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# The performance and associated mechanisms of carbon transformation (PHAs, polyhydroxyalkanoates) and nitrogen removal for landfill leachate treatment in a sequencing batch biofilm reactor (SBBR)

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A modified sequencing batch biofilm reactor (SBBR, adding a pre-anoxic phase before the aeration phase) was used to treat landfill leachate. The overall SBBR operation period was divided into a load-increasing period I (69 days) and a steady operation period II (41 days). In period I, the influent total nitrogen (TN) and chemical oxygen demand (COD) concentrations increased from approximately 60 and 400 mg L<sup>-1</sup> to 1000 and 6500 mg L<sup>-1</sup>, respectively, and these were kept for period II. In period II, the COD and TN removal rates were 83–88% and 95–98%, with effluent COD and TN concentrations of less than 500–600 and 10–20 mg L<sup>-1</sup>, respectively. The end of pre-anoxic phase PHA (polyhydroxyalkanoate) content increased from 0.11 Cmol (start of period I) to 0.22 Cmol (end of period II). The contributions from simultaneous nitrification and denitrification (SND) and endogenous denitrification to the TN removal rate were approximately 60% and 40%, respectively. The mechanisms of carbon transformation and nitrogen removal were: (1) the synthesis of PHAs in the pre-anoxic phase; (2) short-range nitrification; (3) simultaneous nitrification and denitrification (SND); and (4) endogenous denitrification. Microbial diversity analysis revealed that *Proteobacteria* and *Bacteroidetes* accounted for 89.66% of the total bacteria. Ammonia-oxidizing bacteria (AOB, *Nitrosomonas*) and denitrifying bacteria with the ability to transform organic matter into PHAs (*Paracoccus* and *Thauera*) are the dominant bacterial communities.

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## 1. Introduction

The main treatment method for municipal solid waste worldwide involves the use of sanitary landfills. However, sanitary landfills generate landfill leachate, containing high concentrations of organic matter, nitrogen, inorganic salts, and heavy metals,<sup>1–3</sup> resulting in serious pollution of the external aquatic environment.<sup>4</sup> Therefore, treatment technologies for landfill leachate with improved efficiency and low cost are important for sewage treatment strategies.

Currently, the main treatment methods for landfill leachate are physical–chemical and biological methods. Physical–chemical methods include ammonia stripping technology, advanced oxidation, and adsorption;<sup>5–7</sup> these methods are typically used in pre-treatment or post-treatment steps due to

their secondary pollution and high cost. Biochemical methods, including sequencing batch reactors (SBRs), up-flow anaerobic sludge beds (UASBs), anaerobic sequencing batch reactors (ASBRs), membrane bio-reactors (MBRs) and related methods, could treat landfill leachate effectively and environmentally.<sup>8,9</sup> However, anaerobic methods are unable to remove NH<sub>4</sub><sup>+</sup>-N;<sup>10</sup> traditional aerobic methods need the addition of extra carbon sources to remove nitrogen.<sup>11</sup>

PHAs (polyhydroxyalkanoates) composed of polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) have a significant impact on carbon substrate conversion in activated sludge processes.<sup>12</sup> Some microbes can convert organic matter into intracellular PHAs when there is enough organic matter, then use PHAs as a carbon source for denitrification to remove nitrogen.<sup>13</sup> Zhu *et al.* used PHAs as a carbon source for endogenous denitrification to remove nitrogen in SBR systems, therefore the strategy of using PHAs to remove nitrogen is feasible.<sup>14,15</sup>

PHAs can be used as a carbon source for simultaneous nitrification and denitrification (SND) to remove nitrogen.<sup>16,17</sup> The realization of SND depends on the anaerobic reaction zone of the system, so there is no SND in the aeration phase of

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a SBR.<sup>14,15</sup> A SBR can achieve SND through controlling the dissolved oxygen (DO) concentration over a range of 0.5 to 1 mg L<sup>-1</sup>,<sup>18,19</sup> but a lower DO concentration can limit the nitrification reaction rate. Anaerobic reaction zones exist in the biofilm inner layer of a SBBR system because of the dissolved oxygen gradient; therefore, a SBBR could achieve SND when the DO concentration is controlled over the range of 2 to 3 mg L<sup>-1</sup>. If an anoxic phase is added before the aeration phase to modify a SBBR, the biofilm can store PHAs during the pre-anoxic phase and the modified SBBR could achieve SND in the anaerobic reaction zone of the aeration phase. SND facilitates the removal of nitrogen through these properties: (1) the alkali produced by SND makes up for alkali consumed by the nitrification reaction, which maintains a stable pH range; (2) there is reduced inhibition from free ammonia and free NO<sub>2</sub><sup>-</sup>-N of the nitrification reaction, with enhanced nitrification reaction rates; and (3) SND can use PHAs as a carbon source. A survey of the literature shows that no work has been done so far regarding landfill leachate treatment by SND in a modified SBBR, but it is very meaningful to realize SND in a SBBR system.

