluent prepared by tap water amended
PO4 concentration of 0.3 mg/L being obtained. The phosphorus was provided by KH2-
P O4, with a PO43−P concentration of 0.3 mg/L being obtained. The
CH3COONa was added to provide carbon source according to de-
required C/N ratios, and no microelements were added since they were
available in tap water. About 20–30 L of synthetic wastewater was
prepared every 2 days, and its pH was adjusted to about 7.0 using
HCl and NaOH every morning on each day. Such simulated WWTP
effluent was put in the 60-L influent tank, and its pH was adjusted once everyday to about
7.0 with KH2PO4 and K2HPO4. The wastewater was pumped from
the influent tank to flow through the 3D-BER, and was then
drained to the laboratory drainage system. To make microorgan-
isms adapt to the environment, electric current in the 3D-BER
was gradually changing from 0 to 20, 20 to 40, and 40 to 60 mA,
with each current level lasting for about 1 week. The influent and
effluent were run continuously, and the hydraulic retention time
(HRT) was set at 7 h. This phase lasted for about 1 month, and
the effluent and influent concentration of nitrate were also moni-
tored. After this phase, more than 90% of nitrate was steadily re-
moved. A thick layer of dark-brown biofilm was visibly seen to
cover the AC filler, and the effluent of 3D-BER was also seen with
no suspended sludge, indicating that the biofilm had been well
formed. All bacterial inoculation and acclimation were carried
out during winter time at a room temperature of 10–15 °C.

After biofilm immobilization and aclimatization, the 3D-BER
system was operated under different experimental conditions of
C/N ratio and HRT using simulated WWTP effluent as described
in Section 2.1. The simulated WWTP effluent was put in the 60-L
influent tank, and was pumped to flow through the 3D-BER, and
then flew out of the reactor and was discharged to the laboratory
drainage system. A constant current of 40 mA was applied for all
the normal operation experiments. The C/N ratio was tested for
0.5, 1.0, 1.5, 2.0, and 3.0, and the HRT was tested for 5, 7, 10, and
12 h, respectively. The operational conditions (C/N and HRT) for
each run were maintained until steady nitrate removal for at least
6 days. At every 24 h interval, 50 mL of sample was taken from the
reactor effluent for nitrate and nitrite concentration analysis,
each sample was taken for at least two successive HRTs.

2.3. Analytical methods

On a UV–Visible spectrophotometer (Shimadzu, Japan), the ni-
trate (NO3−N), nitrite (NO2−N), and total nitrogen (TN) in water
samples were measured using ultraviolet spectrophotometry,
spectrophotometry based on N-[1-naphthyl]ethylenediamine
dihydrochloride, and ultraviolet spectrophotometry based on alka-
line potassium persulfate digestion, respectively (SEPA, 2002).
CODc was measured using a COD analyzer (model 5B-6C provided
by Lianhua Technology Co., Ltd., Beijing, China). pH was measured
using a pH meter (model E-201-9 provided by Shanghai Yoke
Instrument Co., Ltd., China). The temperature of water sample
was measured using a thermometer that was fixed on the wall of
the 3D-BER reactor. The removal of nitrate or total nitrogen (η)
was calculated using the following formula:

$$
\eta = \frac{C_0 - C_t}{C_0} \times 100\%
$$

where C0 is the initial concentration of nitrate or total nitrogen (mg/L), and Ct (mg/L) is the remaining concentration of nitrate or total
nitrogen at time t.

2.4. Microbiological analysis

Bacterial community in the biofilm formed on the AC filler in
3D-BER was analyzed by DNA-based molecular techniques. About
20 mL of AC filler sample was taken from the 3D-BER after
2 months of biofilm immobilization and aclimatization as well as
normal operation when its operating condition was C/N = 1.0,
was put in the centrifuge for centrifugation at 6000 rpm for 5 min, and the supernatant was removed after centrifugation. The left biofilm sample in the bottom of CT was added with about 7 mL of ultrapure water and was centrifuged again at 6000 rpm for 5 min, and the supernatant was removed. Such washing through centrifugation was conducted for three times. The washed biofilm was then dehydrated with 7 mL of 30%, 50%, 70%, 80%, and 90% ethanol (static dehydration for 10 min for each concentration of ethanol), respectively. The biofilm was finally dehydrated three times with about 7 mL of 100% ethanol (15 min for each time). At last, 7 mL of ethanol and isomyl acetate mixture (100% ethanol:isomyl acetate = 1:1) was poured into the CT staying for 15 min. Pure isomyl acetate was then added for substitution for 15 min. The settled sample was put on a filter paper (10–15 μm) and was dried with a vacuum dryer (Alpha 1–2 LD plus, CHRIST) for 24 h at a pre-cooling temperature of −10 °C. The dried sample was then potted with a 10–20 nm gold layer (Puig et al., 2011). The coated sample was examined with a SEM (HITACHIS-4300, Hitachi), and the images were captured digitally.

