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Effects of additional fermented food wastes on nitrogen removal enhancement and sludge characteristics in a sequential batch reactor for wastewater treatment

Yongmei Zhang[1,2] · Xiaochang C. Wang[1,3] · Zhe Cheng[1,2] · Yuyou Li[4] · Jialing Tang[1,2]

Abstract In order to enhance nitrogen removal from domestic wastewater with a carbon/nitrogen (C/N) ratio as low as 2.2:1, external carbon source was prepared by short-term fermentation of food wastes and its effect was evaluated by experiments using sequencing batch reactors (SBRs). The addition of fermented food wastes, with carbohydrate (42.8 %) and organic acids (24.6 %) as the main organic carbon components, could enhance the total nitrogen (TN) removal by about 25 % in contrast to the 20 % brought about by the addition of sodium acetate when the C/N ratio was equally adjusted to 6.6:1. The fermented food waste addition resulted in more efficient denitrification in the first anoxic stage of the SBR operation cycle than sodium acetate. In order to characterize the metabolic potential of microorganisms by utilizing different carbon sources, Biolog-ECO tests were conducted with activated sludge samples from the SBRs. As a result, in comparison with sodium acetate, the sludge sample by fermented food waste addition showed a greater average well color development (AWCD$_{590}$), better utilization level of common carbon sources, and higher microbial diversity indexes. As a multi-organic mixture, fermented food wastes seem to be superior over mono-organic chemicals as an external carbon source.

Keywords Fermented food wastes · Sodium acetate · Nitrogen removal · Sequential batch reactor

Introduction

In China, many wastewater treatment plants (WWTPs) have the problem of high nitrogen concentration residual in the treated effluent, which is mostly caused by a shortage of carbon source in the influent to sustain effective denitrification. Therefore, external carbon source addition is often required for enhancing nitrogen removal to meet the stringent standards for effluent discharge. The common external carbon sources utilized for this purpose are usually commercial chemicals such as glucose, sodium acetate, methanol, ethanol, etc. (Elefsiniotis and Li 2006; Lee and Welander 1996; Oskar et al. 2007; Peng et al. 2007; Chen et al. 2015). However, the use of commercial chemicals may not only increase the operation costs, but also deteriorate the effluent quality (Liu et al. 2012). In recent years, anaerobic fermentation products from the substrates that are rich in carbonaceous matter, such as food wastes, were found to be useful as external carbon sources (Lim et al. 2000). As food wastes are easily collectable, comparatively clean, free from toxic substances, and with high organic contents, they are considered as very promising substrates for the preparation of carbon source under the principle of turning "wastes" into "treasures."

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The anaerobic fermentation process proceeds in successive stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The conventional fermentation operation usually requires pH adjustment for maximizing the production of readily biodegradable organics, such as volatile fatty acids (VFAs) (Jiang et al. 2013; Lim et al. 2008; Kim et al. 2006), which further leads to higher methane production. Large amounts of chemicals are thus consumed, and longer time (5–10 days) is needed for completing the whole process (Chu et al. 2012). In contrast to this, if the objective of fermentation is mainly for carbon source production, attention can only be paid to the first two stages, namely hydrolysis and acidification (Ramos et al. 2012). In fact, not only the readily biodegradable VFAs but also the acidification intermediates are utilizable by bacteria in biological reactors (Lee and Welander 1996; Elefsiniotis et al. 2004). These intermediates are also recognized as high-quality carbon sources for denitrification (Elefsiniotis and Li 2006; Sage et al. 2006). The transformation of particulate carbon into these intermediates may not require long fermentation time. On the other hand, because VFAs and lactic acid are not the main target products, pH adjustment may not be required. Therefore, the so-called short-term free anaerobic fermentation which needs only 1–3 days may become suitable for the preparation of fermented food wastes to meet the needs for enhancing nitrogen removal in wastewater treatment. However, little information is available so far on the chemical components of fermented food wastes produced by short-term fermentation and its performance in enhancing nitrogen removal as being used as the external carbon source.

This study aims to verify the effect of nitrogen removal enhancement by using short-term free fermentation products from food wastes as alternative external carbon source for treating practical wastewater with low C/N ratio. Attention was also paid to the effect of external carbon source on the characteristics of activated sludge and microbial community diversity in the biological system. Sodium acetate, a typical commercial chemical carbon source, was used in the study for a comparison.