The objectives of this study were: (1) to determine the operating parameters and investigate the variation in biofilm indicators, such as biomass, density and extracellular polymeric substance (EPS) concentration; (2) to monitor the variation in COD, TN, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, COD and TN removal rates, PHA content, pH, DO, oxidation-reduction potential (ORP), *etc.* over the whole operation period and typical cycles of period II; (3) to investigate mechanisms of carbon transformation and nitrogen removal; and (4) to investigate the structures and characteristics of dominant bacterial communities.

## 2. Materials and methods

### 2.1 Experimental setup

A polymethyl methacrylate SBBR reactor was used for the experiments, as shown in Fig. 1a. A laboratory scale cylindrical SBBR reactor with a height of 50 cm, diameter of 20 cm, and working volume of 10 L was used. The SBBR reactor included a Multi 3620 analyzer for the detection of pH, DO and ORP,

mechanical stirrers, an air diffuser, an air compressor, a heating belt, and a temperature control box. The air diffuser is connected to the air compressor for the aeration of the SBBR. The heating belt and temperature control box constitute a temperature control device to regulate the temperature. The biofilm filler consists of polyurethane and other polymer materials, referred to as PPC, and the PPC characteristics are shown in Table 1.

### 2.2 Influent media, sludge and filler

Raw landfill leachate for this study was taken from the Ji Yang MSW Sanitation Landfill Site, Jinan, Shandong Province, China. The characteristics of the landfill leachate are shown in Table 2. The seed sludge was obtained from return sludge from the Everbright Sewage Treatment Plant, Jinan, Shandong Province, China. The concentrations of mixed liquid suspended solids (MLSSs), mixed liquor volatile suspended solids (MLVSSs) and the sludge volume index (SVI) were approximately 9230 mg L<sup>-1</sup>, 7523 mg L<sup>-1</sup> and 123 mL g<sup>-1</sup>, respectively.

### 2.3 Operational procedure

The operation mode of the SBBR system includes: (a) a filling phase (5 min); (b) a pre-anoxic phase (60 min); (c) an aeration phase (aeration time); (d) an anoxic phase (anoxic time); (e) a settling phase (30 min); (f) an effluent phase (5 min); and (g) an idle phase (idle time), as shown in Fig. 1b. The aeration time and anoxic time were determined from the ammonium valley (pH variation) and nitrate knee (ORP variation) real-time control methods.<sup>20</sup>

The volumetric exchange rate was 30%. The temperature was controlled at 25 ± 1 °C. The DO concentration was maintained over a range from 2.0 to 3.0 mg L<sup>-1</sup> during the aeration phase.

### 2.4 Method determination and calculations

The pH, DO, ORP, and temperature were monitored using a Multi 3620 analyzer (WTW Company, Germany). NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, COD, MLSSs, MLVSSs, and the SVI were measured using standard methods.<sup>21</sup> The total nitrogen (TN)

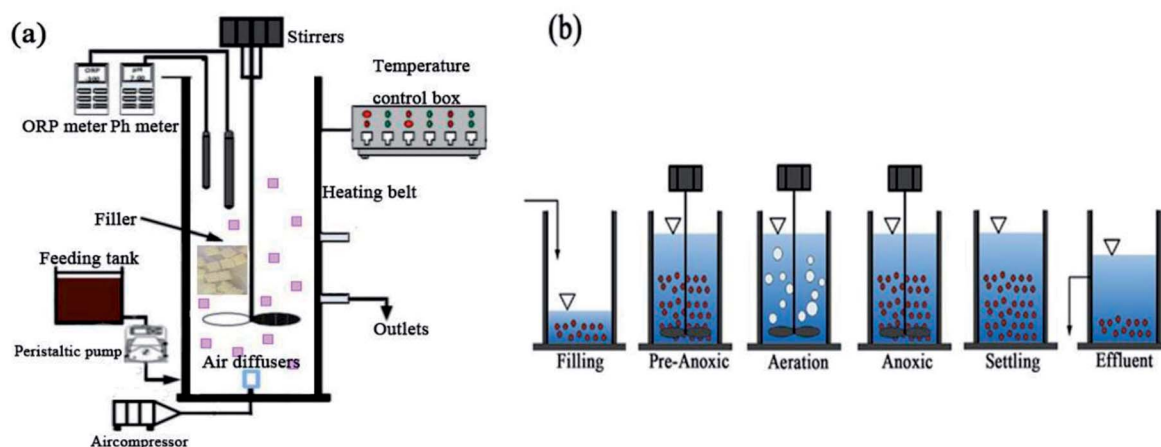


Fig. 1 Schematic diagrams of the SBBR device (a); and the operational mode (b).