3. Results and discussions

3.1. Effect of C/N ratio on denitrification performance

Fig. 2a and b present the variations of average nitrogen concentration and nitrogen removal under different C/N ratio conditions during the 3D-BER treatment process (with HRT = 7 h), while the nitrate (NO₃⁻–N) concentration in the influent was kept as a relatively constant value (i.e. 30 mg/L). The experiment for each C/N ratio was conducted for at least 6 days. It was observed that the C/N ratio greatly affected the accumulation of nitrite and the removal of nitrogen, with low C/N ratio beneficial for nitrite formation. It was observed that the concentration of NO₃⁻–N in the effluent of 3D-BER decreased from 16.69 to 12.15, 12.15 to 8.55, and 8.55 to 0.51 mg/L when the C/N ratio increased from 0.5 to 1.0, 1.0 to 2.0, and 2.0 to 3.0, respectively. Similarly, the concentration of nitrite (NO₂⁻–N) in the effluent decreased from 2.35 to 1.78, 1.78 to 1.39, and 1.39 to 0.09 mg/L when the C/N ratio increased from 0.5 to 1.0, 1.0 to 2.0, and 2.0 to 3.0, respectively. In general, denitrification efficiency increased with C/N ratio, and a high C/N ratio of 3.0 significantly enhanced the removal of nitrogen and the restriction of nitrite accumulation. The removal efficiency of nitrate increased from 48.9% to 62.2% when the C/N ratio increased from 0.5 to 1.0, and a relatively stable nitrate removal efficiency of around 71.7% was maintained for C/N ratio ranging from 1.5 to 2.0. However, a very high nitrate removal of 98.3% was obtained when the C/N ratio increased from 2.0 to 3.0. The removal efficiency of total nitrogen was lower than that of nitrate due to the formation of nitrite during the treatment process. The results obtained in this study were in agreement with results reported in previous studies of BER treatment which indicated that high nitrate removal efficiency (>95%) could be achieved when organic carbon was abundant such as C/N > 2 (Zhao et al., 2012).

