Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.23500

140 (2019) 365–372
February

Variation in bacterial communities during landfill leachate treatment by a modified sequencing batch reactor (SBR)

Wenjun Yin[a,*], Kai Wang[a], Daoji Wu[a,*], Jingtao Xu[a], Xiaorui Gao[a], Xiaoxiang Cheng[a], Congwei Luo[a], Congcong Zhao[b]

[a]*School of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan, 250101, China, Tel. 86 13256741911, 86 13708922504; emails: 1353007350@qq.com (W. Yin), wdj@sdjzu.edu.cn (D. Wu), wangkai@sdjzu.edu.cn (K. Wang), xujingtao@sdjzu.edu.cn (J. Xu), 244022409@qq.com (X. Gao), 2014866780@qq.com (X. Cheng), Tel. 18253590095; email: luocongwei@sdjzu.edu.cn (C. Luo)*

[b]*College of Geography and Environment, Collaborative Innovation Center of Human-Nature and Green Development in Universities of Shandong, Shandong Normal University, Jinan 250014, China, email: zhaocongcong1009@163.com*

Received 11 July 2018; Accepted 20 November 2018

ABSTRACT

A modified sequencing batch reactor, which adds a pre-anoxic phase before the aeration phase, was used to treat toxic landfill leachate, and high-throughput sequencing technology used to study how the classification, distribution and abundance of functional bacterial communities varies during system operation. The system operation results are as follows: in the full load steady operation period (II), influent/effluent chemical oxygen demand (COD) and total nitrogen (TN) concentrations were approximately 6,421/645 and 1,210/19 mg/L, respectively; TN and COD removal rates of the system were above 93% and 87%, respectively. The bacterial community structure of the system is as follows: when compared with the bacterial community structure of the inoculation sludge, the final dominant phyla of the system were Proteobacteria (49.4%) and Bacteroidetes (36.5%), while β-Proteobacteria (30.05%) and α-Proteobacteria (16.4%) under Proteobacteria were the two dominant classes in the full load steady operation period (II). *Nitrosomonas* (22.56%) was the main ammonia-oxidizing bacteria within β-Proteobacteria. The bacterial community associated with endogenous denitrification included *Paracoccus* (α-Proteobacteria, 19.15%), *Thauera* (β-Proteobacteria, 8.36%), and *Truepera* (4.96%). *Paracoccus* and *Thauera* were endogenous denitrifying bacteria that could transform organic matter into polyhydroxyalkanoates (PHA) and use PHA to remove nitrogen under anaerobic conditions, and *Truepera* could resist leachate toxicity.

Keywords: Bacterial community; Endogenous denitrifying bacteria; High-throughput sequencing technology; Landfill leachate; Sequencing batch reactor (SBR)

1. Introduction

Landfill leachate is a type of deep-black toxicity lixivium that produces, rancid odor, multiple heavy metal ions, a high ammonia nitrogen content, unbalanced C/N/P ratios and large changes in water quality indexes indices over time. Landfill leachate can damage the health of environment and human, and so finding a cost-effective disposal process is a global environmental issue that must be urgently solved [1–4].

Some biological treatment process have been successfully applied to the treatment of landfill leachate, for example, Zhu et al. [5] and Wang et al. [6] used a modified sequencing batch reactor (SBR) (adding a pre-anoxic phase before the aeration phase) to treat the landfill leachate, where the system achieves an ideal removal rate of COD and total nitrogen (TN) through

* Corresponding authors.

shortcut nitrification combined with endogenous denitrification without additional carbon sources. However, Zhu et al. [5] and Wang et al. [6] only discussed the removal rate of COD and TN in the SBR system and did not discuss the classification, distribution, and abundance of functional and dominant bacterial communities involved in the denitrification and removal of organic matter. However, the functional and dominant bacterial community characteristics were the key to affecting the biological treatment process. For example, the functional bacterial community in shortcut nitrification was the ammonia-oxidizing bacteria (AOB) that could oxidize NH_4^+-N to NO_2^-N [7,8], and the AOB was mainly classified as Proteobacteria (phylum) and β-Proteobacteria (class) [9], with some AOB under the β-Proteobacteria group mainly divided into *Nitrosomonas* (genus) and *Nitrosospira* (genus). The functional bacterial communities involved in endogenous denitrification were those that had the capacity to store internal carbon sources. The process by which endogenous denitrifying bacteria remove organics and nitrogen is as follows: endogenous denitrifying bacteria first transform organic matter into polyhydroxyalkanoates (PHA) under conditions where organics are abundant, and then use PHA to convert nitrogen under anoxic conditions [10,11]. Therefore, it is very important to analyze and research the classification, distribution and abundance of these functional bacterial communities, which not only provides theoretical foundations for the shortcut nitrification process combined with endogenous denitrification but also improves the reference value for the further process optimization, control processes for leachate treatment and the development of new technology [12,13].

Modern biological molecular techniques such as polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), high-throughput sequencing and clone sequencing have been widely used in aspects of microbial community structure and functional microbial characteristics [14–16]. High-throughput sequencing technology [17,18], as a new type of microbial identification technology, is characterized by its accuracy, high efficiency and high sensitivity, and is suitable for the sequencing of environmental bacterial communities. In this study, high-throughput sequencing technology was used to analyze bacterial communities, to research the classification, distribution, and abundance of short-cut nitrification bacteria (AOB) and endogenous denitrifying bacteria in an SBR system. The study provided a theoretical basis for shortcut nitrification and endogenous denitrification for the treatment of landfill leachate.