Table 1 The characteristics of PPC

Item	Edge length (mm)	Density (kg m <sup>-3</sup> )	Surface area (m <sup>2</sup> m <sup>-1</sup> )	Porosity (%)	Hanging film time (days)	Attachments (mg)
Parameter	10 ± 1	12.5 ± 0.7	>4000	98	3–7	0.02

Table 2 The characteristics of the influent landfill leachate

Compound	pH	NH <sub>4</sub> <sup>+</sup> -N (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )	NO <sub>x</sub> <sup>-</sup> -N <sup>a</sup> (mg L <sup>-1</sup> )	BOD <sub>5</sub> (mg L <sup>-1</sup> )	COD (mg L <sup>-1</sup> )	C/N
Range	8 ± 0.3	1000 ± 100	1100 ± 100	0.5–1	4500 ± 500	6500 ± 500	>6

<sup>a</sup> NO<sub>x</sub><sup>-</sup>-N includes NO<sub>2</sub><sup>-</sup>-N, and NO<sub>3</sub><sup>-</sup>-N.

was analyzed using a TN analyzer (Multi N/C3000, Germany). The extraction and determination of EPS were performed using the method of Zhang *et al.*<sup>22</sup> The extraction of PHAs was performed using the method described in Albuquerque *et al.*<sup>23</sup> The PHA content was determined *via* gas chromatography, calculated using eqn (1) and (2). The determination method for the biofilm indices (biomass and density) comes from the literature;<sup>24</sup> biomass and density were calculated using eqn (3) and (4). The contribution of SND to the TN removal rate was calculated using eqn (5). The nitrite accumulation rate was calculated using eqn (6). Eqn (7) shows the reduction process of NO<sub>2</sub><sup>-</sup>-N. DNA extraction and Illumina high-throughput sequencing analysis of the SBBR and seed-sludge were performed by Novogene Co., Ltd (Beijing, China).

$$Y(\text{PHA}) = \frac{\text{PHA (mg COD)}}{X(\text{g COD}) + \text{PHA (g COD)}} \quad (1)$$

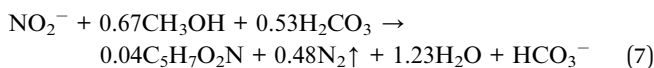
$$X = D_W(\text{g COD}) - \text{PHA (g COD)} \quad (2)$$

$$Q(\text{biofilm}) = \frac{\text{MLVSS (g L}^{-1}\text{)}}{A(\text{m}^2)} \quad (3)$$

$$P(\text{biofilm}) = \frac{Q(\text{biofilm})}{S} \quad (4)$$

$$\text{SND contribution (\%)} = \left( \frac{C(\text{TN}_{\text{end aeration}}) - C(\text{TN}_{\text{eff}})}{C(\text{TN}_{\text{inf}}) - C(\text{TN}_{\text{eff}})} \right) \times 100\% \quad (5)$$

$$\text{Nitrite accumulation rate (\%)} = \frac{C(\text{NO}_2 \text{ end aeration})}{C(\text{TN}_{\text{end aeration}})} \times 100\% \quad (6)$$



where  $Y$  and  $D_W$  are the PHA content and dry biomass weight;  $Q_{\text{biofilm}}$ ,  $A$ , and  $P_{\text{biofilm}}$  are the biomass, surface area and density; and end aeration represents the end of the aeration phase.

## 2.5 Statistical analysis

All statistical analyses were performed using the statistical program SPSS 19.0 (SPSS, Chicago, USA). Three-sample  $t$ -tests were used to evaluate the significance difference for each group. The data are all averages of two or more replicates. The results were analyzed to be statistically significant when  $p < 0.05$  (significance above 95%).

## 3. Results and discussion

### 3.1 Variation of biofilm indices

The biomass, density and EPS concentration are important biofilm indices, and the stability of the biofilm determines the stability of the SBBR system. The overall SBBR operation period was divided into the load-increasing period I (69 days) and the steady operation period II (41 days). As shown in Fig. 2, the biomass and density increased rapidly in period I and then gradually stabilized in period II as the influent load increased. The density of the internal and surface biofilm layers increased from  $5 \pm 0.5$  to  $16 \pm 1$  mg cm<sup>-3</sup> and from  $3 \pm 0.5$  to  $7 \pm 1$  mg cm<sup>-3</sup>, respectively, which demonstrates that the density increases significantly with depth. The biomass increased with