Materials and methods

Wastewater and seed sludge

In this study, wastewater came from the influent to biological treatment units after a fine screen (1 mm) in a campus WWTP in Xi'an, China, where an anaerobic-anoxic-oxic (A^2O) biological unit was under operation for secondary treatment, followed by a submerged MBR unit for water reclamation. The main characteristics of the wastewater are as follows: total chemical oxygen demand (TCOD) 117.7 ± 45.2 mg/L, soluble COD (SCOD) 68.4 ± 30.6 mg/L, total nitrogen (TN) 31.5 ±17.3 mg/L, ammonia nitrogen (NH_4-N) 29.1 ±13.5 mg/L, nitrite nitrogen (NO_2-N) 0.03 ± 0.01 mg/L, nitrate nitrogen (NO_3-N) 2.31 ± 1.1 mg/L, total phosphorus (TP) 3.2 ± 0.3 mg/L, and pH 7.8 ± 0.5. Based on the average SCOD and TN, the C/N ratio of the wastewater was only about 2.2, indicating the shortage of sufficient carbon for effective nitrogen removal. Sequencing batch reactors (SBRs) were operated for the experiment, and the seed sludge was the sediment of the sludge collected from the oxic tank of the A^2O unit under operation.

Fermented food wastes

The food wastes used in this study were collected from the student canteen of the university campus. They were mainly composed of leftovers of cooked foods, such as rice, noodles, meats, eggs, and vegetables. In each collection, bones and insert materials (paper and plastic) were discarded; only the fermentable materials were preserved. After selection, the wastes were crushed and homogenized in a blender to ensure a particle size of less than 5 mm. The main characteristics of the food wastes used in the study are as follows: total solid (TS) 17.4 ± 1.6 %, volatile solid (VS) 16.1 ± 2.6 %, carbohydrate 61.5 ± 11.1 g/L, protein 9.8 ± 2.6 g/L, lipid 9.2 ± 1.2 g/L, TCOD 229.7 ± 15.9 g/L, and SCOD 55.4 ± 4.2 g/L.

The anaerobic fermentation process referred to the authors' previous study (Zhang et al. 2016). It was proceeded in a sealed fermenter with a working volume of 10 L under a reaction temperature controlled at 25 °C through a water jacket connected to a water bath. The initial solid concentration was controlled by adding mass ratio of raw food waste and tap water as 3:1 (*w/w*). The fermenter was run in batch for 60 h until the SCOD and organic acids in the effluent became stable. No pH adjustment was conducted in the whole fermentation process. At the end of the batch fermentation, the mixture was moved to a centrifugal separator to remove most of the solids, and the liquid was filtered by a 0.45-μm membrane filter to obtain the fermented food wastes.

As will be discussed later, the fermented food wastes prepared by the above operation were with low organic acids, but a large portion of the organics is in the form of carbohydrates and other unidentified components. Due to the low protein content (about 3.3 %) of the original food wastes, the nitrogen concentration in the fermentation liquid was measured as 371.2 mg/L which was only about 0.6 % of the SCOD concentration as 62.6 g/L. Therefore, when the fermentation liquid was added to the influent of the SBR as external carbon source, the increase of the nitrogen content was insignificant (no more than 1 mg/L increase in TN concentration when 154 mg/L SCOD was added to adjust the C/N ratio to about 6:1).

Nitrate uptake rate tests

Batch nitrate uptake rate (NUR) tests were conducted to investigate the denitrification performances of the fermented food wastes in comparison with sodium acetate following the reported method (Kujawa and Klapwijk 1999; Zhang et al. 2016). The denitrifying sludge was first loaded into the 1-L sealed conical flask reactors to ensure that the sludge concentration was 2500 ± 200 mg volatile suspended solid /L, and then, 50 mg/L $NaNO_3$ and 300 mg/L carbon sources (fermented food wastes and sodium acetate, both measured as SCOD) were simultaneously added into the reactors so that the initial C/N ratio was 6:1. Nitrogen gas was immediately pumped into the reactors to establish an anoxic environment. The reactors were placed on stirring plates in an incubator at constant temperature (25 ± 1 °C). The initial pH was adjusted to 7 ± 0.2 using 0.5-M NaOH or 0.5-M HCl solution.