2. Materials and methods

2.1. Quality of landfill leachate and inoculation sludge

The landfill leachate in this study was obtained from the Jiyang landfill, established in Jinan, Shandong, Northern China (117°12′N, 36°58′E). The landfill was built and put into use in December 2011, with a total storage capacity of 2,723,400 m^3, and is one of several large-scale landfills in Jinan. The quality of landfill leachate is shown in Table 1. The inoculation sludge is the whole-process-nitration-reaction sludge used for treating domestic sewage (influent TN and COD concentration was approximately 63 and 500 mg/L, respectively) that obtained from Everbright Sewage Treatment Plant (Jinan, Shandong, China). MLSS (mixed liquid suspended solids), sludge volume index (SVI), mixed liquor volatile suspended solids (MLVSS) values of inoculation sludge were approximately 8,569; 105 and 6,217 mg/L, respectively.

2.2. Experimental equipment and procedure

As shown in Fig. 1, the SBR reactor was made of a polymethyl methacrylate reaction column with a total volume of 13 L, and an effective volume is 10 L. Two sampling ports with a spacing of 10 cm were placed on the reaction column. The SBR system includes: (1) an agitation subsystem with a mechanical agitator and impeller to ensure that the activated sludge is well mixed; (2) an aeration subsystem with an air pump, air flow meter and porous aeration head to control dissolved oxygen (DO) approximately 2.5 mg/L; (3) a temperature control subsystem with a heating belt, temperature control box and temperature probe to control the temperature approximately 25°C; (4) a detection subsystem with a pH, DO and oxidation–reduction potential (ORP)-detecting integrated on-line analyzer (WTW Multi340i, Germany) to determine the end of nitrification and denitrification reaction accurately by performing real-time monitoring. The system's drainage ratio was 30%.

The operation mode is shown in Fig. 2, including the (1) filling stage (5 min); (2) pre-anoxic stage (1 h); (3) aeration stage (aeration time); (4) anoxic stage (anoxic time) and (5) settling stage (0.5 h). Aeration time and anoxic time were determined

Table 1
Quality of landfill leachate

Compound	Range
pH	8 ± 0.3
NH_4^+-N (mg/L)	1,000 ± 100
TN (mg/L)	1,100 ± 100
NO_x^-N (mg/L)	0.5–1
BOD_5 (mg/L)	4,500 ± 500
COD (mg/L)	6,500 ± 500
C/N	>6

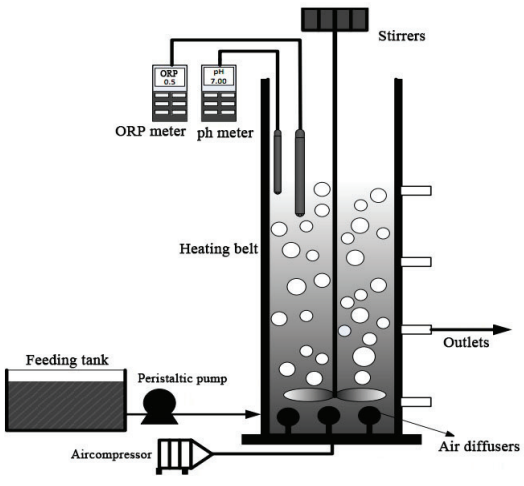

Fig. 1. Experimental equipment of SBR.

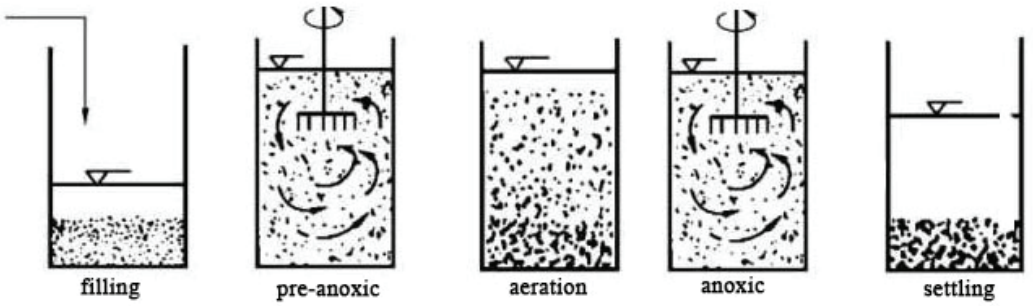

Fig. 2. Operational mode of SBR.

by the ammonium value (pH variation) and nitrate knees (ORP variation) real-time control method. Studies by Aziz et al. [19] have shown that adding a pre-anoxic stage to the traditional SBR can achieve a higher TN removal rate that of traditional SBR.

2.3. Measuring methods

2.3.1. Wastewater analysis methods

The pH, DO, ORP and temperature were measured by using the integrated on-line analyzer (WTW Multi340i). NH_4^+-N, NO_2^--N, NO_3^--N, COD, MLSS, MLVSS and SVI were measured using standard methods [20]. TN analyzer (Multi N/C3000, Germany) was used to measure TN. Extraction and measurement methods of PHA refer to the literature [21].