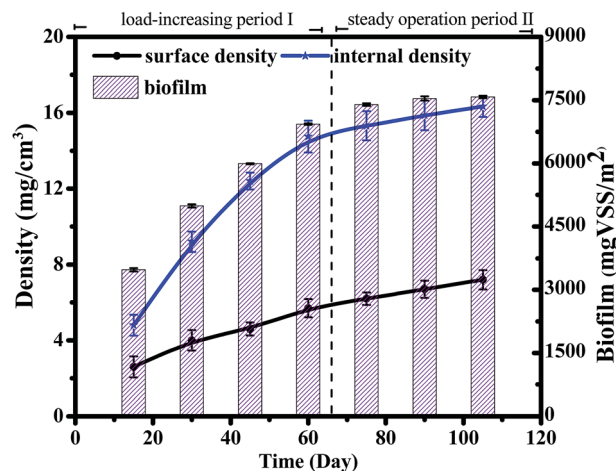


Fig. 2 The variation of biofilm indices.



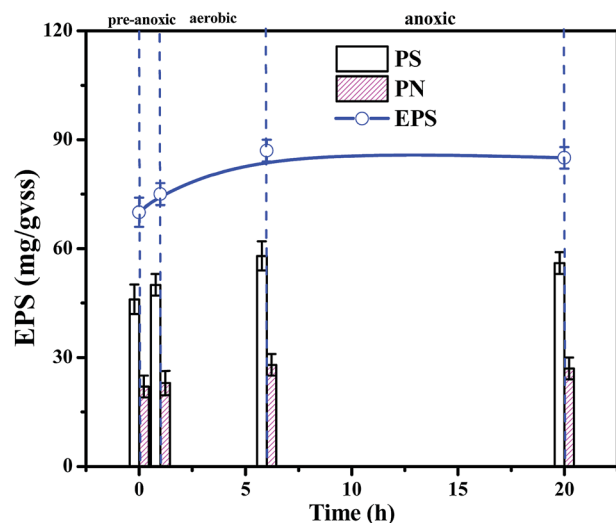


Fig. 3 The variation of EPS over typical cycles of stability period II.

the increase in density; biomass increased from  $3026 \pm 20$  to  $7504 \pm 30$  VSS/m<sup>2</sup>.<sup>25</sup>

EPS consists of polysaccharides (PS), proteins (PN), and nucleic acids.<sup>26</sup> As shown in Fig. 3, the EPS content is basically maintained at 70–80 mg/VSS during typical cycles of the stability period II, indicating that the biofilm system is stable and that EPS forms and stabilizes the biofilm structure together with filler, sludge, and biofilm.<sup>27</sup> And polysaccharides (PSs) were the main EPS component (60%) in the SBBR system. The stability of the biomass, density and EPS concentration during period II indicated that this operational set-up of the SBBR system is stable.

### 3.2 Treatment performance

**3.2.1 Carbon transformation.** As shown in Fig. 4b and c, influent and effluent COD concentrations (COD<sub>inf</sub> and COD<sub>eff</sub>) increased from  $350 \pm 10$  and  $20 \pm 2$  mg L<sup>-1</sup> to  $1100 \pm 50$  and  $550 \pm 50$  mg L<sup>-1</sup> during the load-increasing period I. In the steady operation period II, COD<sub>inf</sub> and COD<sub>eff</sub> were maintained at  $1100 \pm 50$  and  $550 \pm 50$  mg L<sup>-1</sup>, respectively, with a COD removal rate of  $85 \pm 3\%$ . The organic matter contained in the effluent is mainly refractory humic acid, humus, etc.,<sup>14</sup> so the COD<sub>eff</sub> value was maintained at approximately 600 mg L<sup>-1</sup>.

In a conventional SBBR, the removal of organic matter depends on the oxidation of aerobic heterotrophic bacteria in the aerobic phase; aerobic heterotrophic bacteria compete for oxygen (O<sub>2</sub>) more than nitrifying bacteria, and the nitrification reaction begins after the organic matter is removed.<sup>28</sup> As shown in Fig. 5b, the little variation between the COD<sub>eff</sub> and the end of pre-anoxic phase COD concentrations (COD<sub>end pre-anoxic</sub>) explained that the removal of organic matter mainly occurred in the pre-anoxic phases. There is no biodegradable organic matter present in the aeration phase; aerobic heterotrophic bacteria are gradually eliminated and nitrifying bacteria become the dominant bacteria. Therefore, the addition of pre-anoxic phases could not only remove organic matter, but

could also promote the rapid occurrence of the nitrification reaction.