The denitrification effect can also be described using the ratio of ΔC/ΔN, where ΔC and ΔN represent the difference of CODcr, and total nitrogen (TN) between the 3D-BER influent and effluent, respectively. As a result, ΔC/ΔN ratio can be used to represent the approximate COD consumption per unit mass of nitrate removal. Fig. 2c presents the variation of ΔC/ΔN ratio under different influent C/N conditions. The heterotrophic denitrification theoretically requires 2.86 g of COD to remove 1 g of NO₃⁻–N, although the actual ΔC/ΔN ratio for complete nitrate removal is usually above 3.0 due to the presence of oxygen and cell synthesis of microorganisms (Lee et al., 2001). In this study, sodium acetate (CH₃COONa) was added into the simulated WWTF effluent as organic carbon
It was observed from Fig. 2c that $\Delta C/\Delta N$ increased from 0.77 to 2.72 when the influent C/N increased from 0.5 to 3.0. All of these $\Delta C/\Delta N$ ratios are below the theoretical value of 2.86 for complete nitrate removal by heterotrophic denitrifying microorganisms. This may indicate that both heterotrophic and autotrophic denitrification microorganisms existed during the 3D-BER treatment process. A high C/N ratio can accelerate the growth of heterotrophic denitrifying bacteria and thus promote the total denitrification rate (Zhao et al., 2012). A study by Pei et al. (2010) found that a wetland based biological denitrification removed 70% of NO$_3^-$-N at a C/N ratio of 7.0, but a complete removal of NO$_3^-$-N and NO$_2^-$-N was achieved at a C/N ratio of 8. In this study, when the C/N ratio was 3.0, a $\Delta C/\Delta N$ ratio of 2.72 was achieved, and the nitrate removal and total nitrogen removal reached 98.3% and 98.0%, respectively, while the nitrite production was minimal. As a result, the heterotrophic denitrification may have played a major role at a relatively high C/N ratio when using the 3D-BER process. However, when the organic carbon content was gradually decreasing in the reactor, the heterotrophic denitrifying bacteria could be gradually domesticated into autotrophic denitrifying bacteria, and the original autotrophic denitrifying bacteria could gradually become the main microorganisms for nitrate removal. The autotrophic microorganisms usually need a long adaptive phase with slow growth, and thus may lead to a lower denitrification rate (Zhao et al., 2011). For example, when the C/N ratio was 0.5, a $\Delta C/\Delta N$ ratio of 0.77 was achieved, and the nitrate removal of only 48.9% was obtained, with a considerable amount of nitrate being accumulated. Thus, the autotrophic microorganisms may have played a major role at a very low C/N ratio (i.e. 0.5). When the influent C/N ratio ranged from 1.0 to 2.0, both heterotrophic and autotrophic denitrification microorganisms may play important roles in nitrate removal when using the 3D-BER, with a less considerable amount of nitrite being generated (Zhao et al., 2012). For example, when the C/N ratio was 1.0, 1.5, and 2.0, a $\Delta C/\Delta N$ ratio of 0.99, 1.49, and 2.21 was achieved, and the average nitrate removal of 62.2%, 70.4%, and 72.9% was obtained, respectively. The nitrate removal was nearly stable especially for C/N ratio of 1.5 and 2.0. Such cooperative heterotrophic and autotrophic denitrification in 3D-BER system can lead to significant advantages (e.g., high nitrogen removal efficiency and thus larger treatment capacity) as compared with only autotrophic denitrification (Zhao et al., 2011).

Fig. 2. Impact of C/N ratio on the denitrification effect of 3D-BER (experimental condition: flow rate = 504 mL/h; HRT = 7 h; $T = 19 \pm 2^\circ C$; influent pH = 7.0; $I = 40$ mA).

Fig. 3. Impact of hydraulic retention time (HRT) on nitrogen removal in 3D-BER (experimental conditions: C/N = 1.5; $T = 24 \pm 2^\circ C$; influent pH = 7.0; $I = 40$ mA.)
3.2. Effect of HRT on denitrification performance

It was found from Fig. 2a that an influent C/N ratio of 1.5 was associated with a relatively satisfactory denitrification performance (i.e. nitrate and total nitrogen removal of 70.4% and 65.2%). This C/N ratio was then used to examine the effect of HRT on the denitrification performance of 3D-BER, and Fig. 3 presents the results. The experiment for each HRT was conducted for at least 8 days. It was observed that the nitrate and total nitrogen removal reached 75.9% and 70.5% at a HRT of 7 h and a temperature of 24 ± 2 °C, respectively. However, the corresponding removal was 70.4% and 65.22% (Fig. 2a) at a HRT of 7 h and a temperature of 19 ± 2 °C, respectively. Such difference might mainly be caused by temperature variation during different experimental period, with higher temperature promoting nitrate removal. It was found from Fig. 3 that HRT could greatly affect the removal of nitrogen and the accumulation of nitrite. At low HRT condition such as HRT = 5 h, a relatively lower denitrification rate (e.g., nitrate and total nitrogen removal of 66.0% and 54.6%) was achieved, and a NO3−/N and NO2−N concentration of above 10 and 3.5–4 mg/L was observed in the effluent of 3D-BER, respectively. The denitrification rate increased with HRT, but it reached a maximum value (e.g., average nitrate removal of 88.4%, ranging from 85.0% to 90.0%) when the HRT was 10 h. Further increase of HRT beyond 10 h resulted in the decrease of denitrification rate. Longer HRT (i.e. 10–12 h) also led to less accumulation of nitrite in the 3D-BER effluent (e.g., less than 1 mg/L). In the environment with relatively poor organic carbon source (i.e. C/N = 1.5), both heterotrophic and autotrophic bacterial activities may be important, but the autotrophic bacteria need more time to degrade nitrogen due to their relatively slow growth. As a result, a longer HRT could facilitate the nitrogen removal by such denitrifying bacteria and result in a lower nitrite accumulation. However, in the continuous running mode of 3D-BER, too long HRT under relatively poor organic carbon source condition could result in the endogenous respiration phase of heterotrophic bacteria (i.e. a phase when bacteria live with nutrition in their bacterial body) for a long time. This would result in the reduction of heterotrophic bacteria and thus the poor denitrification effect, such as the reduced nitrate and total nitrogen removal of 79.0% and 76.0% at HRT = 12 h as shown in Fig.3. Consequently, when using 3D-BER for treating WWTP effluent with poor organic carbon source, the extension of HRT could enhance the nitrogen removal and restrict the nitrite accumulation, but too long HRT is not necessary due to the cooperative heterotrophic and autotrophic denitrification mechanisms.