2.3.2. High-throughput sequencing methods

Sludge samples that were taken from inoculation sludge were defined as A_x and samples taken from sludge in period (II) were defined as B_x. The extraction, purification and concentration of DNA in these sludge samples were performed by using the CTAB method and agarose gel electrophoresis. Each DNA sludge sample was diluted to 1 ng/μL in the centrifuge tube and denoted as template DNA, and the primers and the section primers corresponding to of 16SV4 domain were the specific primers with barcode and the 16SV4 domain (515F and 806R). PCR amplification used high-efficiency and high-fidelity enzymes to ensure the amplification efficiency and accuracy of the PCR tester (Phusion® High-Fidelity PCR Master Mix with GC Buffer from Biolabs, New Zealand), and we then used a 1 × Tris-acetate-EDTA concentration 2% agarose gel electrophoresis and purification kit (Thermo Scientific Gene JET gel recovery reagent (Box)) to purify equal quantities of mixed PCR products, and then cut and recovered the target bands. The library building kit (Thermo Fisher's Ion Plus Fragment Library Kit, 48 runs) and IonS5TMXL (Thermo Fisher Inc.) were used to build a library and make a computer sequence. After Qubit quantification and library detection, the single-end sequencing method was used to sequence small fragment libraries. Reads were cut and filtered, OTUs were clustered and species annotation and abundance analysis were performed to reveal, classification, distribution characteristics and abundance of the sludge sample functional microorganisms. Alpha diversity analysis was conducted on individual samples to reflect the abundance and diversity of bacterial colonies, using methods such as the Chao index, ACE index, Shannon index, Simpson index and coverage index. Finally, the bacterial communities of the sludge samples were analyzed statistically at the levels of phylum, class, genus and species.

2.3.3. Statistical analysis

Three sample t-tests were used to evaluate the significant difference of each groups. The data were all averages of two or more replicates. The results were considered to be statistically significant when $p < 0.05$ (the level of significance above 95%).

3. Results and discussion

3.1. Landfill leachate removal performance

As shown in Fig. 3, the SBR system ran for approximately 110 d, and the entire operation period was divided into a load raising period (I) and full load steady operation period (II). Final influent and effluent TN concentrations were maintained at approximately 1,210 and 19 mg/L, respectively, and the removal rate of TN was maintained at over 93%. During the operation period, the activity of nitrite-oxidizing bacteria (NOB) was gradually inhibited as the concentration of free ammonia increased, and AOB gradually became the dominant bacteria in the system [22]. $NO_3^-N_{(\text{after aerobic})}$ (the concentration of NO_3^--N after the aeration period) gradually decreased, $NO^{2-}N_{(\text{after aerobic})}$ (the concentration of NO_2^--N after the aeration period) gradually increased and exceeded the concentration of $NO_3^-N_{(\text{after aerobic})}$ at the 44th day, and the concentration of $NO_3^-N_{(\text{after aerobic})}$ finally decreased below 1 mg/L. Within the aeration period during period (II), essentially all of the nitrogen after the aeration period was NO_2^--N, and the nitrite accumulation rate, the nitrification time and final $NO_2^-N_{(\text{after aerobic})}$ were finally maintained at approximately 99%, 4.5 h and 120 mg/L, respectively. Shortcut nitrification was the main nitrification reaction, representative of the shortcut nitrification functional bacterial community.

The nitrogen produced in the aeration stage was mainly removed by endogenous denitrification, and the process of endogenous denitrifying bacteria storing PHA also removed organic matter. As shown in Fig. 3, the final $COD_{(\text{inf})}$ (the influent COD concentration) was maintained at approximately 6,421 mg/L, while the $COD_{(\text{after pre-anoxic})}$ (the COD concentration at the end of pre-anoxic phase) and $COD_{(\text{eff})}$ (the effluent COD concentration) had similar values at 645 mg/L, the final COD removal rate was maintained above 85%, and the content

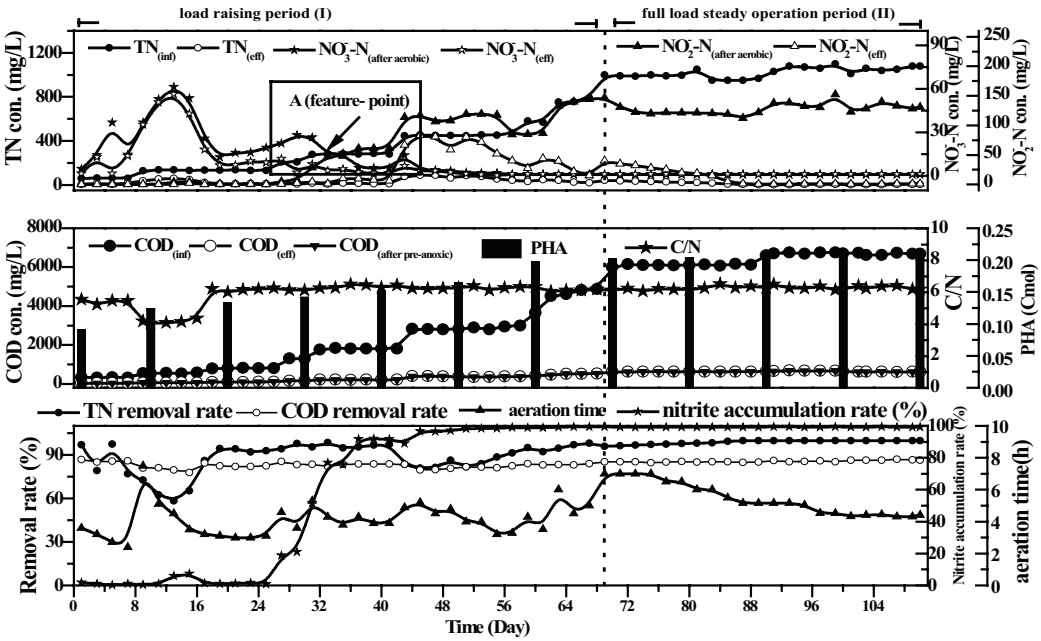

Fig. 3. Treatment performance of the SBR over the whole operation period.