As shown in Fig. 5b, the PHA content increased with decreasing COD<sub>inf</sub> in the pre-anoxic phase, which indicated that biofilm converted organic matter into PHAs. The PHA content (start of the pre-anoxic phase) increased from  $0.065 \pm 0.001$  Cmol to a maximum content (end of the pre-anoxic phase) of  $0.223 \pm 0.005$  Cmol, then decreased sharply to  $0.125 \pm 0.005$  Cmol (end of the aeration phase), and slowly reduced to  $0.065 \pm 0.005$  Cmol (end of the anoxic phase). The NO<sub>2</sub><sup>-</sup>-N concentration decreased as the PHA content decreased synchronously in the aeration and anoxic phases, which indicates that the removal of NO<sub>2</sub><sup>-</sup>-N requires the consumption of PHAs. The NH<sub>4</sub><sup>+</sup>-N concentration was not obviously increased during the aeration and anoxic phases, which explains that the biofilm used PHAs for the removal of NO<sub>2</sub><sup>-</sup>-N, rather than carbon sources from cell lyses. The synthesis of PHAs occurs in the pre-anoxic phase, and the consumption of PHAs occurs in the aeration and anoxic phases, indicating that biofilm converts organic matter into intracellular PHAs in the pre-anoxic phase and consumes PHAs for the removal of NO<sub>2</sub><sup>-</sup>-N in the aeration phase and the anoxic phase.

Fig. 4b shows that the end of pre-anoxic phase PHA content (PHA<sub>end pre-anoxic</sub>) increased from 0.1 Cmol to 0.22 Cmol, as the improvement in COD<sub>inf</sub> demonstrated that the PHA content threshold in the microbial cells can be increased. A theoretical COD conversion value of 1 Cmol PHA is approximately 36 g COD, so the theoretical COD conversion value of PHA<sub>end pre-anoxic</sub> (0.22 Cmol) is 5.38 g COD; the reduction value of COD was  $6.0 \pm 0.3$  g COD, so the theoretical COD conversion value was less than the reduction value of COD, which suggests that part of the organic matter was converted to PHAs and part of it was used for metabolism or other aspects.<sup>29,30</sup>

**3.2.2 Nitrogen removal.** As shown in Fig. 4a and c, the influent and effluent TN concentrations (TN<sub>inf</sub> and TN<sub>eff</sub>) increased from  $60 \pm 5$  and  $20 \pm 2$  mg L<sup>-1</sup> to  $1100 \pm 50$  and  $10 \pm 50$  mg L<sup>-1</sup> during the load-increasing period I. In the steady operation period II, TN<sub>inf</sub> and TN<sub>eff</sub> were  $1100 \pm 50$  and  $10 \pm 2$  mg L<sup>-1</sup>, respectively, with the TN removal rate maintained at 98%. The contributions of simultaneous nitrification and denitrification (SND) and endogenous denitrification to the TN removal rate were approximately 60% and 40%, respectively.

Fig. 5b shows that the NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations quickly increase as the NH<sub>4</sub><sup>+</sup>-N concentration decreases during the whole aeration phase. The nitrification reaction becomes the dominant aerobic reaction in the aeration phase and aerobic heterotrophic bacteria were gradually eliminated due to a lack of organic matter; this result is consistent with the conclusions when discussing carbon transformation. With the increase in the influent load, the end of aeration phase NO<sub>2</sub><sup>-</sup>-N concentration (NO<sub>2</sub><sup>-</sup>-N<sub>aeration</sub>) started to exceed the end of aeration phase NO<sub>3</sub><sup>-</sup>-N concentration (NO<sub>3</sub><sup>-</sup>-N<sub>aeration</sub>) at point A (Fig. 4a), then the NO<sub>3</sub><sup>-</sup>-N<sub>aeration</sub> concentration gradually decreased below 1 mg L<sup>-1</sup> and the nitrite accumulation rate increased to over 98%. It is indicated that the SBBR system realizes the short-range nitrification reaction in the aeration phase. The achievement of the short-range nitrification reaction