3.3. Bacterial community in 3D-BER

The analysis of 16S rDNA gene clones was conducted to assess the bacterial community in the biofilm of 3D-BER. A total of 102 clones were obtained and sequenced without dividing operational taxonomic units (OTUs). Table 1 lists the phylogenetic groups of all of the analyzed bacterial clones. It can be found that the 3D-BER biofilm was dominated by Proteobacteria phylum in which the β- and γ-Proteobacteria were the most abundant, accounting for 61.77% and 30.39% of the total clones, respectively. Only a small percentage (4.90%) of the total clones were related to other Gram-positive phyla of Firmicutes and Actinobacteria which were usually detected in anaerobic sludge digester and methanogenic inclusions (Ndez et al., 2008).

Proteobacteria was found to be dominant in many sulfur-based chemolithotrophic denitrification bioreactor studies (Ndez et al., 2008). Park et al. (2006) reported that Proteobacteria and flavobacteria were the dominant bacteria in a conventional biofilm-electrode reactor where the β-Proteobacteria played key roles in the denitrification performance of biofilm reactor. However, the flavobacteria was not detected in the biofilm of 3D-BER in this study. It was reported that β-Proteobacteria bacteria tend to survive in the anaerobic environment utilizing organic nutrients, while some of them may also utilize hydrogen, ammonia, methane and volatile fatty acids (Xia et al., 2010). Thus, the anaerobic operating environment in 3D-BER may facilitate the survival of β-Proteobacteria in the biofilm (i.e. 61.77% of the total clones). The β-Proteobacteria detected in the biofilm sample was further subdivided into classes and genera as shown in Fig. 4. A significant microbial diversity in the biofilm was observed. Fig. 4a shows that 57.15% of the total clones related to β-Proteobacteria were pertaining to the family Rhodocyclaceae class (i.e. the genera of Thauera, Sulfuritalea and Azospira). Among them, 83.0% of the total clones belonged to genus Thauera, which have been reported in many denitrifying bioreactor studies (Osaka et al., 2008; Daniel et al., 2009). Mao, 2009 examined 8 Thauera bacteria (Thauera aminooaromatica, Thauera linalooliis, Thauera phenylacetica, Thauera terpenica, Thauera sp. DNT-1, Thauera sp. 27, Thauera sp. 28, and Thauera sp. 63), and found that all of them had denitrification function. It was found from Fig. 4a that 33.33% of the β-Proteobacteria consisted of the family class of Comamonadaceae (Acidovorax, Albidiferax, Aquincula, Giesbergenia, Hydrogenophaga, Pelomonas, Polaromonas, Simplicispira, unclassified Comamonadaceae), which had been found in denitrifying reactors with acetate as the organic carbon source for microorganism (Ginige et al., 2005; Osaka et al., 2008). In this study, the organic carbon source in the 3D-BER was supplemented with sodium acetate, and this would lead to a relatively high percentage of Comamonadaceae bacteria in the biofilm. The Burkholderiales-like bacteria (Aquabacterium, Ideonella, Rubrivivax, unclassified Burkholderiales) accounted for 9.52% of the β-Proteobacteria in the biofilm of 3D-BER, while this family class of bacteria have been reported in the studies of anoxic/aerobic digestion bioreactors (Zheng, 2012).