of $PHA_{(after\ pre\text{-}anoxic)}$ (the PHA content at the end of pre-anoxic phase) was finally maintained at approximately 0.22 Cmol. These results showed that the removal of COD mainly occurred in the pre-anoxic phase, and the endogenous denitrifying bacteria stored enough internal carbon sources to remove nitrogen. Fig. 4 shows that variation of indices in typical cycles of the stability period (II). The content of PHA increased with the decrease in COD concentration in the pre-anoxic stage and then decreased gradually in the aeration stage and anoxic stage. This also indicated that the removal of COD and the synthesis of PHA by endogenous denitrifying bacteria occurred in the pre-anoxic stage. In the anoxic stage, NO_2^--N decreased in proportion with the decrease in PHA content, but other material indicators essentially did not change, indicating that endogenous denitrifying bacteria uses the internal carbon source for the removal of NO_2^--N at this stage. In general, the system relies on shortcut nitrification and endogenous denitrification to achieve better removal of TN and COD by using organic matter contained in raw water. AOB and endogenous denitrifying bacteria were the functional and dominant microorganism in the system. Therefore, further study on the classification, distribution and abundance of AOB and endogenous denitrifying bacteria flora was very important to further improve the removal efficiency of total nitrogen and COD.

Table 2 summarizes the overall performance of COD and TN removal in landfill leachate treatment using biological processes, as reported from the recent literatures. In general, nitrogen can be removed through the natural pathway of nitrification/denitrification, which means that the organic matter is completely degraded during the nitrification stage, so the denitrification process will not be carried out thoroughly. The results from the study by Wei et al. [23] suggested that an extra carbon source addition seems to be necessary in the anoxic stage, in order to reduce the TN concentration in the effluent. However, it appears that this would raise the operational cost drastically for a real landfill leachate treatment plant. On the other hand, although Zhu et al. [5] and Wang et al. [6] realized relatively effective nitrogen removal without the extra carbon source addition, they did not study microbial community characteristics of the operating system, and many studies have further analyzed the wastewater treatment mechanism through microbial population characteristics [24]. Based on the deep treatment of landfill leachate, this study further explored the mechanism of organic matter and TN removal using microbial population characteristics.

3.2. High-throughput bacterial community analysis

16S rRNA is located in the small ribosomal subunit of bacteria, including 10 conserved regions and 9 hypervariable regions. Among them, the conserved region shows little difference among bacteria, and the hypervariable region is specific to genus with certain differences determined by the kinship. Therefore, 16S rDNA can be used as a characteristic nucleic acid sequence to reveal biological species, and is considered to be the most suitable indicator for bacterial phylogeny development and taxonomic identification.

3.2.1. Analysis of bacterial diversity

A_x (A_1, A_2 and A_3) and B_x (B_1, B_2 and B_3) were sequenced using the IonS5TMXL sequencing platform. The optimized sequences were clustered under a similarity of 97%, and the results are shown in Table 3. Sludge samples A_1, A_2, A_3, B_1, B_2 and B_3 obtained 704; 629; 646; 1,462; 1,520 and 1,530 OTU numbers, respectively, and the species coverage index of all sludge samples was greater than 99%, indicating that this sequencing result appropriately described the real situation of all microorganisms in the sample [25]. The ACE index and Chao index were used to analyze the number of OTU in the bacterial community and reflected the abundance of the

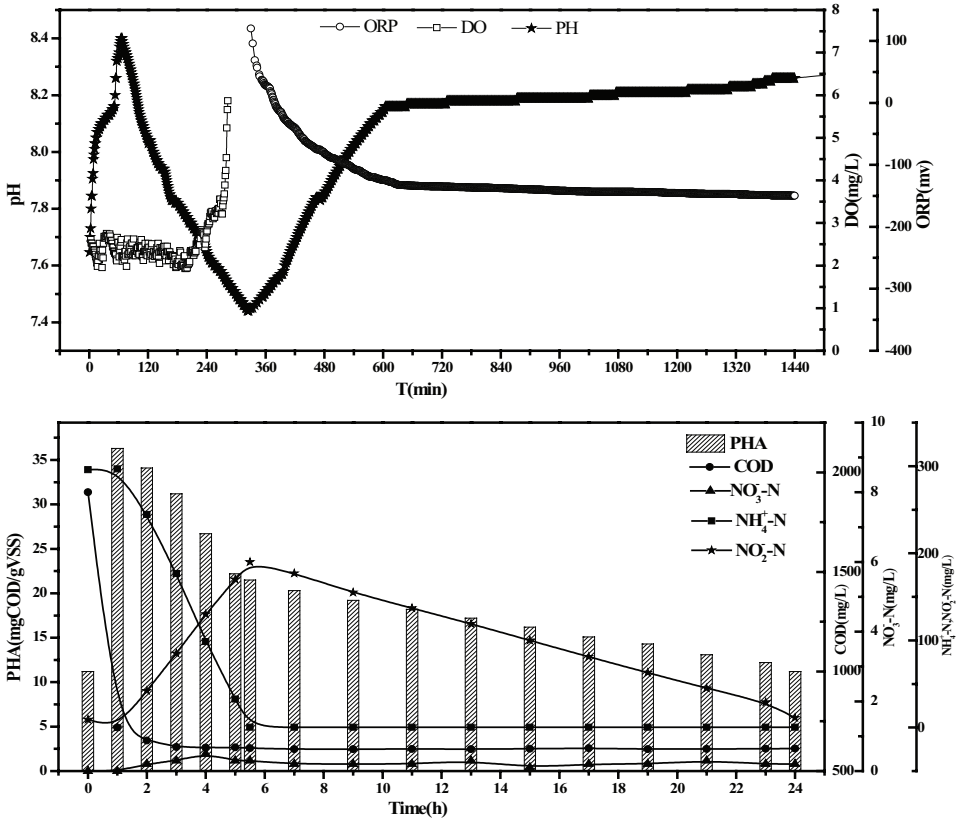

Fig. 4. Variation of indices in typical cycles of period II.