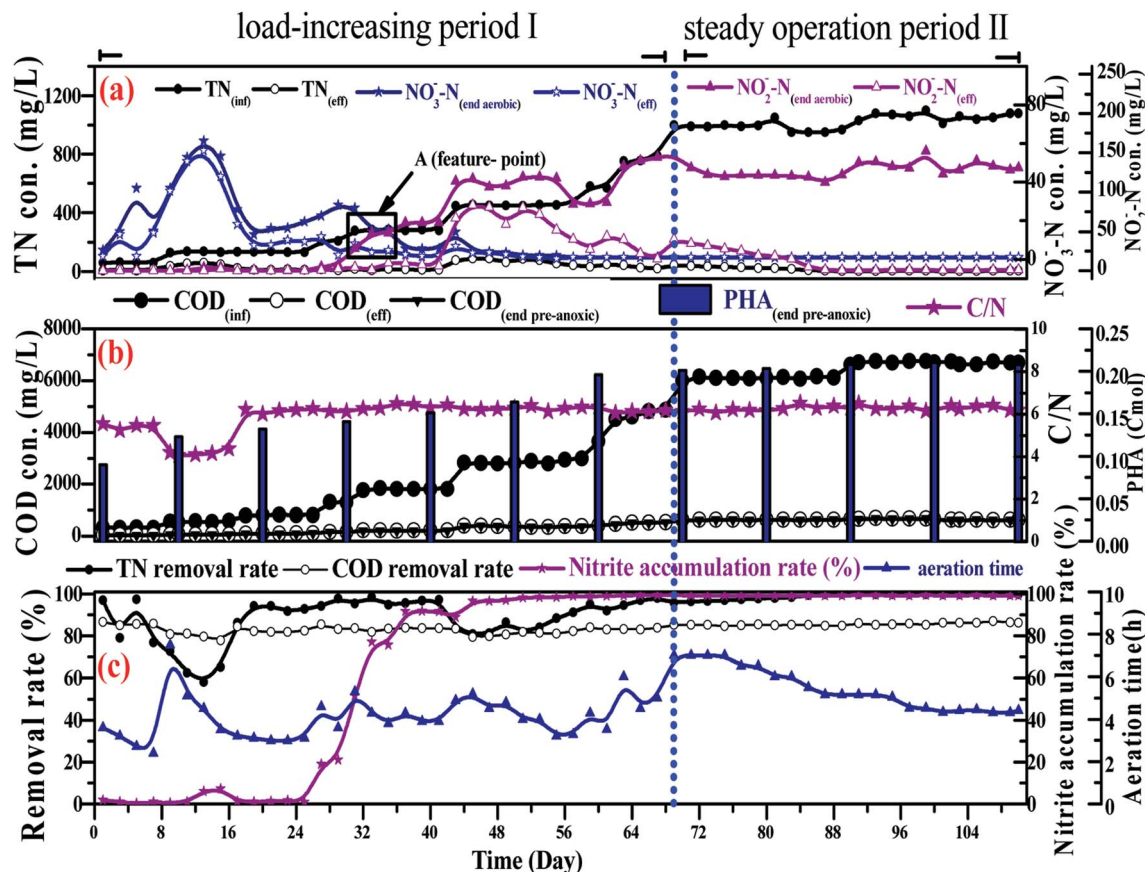


Fig. 4 The treatment performance of the SBBR over the whole operation period.

depends on the influent load; a high influent load has high free ammonia (FA) and leachate toxicity concentrations. High FA and leachate toxicity concentrations can inhibit the activity of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), but the inhibition thresholds of AOB ( $10\text{--}150\text{ mg L}^{-1}$ ) were smaller than those of NOB ( $0.1\text{--}1.0\text{ mg L}^{-1}$ ), and AOB shows stronger adaptability and resistance than NOB.<sup>31</sup> In this study, NOB was gradually eliminated and AOB eventually became the dominant nitrobacteria, resulting in the short-cut nitrification reaction becoming the dominant nitrification reaction, with the product after the end of the aeration phase being basically all  $\text{NO}_2^-$ -N. The short-cut nitrification reaction can increase the TN removal rate through the following properties: (1) economizing carbon source and oxygen consumption; (2) reducing the aeration time; (3) reducing alkali consumption; and (4) increasing the PHA content of the initial anoxic phase.

Biofilm has a large amount of PHAs at the beginning of the aeration phase; the PHAs could be used as a carbon source for simultaneous nitrification and denitrification (SND) to remove  $\text{NO}_2^-$ -N. As shown in Fig. 5b, the theoretical  $\text{NO}_2^-$ -N value produced by nitrification is  $3 \pm 0.3\text{ g N}$ , but the actual end of aeration phase  $\text{NO}_2^-$ -N value (in the range of 1.3 to 1.5 g N) is less than the theoretical value; the PHA content is reduced from the aeration stage, indicating that some  $\text{NO}_2^-$ -N is reduced through SND. SND only exist in an anaerobic area:

a traditional activated sludge process achieves SND through controlling the DO concentration in the range of  $0.5$  to  $1\text{ mg L}^{-1}$ . However, this SBBR system could achieve SND when the DO concentration was controlled in the range of  $2\text{--}3\text{ mg L}^{-1}$ , which was due to the presence of anaerobic zones in the biofilm inner layer. SND could increase the TN removal rate through the following properties: (1) alkalis produced by SND made up for alkalis consumed by short-cut nitrification, which maintained a stable pH range; (2) the reduced inhibition of short-range nitrification by free nitrite; and (3) enhanced nitrification reaction rates.