Unlike the prominent diversity of β-Proteobacteria in the 3D-BER biofilm, γ-Proteobacteria contained three genera (Enterobacter, Citrobacter and Erwinia) (Fig. 4c), where the Enterobacter-like bacteria of Enterobacteriaceae took up 88.89% of the clones belonging to γ-Proteobacteria. Enterobacteriaceae-like bacteria were found to have the physiological function of denitrification and polyphosphate accumulation even under anoxic condition, although their denitrification capacity and aerobic phosphorus accumulation capacity were excellent (Bao et al., 2007). This can explain the removal of both nitrate and phosphorus in 3D-BER. Fig. 5 presents the phylogenetic tree of gene sequences in 3D-BER biofilm, where M represents a clone. For example, clone MB9 represents the Enterobacter-like bacteria. Such significant diversity of microbial community in the biofilm as shown in Fig. 5 could enhance the denitrification performance of 3D-BER for wastewater with low C/N ratio.

Table 1

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Number of clones</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Proteobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-proteobacteria</td>
<td>1</td>
<td>0.98</td>
</tr>
<tr>
<td>β-proteobacteria</td>
<td>63</td>
<td>61.77</td>
</tr>
<tr>
<td>γ-proteobacteria</td>
<td>31</td>
<td>30.39</td>
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<td>ε-proteobacteria</td>
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<td>1.96</td>
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<td>Firmicutes</td>
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<td></td>
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<tr>
<td>Clostridia</td>
<td>3</td>
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</tr>
<tr>
<td>Bacilli</td>
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<td>0.98</td>
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<tr>
<td>Actinobacteria</td>
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<td></td>
</tr>
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<tr>
<td>Total</td>
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<td>100</td>
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Fig. 4. Distribution of $\beta$- and $\gamma$-Proteobacteria in the biofilm of 3D-BER (a) percentage of $\beta$-proteobacteria classes; (b) percentage of genera in $\beta$-proteobacteria; (c) percentage of genera in $\gamma$-proteobacteria (experimental condition: C/N = 1, HRT = 7 h, $T = 19 \pm 2^\circ$C, $f = 40$ mA).

Fig. 5. Phylogenetic tree of gene sequences in 3D-BER (note: M represents a clone).
3.4. Surface morphology of biofilm in 3D-BER

The surface morphology and structure of biofilm in the 3D-BER under different influent C/N ratio conditions were obtained (see Supplementary Material). It was found that the microorganisms in the 3D-BER biofilm at relatively low C/N ratio (i.e. 1.0) were mainly short rod (1–2 μm) shaped bacteria, while those at higher C/N ratio consisted of both short rod (1–2 μm) and axiolitic shaped (0.5–1 μm) bacteria. This is in agreement with the results of 16S rDNA analysis which indicated that the biofilm was abundant with Thauera-like and Enterobacter-like bacteria, while such bacteria were mostly short rod shaped (Buchanan and Gibbone, 1984; Mao, 2009). It can also be found that the biofilm contained a much less amount of bacteria at relatively low influent C/N ratio than that at a higher C/N ratio (see Supplementary Material). The reason for this can be explained that the growth of heterotrophic bacteria depends on organic carbon source. The normal operation experiments of 3D-BER in this study started from a high C/N ratio (i.e. 3.0) to a low C/N ratio (i.e. 0.5). The low C/N ratio could lead to the slow growth of bacteria and thus decrease the amount and type of heterotrophic bacteria (e.g., mainly short rod bacteria, 1–2 μm) as compared to environment condition with higher influent C/N (e.g., both short rod and axiolitic bacteria, 1–2 and 0.5–1 μm). In fact, it was visibly seen during the experiments that the C/N ratio directly affected the amount of biofilms, and the biofilms at C/N ratio of 0.5 and 1.0 were thinner and less intensive as compared to those at a C/N ratio of 3.0. It was also found that an extensive pore structure existed in the biofilm, and this could facilitate the sufficient contact of bacteria with nitrogen components and the easy flow of nitrogen gas during the denitrification process, leading to enhanced denitrification rate. However, for the biofilm with low C/N ratio, such pore structure was not obvious.

4. Conclusion

The 3D-BER technology was effectively used for removing nitrate from simulated WWTP effluent. An average nitrate removal of 98.3% and 88.4% was obtained at C/N ratio of 3.0 and HRT of 7 h as well as at C/N ratio of 1.5 and HRT of 10 h, respectively. The biofilm on the AC filler of reactor was dominated by Proteobacteria in which the β- and γ-Proteobacteria were the most abundant. The diverse and dynamic bacterial community composition during treatment could greatly improve the performance of 3D-BER and make it an attractive denitrification technology for wastewater with poor organic carbon source.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.06.001.

References