Table 2
Comparison of COD and nitrogen removal from landfill leachate treatment reported in the literatures using biological process

Treatment process	Characteristic of influent	Characteristic of effluent	Note
ASBR + PSBR [6]	COD = 7,341–10,448; TN = 1,450–2,450	COD < 900; TN = 170–250	
AO/AO-MBR [24]	COD = 3,516–21,080; TN = 1,308–2,835	COD ≈ 2,584; TN < 20	Microbial community analysis
GSBR [23]	COD = 4,298–5,992; TN = 855–1,453	TN > 674	Carbon sources added
ASBR + SBR [5]	COD =5,890–6,800; TN = 1,030–1,180	COD ≈ 1,056; TN = 1,030–1,180	
This study	COD = 6,500 ± 500; TN = 1,100 ± 100	COD ≈ 650; TN < 20	Microbial community analysis

Table 3
Analysis of bacterial flora abundance and diversity

Samples	Number of sequences	OTU_{number}	Shannon	Simpson	Chao	ACE	Coverage (%)
A_1	68,216	704	5.886	0.956	924.277	916.146	99.60%
A_2	64,623	586	5.607	0.954	648.523	659.318	99.80%
A_3	69,737	575	5.598	0.956	626.663	635.462	99.80%
B_1	80,196	1,374	8.022	0.989	1,450.712	1,459.622	99.70%
B_2	80,295	1,451	8.189	0.988	1,537.034	1,547.265	99.70%
B_3	80,183	1,449	8.187	0.989	1,531.098	1,528.481	99.70%

bacterial communities in the sludge samples: the higher the index value, the higher the abundance of the bacterial community. The Shannon index and the Simpson index were used to analyze the diversity of bacterial communities of samples: the larger the Shannon value, the higher the diversity of the bacterial community, the higher the Simpson index value, the lower the diversity of bacterial community, indicating that the abundance of the dominant bacterial community in each sludge sample was larger [26]. The values of the OTU, ACE index, Chao index and Shannon index of samples B_x were larger than those of the sludge samples A_x ($B_2 > B_3 > B_1 > A_1 > A_2 > A_3$) and indicated that the abundance and diversity of A_x were lower

than B_x. The Simpson index of A_x and B_x also indicated that the community diversity and equilibrium degree of B_x were higher than that of A_x. The results also showed that the microbial community in the inoculated sludge had undergone significant change, and the high concentration of toxic leachate and the operating mode of the SBR system were the probable causes of this result. Only the functional bacterial communities with strong resistance to and suitability for the system's operating environment could survive gradually become the dominant bacterial community, while the inferior bacteria in the seed sludge were gradually eliminated.

3.2.2. Analysis at the phylum level

As shown in Fig. 5, bacterial communities from sludge samples A_x and B_x were composed of Bacteroidetes, Proteobacteria, Nitrospirae, Acidobacteria, Planctomycetes, Chloroflexi, Deinococcus-Thermus, Chlorobi, Firmicutes and Armatimonadetes at the phylum level. Sludge samples A_x and B_x both showed Bacteroidetes and Proteobacteria as the dominant phyla, and the average abundance values of the two phyla in sludge samples A_x and B_x were 85% and 55%, respectively. The average abundance of Proteobacteria in sludge samples B_x (the abundance of B_1, B_2, B_3 were 41.41%, 35.55%, 33.14%, respectively) was higher than that of Bacteroidetes (the abundance of B_1, B_2 and B_3 were 17.57%, 19.75% and 20.19%, respectively); the average abundance of Proteobacteria (the abundance of A_1, A_2 and A_3 were 41.39%, 55.73% and 51.09%, respectively) in the sludge samples A_x were higher than the abundance of Bacteroidetes (the abundance of A_1, A_2, A_3 were 42.90%, 31.31%, 35.30%, respectively), and the abundance of the two phyla in the sludge samples B_x was less than that in sludge samples A_x. The results were consistent with the observation that the abundance of Proteobacteria in the conventional activated sludge was higher than that of Bacteroidetes. The majority of Proteobacteria were involved in denitrification and organic removal [27]. Bacteroidetes (phylum) were chemoorganotrophic bacteria that could metabolize carbohydrates and degrade complex organic matters [28]. The abundance of Deinococcus-Thermus (phylum) increased in sludge samples A_x, where *Deinococcus* (genus) and *Truepera* (genus) of Deinococcaceae (family) from Deinococcus-Thermus (phylum) include several bulbous bacteria that can swallow pollutants and toxins and are resistant to harsh environments. The average abundance of Nitrospirae (phylum) in sludge samples B_x was 9.09%, and the *Nitrospira* (genus) in Nitrospirae (phylum) was an important NOB in the biological denitrification process [29]. However, Nitrospirae (phylum) was almost absent in the sludge samples A_x, while the results also showed that NOB was almost absent in period (II), and that shortcut nitrification was the main nitrification reaction. The Proteobacteria (phylum) contains a large amount of AOB: in order to further research the bacterial genera and abundance of AOB in period (II), bacterial communities in sludge samples A_x and B_x need to be compared at the class and genus level.