The concentration of  $\text{NO}_2^-$ -N decreased as the PHA content decreased during the anoxic phase, which indicated that endogenous denitrification used PHAs as a carbon source for nitrogen removal. Van Aalst *et al.* showed that cells preferentially used PHAs as a metabolic carbon source rather than a denitrification carbon source when the PHA content was at a critical level,<sup>32</sup> suggesting that the PHA content determined the contribution of endogenous denitrification to the TN removal rate. Given that: (1) the  $\text{NO}_2^-$ -N reduction value was  $3 \pm 0.3\text{ g N}$ ; (2) each reduction of 1 g of  $\text{NO}_2^-$ -N could consume 1.71 g COD, theoretically (eqn (7)); (3) the theoretical COD value (COD-1) for the reduction of  $\text{NO}_2^-$ -N was 5.13 g COD; (4) the  $\text{PHA}_{\text{pre-anoxic}}$  content was above 0.2 Cmol; (5) the theoretical COD value corresponding to the PHA content was approximately 7.2 g COD (COD-2); and (6) COD-2 is more than



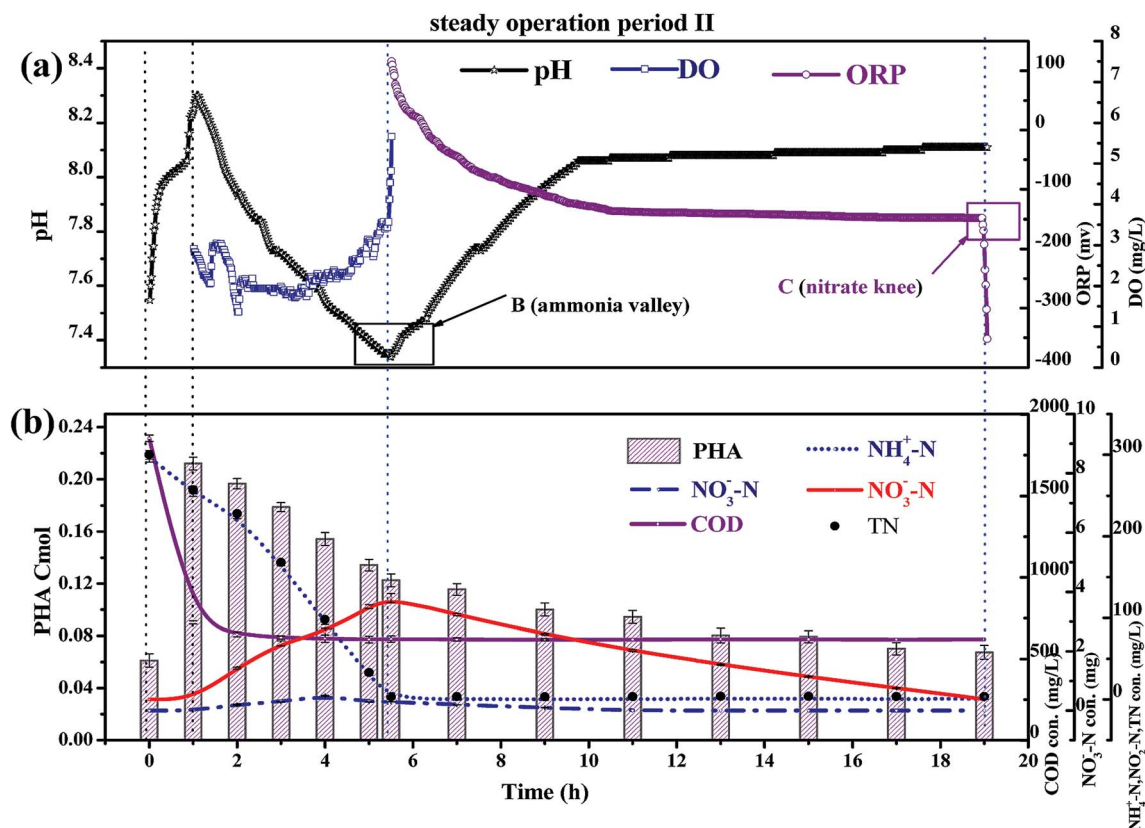


Fig. 5 The variation of indices over typical cycles of stability period II.

COD-1, the PHA content was sufficient to achieve the reduction of  $\text{NO}_2^-\text{-N}$ . In other words, the biofilm has sufficient PHA content to act as a carbon source for SND and endogenous denitrification to remove TN. Endogenous denitrification could use PHAs as a carbon source for nitrogen removal to guarantee a lower effluent TN concentration.

The mechanisms for carbon transformation and nitrogen removal during landfill leachate treatment are: (1) biofilm converts influent organic matter into intracellular PHAs during the pre-anoxic phase; (2) the realization of short-range nitrification saves carbon sources and aeration time during the aeration phase; (3) the synergistic effects of SND and short-range nitrification promote the nitrification reaction rate and maintain a constant pH; and (4) SND and endogenous denitrification use PHAs as a carbon source to remove nitrogen.

### 3.3 diversity of microbial communities

Statistics showing the alpha diversity of the SBBR and seed-sludge at a 97% consistency threshold are shown in Table 3. The coverage index indicates the good coverage of the sequencing. The

community composition, species richness, and diversity of the seed-sludge were higher than those of the SBBR. At the phylum level for the SBBR, as shown in Fig. 6a, *Proteobacteria* (59.38%) and *Bacteroidetes* (30.28%) were the dominant communities, accounting for 89.66% of the total bacteria. The abundance of *Bacteroides* in the SBBR was higher than that in the seed sludge, and this may be related to the quality of the landfill leachate.<sup>33</sup> At the genera level for the SBBR, as shown in Fig. 6b, the top four genera (with relative abundances higher than 1%) were *Paracoccus* (28.60%), *Thauera* (10.34%), *Nitrosomonas* (5.89%) and *Truepera* (3.42%), accounting for 48.25% of the total bacteria.

In the nitrobacteria community, unidentified-Nitrospiraceae (NOB) presented in the seed sludge finally disappears; *Nitrosomonas* (AOB) becomes the dominant nitrobacteria and *Nitrosomonas europaea* (AOB, 5.57%) becomes the dominant species with the most abundance. This explains that the short-cut nitrification reaction is the dominant nitrification reaction, and this result is consistent with the conclusions made when discussing nitrogen removal. *Paracoccus*, *Thauera*, and *Truepera* belong to the denitrifying bacteria community, and account for 42.36% of the total

Table 3 Alpha diversity indices for 97% sequence similarity

Group	Observed species	Shannon	Simpson	Chao	Coverage
SBBR	572	4.80	0.889	655.59	0.998
Seed-sludge	1424	8.09	0.995	1506.28	0.997



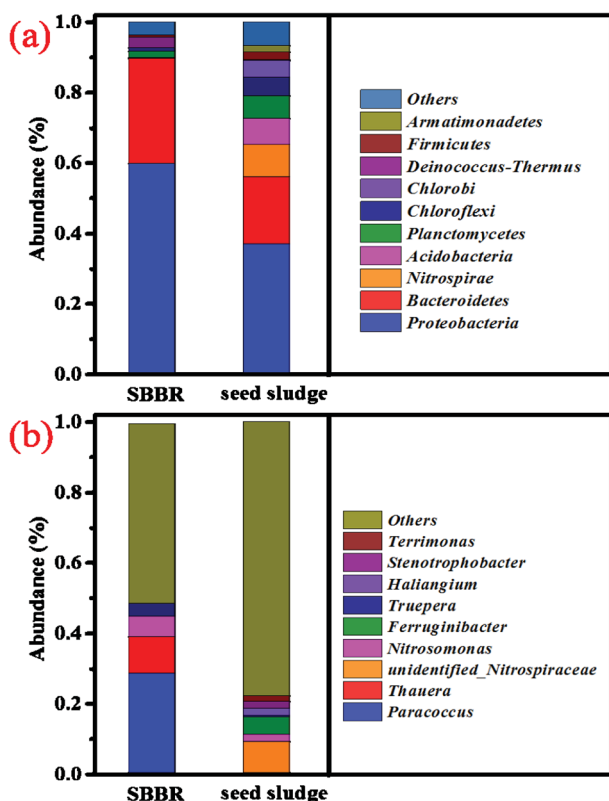


Fig. 6 The microbial diversity of the top 10 examples at the phylum level (a); and the microbial diversity of the top 9 examples at the genus level (b).

bacteria. *Paracoccus*<sup>34</sup> and *Thauera*<sup>35</sup> have the ability to transform organic matter into PHAs, accounting for 38.94% of the total bacteria. *Thauera* and *Truepera* can degrade bio-refractory organic matter and resist harsh environments.<sup>36</sup> This shows that ammonia-oxidizing bacteria and denitrifying bacteria with the ability to transform organic matter into PHAs become the dominant bacterial communities.

## 4. Conclusions

A modified SBBR, with the addition of a pre-anoxic phase before the aeration phase in a conventional SBBR, was used to treat landfill leachate. The system achieves stable operation with 110 days of operation, and COD and TN removal rates were 83–88% and 95–98%. In the pre-anoxic phase, biofilm converts organic matter into intracellular PHAs. In the aeration phase, short-range nitrification removes  $\text{NH}_4^+\text{-N}$  to produce  $\text{NO}_2^-\text{-N}$ , and SND uses PHAs as a carbon source to remove  $\text{NO}_2^-\text{-N}$ . In the anoxic phase, endogenous denitrification uses PHAs as a carbon source to remove residual  $\text{NO}_2^-\text{-N}$ . Ammonia-oxidizing bacteria and denitrifying bacteria with the ability to transform organic matter into PHAs become the dominant bacterial communities.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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