3.2.3. Analysis at the class level

As shown in Fig. 6, β-Proteobacteria were the most abundant class in sludge samples A_x and B_x (the abundance of A_1, A_2, A_3, B_1, B_2 and B_3 were 26.88%, 33.76%, 29.52%, 15.81%, 10.18% and 8.41%, respectively); *Nitrosomonas* (genus), *Nitrosospira* (genus) and other bacteria in the Nitrosomonadales (order) contained in β-Proteobacteria (class) were common AOB, and β-Proteobacteria (class) also contained some denitrifying bacteria community such as *Thauera* (genus), *Thiobacillus* (genus), along with others [30]. The abundance of β-proteobacteria in sludge samples A_x was higher than sludge samples B_x, indicating that the system contained highly abundant AOB and denitrifying bacteria in the full load stability period (II). The average abundance of α-proteobacteria (class) in sludge samples A_x was higher (16.4%) than that in B_x (10.6%), indicating that both β-Proteobacteria (class) and α-Proteobacteria (class) were dominant classes in sludge samples A_x. The reason that α-Proteobacteria (class) was the dominant class was that α-Proteobacteria contained *Truepera* (genus) and other denitrifying bacteria. The abundances of Gammaproteobacteria (class) and Deltaproteobacteria (class) within sludge sample A_x were all less than B_x, indicating that Gammaproteobacteria and Deltaproteobacteria had been phased out during the operation of the system.

3.2.4. Analysis at the genus level

To further research the classification and abundance contained in β-Proteobacteria (class) and α-Proteobacteria (class), six species from sludge samples were analyzed at the genus level. As shown in Table 4, at the genus level, *Paracoccus*,

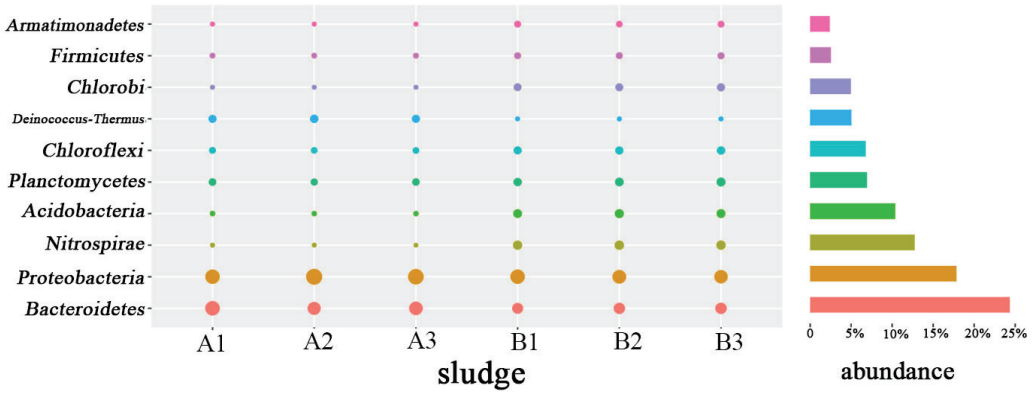

Fig. 5. Bacterial diversity of top 10 phyla.

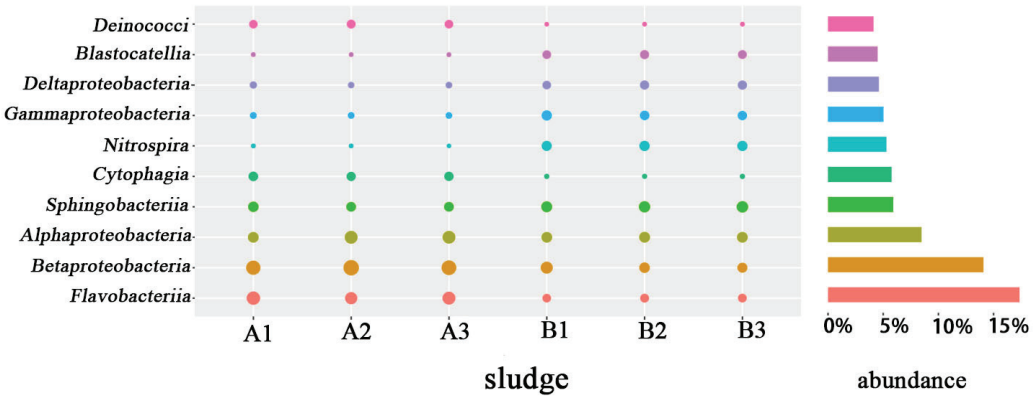

Fig. 6. Bacterial diversity of top 10 classes.

Table 4
Bacterial level abundance statistics

Genera	Phylum and class	A_x	B_x
Paracoccus	P-Proteobacteria; c-Alphaproteobacteria	16.15%	0.28%
Nitrosomonas	P-Proteobacteria; c-Betaproteobacteria	8.47%	0.41%
Nitrospira	P-Nitrospirae; c-Nitrospiria	0.02%	9.09%
Thauera	P-Proteobacteria; c-Betaproteobacteria	8.36%	1.96%
Truepera	P-Deinococcus-Thermus; c-Deinococci	4.96%	0.02%
Ferruginibacter	P-Bacteroidetes; c-Sphingobacteriia	0.02%	5.10%
Haliangium	P-Proteobacteria; c-Deltaproteobacteria	0.01%	2.00%
Stenotrophobacter	P-Acidobacteria; c-Blastocatellia	0.01%	2.02%

Nitrosomonas, *Nitrospira*, *Thauera*, *Truepera*, *Ferruginibacter*, *Haliangium*, *Stenotrophobacter* and other species constitute the bacterial genera of sludge samples A_x and B_x. The Nitrospiraceae (genus, *Nitrospira* is NOB) in B_x disappeared within A_x, while the abundance of *Nitrosomonas* (AOB) in A_x was higher than in B_x, and the abundance of *Nitrosomonas* (8.47%) was the second most dominant genera in period (II), indicating that the nitrification-based functional and dominant bacterial communities were *Nitrosomonas* (genus); *Nitrosomonas* (genus) was involved in shortcut nitrification. The abundance of *Thauera* (genus) within sludge samples A_x (8.36%) was higher than in B_x (1.96%), indicating that β-Proteobacteria (class) indeed contained a higher abundance of AOB and denitrifying bacteria. *Paracoccus*, *Thauera* and *Truepera* are denitrifying bacteria, and the total abundance (35%) in the sludge samples of A_x was higher than the abundance (3%) in B_x, indicating that there are significant quantities of denitrifying bacteria in period (II). *Paracoccus* [31] and *Thauera* [32] are endogenous denitrifying bacteria, and convert organic matter into internal carbon sources, using internal carbon to remove nitrogen. *Thauera* could also degrade macromolecular organic matter into small molecular organic matter [33]. The total abundance of *Paracoccus* and *Thauera* in the sludge samples of A_x was nearly 27.51%, which showed that *Paracoccus* and *Thauera* were the main endogenous denitrifying bacteria in this system. *Paracoccus* was classified as α-Proteobacteria (class) and its abundance was similar to the abundance of α-Proteobacteria, indicating that α-Proteobacteria only contained *Paracoccus* during period (II). *Thauera* was classified as Deinococcus-Thermus (phylum) and Deinococcaceae (family), which had the ability to resist harsh environmental factors, and the abundance of *Thauera* in A_x was greater than B_x, indicating that activated sludge in the period (II) could resist leachate toxicity and remove nitrogen.

4. Conclusion

After continuous operation for 110 d, the influent concentration of TN and COD was approximately 1,210 and 6,421 mg/L, respectively, in the SBR system, the removal rate of TN and COD of leachate was approximately 93% and 85%, and the accumulation rate of nitrite was close to 100%. NOB eventually disappeared in the full load steady operation period (II), while *Nitrosomonas* (Proteobacteria, β-Proteobacteria) was AOB involved in shortcut nitrification. Proteobacteria and β-Proteobacteria were the dominant phylum and class in the system, and in addition, Bacteroidetes and Deinococcus-Thermus were also dominant phyla in the system. The bacterial genera involved in denitrification were mainly *Paracoccus* (α-Proteobacteria), *Thauera* (β-Proteobacteria) and *Truepera*. The former two are endogenous denitrifying bacteria that can store carbon sources, and *Thauera* is a highly resistant bacterial genus.

Acknowledgments

This work is supported by Shandong Provincial Natural Science Foundation, China (ZR2017BEE067), Science and Technology Planning of Ministry of Housing and Urban-Rural Development of the People's Republic of China (UDC2017031712), National Key Research and Development Program of China (2017YFF0209903), Natural Science Foundation of Shandong Province of China (ZR2018BEE036), National Natural Science Foundation of China (21307078).

References

[1] J. Liu, P. Zhang, H. Li, Y. Tian, S. Wang, Y. Song, G. Zeng, C. Sun, Z. Tian, Denitrification of landfill leachate under different hydraulic retention time in a two-stage anoxic/oxic combined membrane bioreactor process: performances and bacterial community, Bioresour. Technol., 250 (2018) 110–116.

[2] J. Liu, Z. Tian, P. Zhang, G. Qiu, Y. Wu, H. Zhang, R. Xu, W. Fang, J. Ye, Y. Song, G. Zeng, Influence of reflux ratio on two-stage anoxic/oxic with MBR for leachate treatment: performance and microbial community structure, Bioresour. Technol., 256 (2018) 69–76.

[3] A. Mojiri, H.A. Aziz, N.Q. Zaman, S.Q. Aziz, M.A. Zahed, Powdered ZELIAC augmented sequencing batch reactors (SBR) process for co-treatment of landfill leachate and domestic wastewater, J. Environ. Manage., 139 (2014) 1–14.

[4] G. Insel, M. Dagdar, S. Dogruel, N. Dizge, E. Ubay Cokgor, B. Keskinler, Biodegradation characteristics and size fractionation of landfill leachate for integrated membrane treatment, J. Hazard. Mater., 260 (2013) 825–832.

[5] R. Zhu, S. Wang, J. Li, K. Wang, L. Miao, B. Ma, Y. Peng, Biological nitrogen removal from landfill leachate using anaerobic-aerobic process: denitritation via organics in raw leachate and intracellular storage polymers of microorganisms, Bioresour. Technol., 128 (2013) 401–408.

[6] K. Wang, S. Wang, R. Zhu, L. Miao, Y. Peng, Advanced nitrogen removal from landfill leachate without addition of external carbon using a novel system coupling ASBR and modified SBR, Bioresour. Technol., 134 (2013) 212–218.

[7] C. Mota, M.A. Head, J.A. Ridenoure, J.J. Cheng, F.L. De Los Reyes, Effects of aeration cycles on nitrifying bacterial populations and nitrogen removal in intermittently aerated reactors, Appl. Environ. Microbiol., 71 (2005) 8565–8572.

[8] Y. Peng, S. Zhang, W. Zeng, S. Zheng, T. Mino, H. Satoh, Organic removal by denitritation and methanogenesis and nitrogen removal by nitritation from landfill leachate, Water Res., 42 (2008) 883–892.

[9] U. Purkhold, A. Pommerening-Röser, S. Juretschko, M.C. Schmid, H.P. Koops, M. Wagner, Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys, Appl. Environ. Microbiol., 66 (2000) 5368–5382.

[10] Q. Wen, Z. Chen, C. Wang, N. Ren, Bulking sludge for PHA production: energy saving and comparative storage capacity with well-settled sludge, J. Environ. Sci. (China), 24 (2012) 1744–1752.

[11] J.J. Beun, F. Paletta, M.C.M. Van Loosdrecht, J.J. Heijnen, Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures, Biotechnol. Bioeng., 67 (2000) 379–389.

[12] Y. Liu, F. Liu, L. Ni, M. Meng, X. Meng, G. Zhong, J. Qiu, A modeling study by response surface methodology (RSM) on Sr(ii) ion dynamic adsorption optimization using a novel magnetic ion imprinted polymer, RSC Adv., 6 (2016) 54679–54692.

[13] G. Wang, Y. Wang, Optimization of Additives of Intumescent Fire Resistant Coating for Steel Structure by Response Surface Methodology, Huagong Xuebao/CIESC J., 63 (2012) 978–984.

[14] P. Wu, X. Ji, X. Song, Y. Shen, Nutrient removal performance and microbial community analysis of a combined ABR-MBR (CAMBR) process, Chem. Eng. J., 232 (2013) 273–279.

[15] E. Lyautey, B. Lacoste, L. Ten-Hage, J.L. Rols, F. Garabetian, Analysis of bacterial diversity in river biofilms using 16 S rDNA PCR-DGGE: methodological settings and fingerprints interpretation, Water Res., 39 (2005) 380–388.

[16] J.C. López-Gutiérrez, S. Henry, S. Hallet, F. Martin-Laurent, G. Catroux, L. Philippot, Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR, J. Microbiol. Methods, 57 (2004) 399–407.

[17] A. Gribble, G. Brightwell, Spoilage characteristics of *Brochothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR, Meat Sci., 94 (2013) 361–368.

[18] W. Rutvisuttinunt, P. Chinnawirotpisan, S. Simasathien, S.K. Shrestha, I.K. Yoon, C. Klungthong, S. Fernandez, Simultaneous and complete genome sequencing of influenza A and B with high coverage by Illumina MiSeq Platform, J. Virol. Methods, 193 (2013) 394–404.

[19] S.Q. Aziz, H.A. Aziz, M.S. Yusoff, Powdered activated carbon augmented double react-settle sequencing batch reactor process for treatment of landfill leachate, Desalination, 277 (2011) 313–320.

[20] L.S. Clesceri, A.D. Eaton, A.E. Greenberg, Standard Methods for the Examination of Water and Wastewater American Public Health Association, 1998.

[21] M.G.E. Albuquerque, M. Eiroa, C. Torres, B.R. Nunes, M.A.M. Reis, Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses, J. Biotechnol., 130 (2007) 411–421.

[22] J.J. Beun, K. Dircks, M.C.M. Van Loosdrecht, J.J. Heijnen, Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures, Water Res., 36 (2002) 1167–1180.

[23] Y. Wei, M. Ji, R. Li, F. Qin, Organic and nitrogen removal from landfill leachate in aerobic granular sludge sequencing batch reactors, Waste Manage., 32 (2012) 448–455.

[24] W. Yin, K. Wang, J. Xu, D. Wu, C. Zhao, The performance and associated mechanisms of carbon transformation (PHAs, polyhydroxyalkanoates) and nitrogen removal for landfill leachate treatment in a sequencing batch biofilm reactor (SBBR), RSC Adv., 8 (2018) 42329–42336.

[25] A.K. Bartram, M.D.J. Lynch, J.C. Stearns, G. Moreno-Hagelsieb, J.D. Neufeld, Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads, Appl. Environ. Microbiol., 77 (2011) 3846–3852.

[26] M. Hu, X. Wang, X. Wen, Y. Xia, Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis, Bioresour. Technol., 117 (2012) 72–79.

[27] Y.M. Chang, Q. Yang, C.B. Hao, H.T. Shang, T.S. Jiang, Experimental study of autotrophic denitrification bacteria through bioaugmentation of activated sludge from municipal wastewater plant, Huan Jing Ke Xue, 32 (2011) 1210–1216.

[28] C. Kragelund, C. Levantesi, A. Borger, K. Thelen, D. Eikelboom, V. Tandoi, Y. Kong, J. Krooneman, P. Larsen, T.R. Thomsen, P.H. Nielsen, Identity, abundance and ecophysiology of filamentous bacteria belonging to the Bacteroidetes present in activated sludge plants, Microbiology, 154 (2008) 886–894.

[29] H.D. Park, D.R. Noguera, Nitrospira community composition in nitrifying reactors operated with two different dissolved oxygen levels, J. Microbiol. Biotechnol., 18 (2008) 1470–1474.

[30] T.R. Thomsen, Y. Kong, P.H. Nielsen, Ecophysiology of abundant denitrifying bacteria in activated sludge, FEMS Microbiol. Ecol., 60 (2007) 370–382.

[31] S.S. Sawant, B.K. Salunke, B.S. Kim, Degradation of corn stover by fungal cellulase cocktail for production of polyhydroxyalkanoates by moderate halophile *Paracoccus* sp. LL1, Bioresour. Technol., 194 (2015) 247–255.

[32] G. Kor-Bicakci, I. Pala-Ozkok, A. Ural, D. Jonas, D. Orhon, E. Ubay-Cokgor, Is the chronic impact of sulfamethoxazole different for slow growing culture? The effect of culture history, Bioresour. Technol., 206 (2016) 65–76.

[33] T. Mechichi, E. Stackebrandt, N. Gad'on, G. Fuchs, Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions, and description of Thauera phenylacetica sp. nov., Thauera aminoaromatica sp. nov., and Azoarcus buckelii sp. nov., Arch. Microbiol., 178 (2002) 26–35.