SBR setup and operation

Two lab-scale SBRs, each with a working volume of 4.5 L, were operated for investigating the effect of external carbon source addition (fermented food wastes and sodium acetate) on nitrogen removal performance. The reaction temperature was controlled at 25 ± 1 °C through water bath. The operation was in a multi-stage sequence of 2-h anaerobic (An), 2.5-h aerobic (OI), 2.5-h anoxic (AI), 1-h aerobic (OII), 1-h anoxic (AII), and 0.5-h aerobic (OIII) stages, followed by 1-h settling, 5-min decanting, and an 85-min idle period, so the total time for one operation cycle was 12 h (Fig. 3). However, in each operation cycle, only 2.25 L of the supernatant (half of the working volume) was decanted after settling, and equivalent volume (2.25 L) of wastewater was fed to the SBR in the initial stage of the next cycle. Therefore, the real hydraulic retention time (HRT) became 24 h. In the aerobic stages (OI, OII, and OIII), aeration was provided to keep the dissolved oxygen (DO) concentration at about 6 mg/L. Ten minutes before the end of OIII stage, part of the sludge was discharged to keep the solid retention time (SRT) at about 20 days. Gentle agitation was provided using a two-blade impeller to keep the liquor in a well-mixed condition. The operation cycle was controlled using an automatic controller under prescribed conditions.

For cultivating the seeded activated sludge, the SBRs were firstly operated for 30-day feeding with wastewater only and then with addition of external carbon sources (fermented food wastes or sodium acetate for each SBR) to half of the prescribed concentration (70 mg/L as SCOD) for another 15 days before the full concentration of external carbons (140 mg/L) was added to adjust the C/N ratio of the wastewater in the range of 6–7 (C as SCOD concentration and N as TN

concentration). Sampling and analysis started when the two SBRs reached stable conditions.

Sampling and chemical analysis

Water samples were analyzed after filtration through a 0.45-μm pore size Millipore filter. Ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were measured following standard methods (APHA 1999). COD was measured with a HACH apparatus (DBR 200, HACH, USA). Other parameters, such as temperature, DO, and pH, were measured using a HACH portable meter.

Mixed liquors were directly collected from the SBRs under well-mixed condition, and the analysis of mixed liquor suspended solids (MLSS) and mixture liquor volatile solids (MLVSS) was measured using standard methods (APHA 1999).

Regarding the organic components of the fermented food wastes, carbohydrate was measured by the phenol-sulfuric acid method using glucose as the standard (Herbert et al. 1971), protein was measured by the Lowry-Folin method with bovine serum albumin (BSA) as the standard (Lowry et al. 1951), and lipid was measured by the Soxhlet extraction method (Kobayashi et al. 2012).

VFA concentration was determined using a gas chromatograph (GC, Agilent 6890N) equipped with a flame ionization detector (FID) and a WAXETR column (30 m × 0.25 mm × 0.25 μm). N_2 was the carrier gas fed at a flux of 20 ml/min. The temperatures of the injection port and the detector were maintained at 200 and 250 °C, respectively. The GC oven process was conducted according to the column temperature ramp program (100 °C, 2 min; 3 °C/min; 160 °C; 2 min; 250 °C), and the sample injection volume was 1 μL.

Lactic acid concentration was determined using a liquid chromatograph (LC, Shimadzu Co., Ltd.) equipped with an ultraviolet detector. COSMOSIL 5C18-II was used for the column, and 0.05 mM phosphoric acid buffer liquid (50 mM NaH_2PO_4/50 mM H_3PO_4 = 9:1) was used as the carrier liquid. The analysis was conducted with detector temperature of 40 °C, flow velocity of 1 ml/min, and UV value of 210 nm.

From the mixed liquor samples, the particle size distribution (PSD) of the activated sludge particles was analyzed using a laser granularity distribution analyzer (LS 230/SVM+, Beckman Coulter Corporation, USA) with a detection range of 0.4–2000 μm.

For extracellular polymeric substance (EPS) analysis, the sampled mixed liquor was first centrifuged at 6000 rpm and 4 °C for 10 min using a centrifuge (Model TGL-16M, Shanghai, China). Then, 25 ml of supernatant was filtered through a 0.22-μm filter to obtain the soluble EPS (SEPS). The residues were removed and re-suspended to the original volume in a buffer solution (2 mM Na_3PO_4, 4 mM

NaH$_2$PO$_4$, 9 mM NaCl, 1 mM KCl, pH = 7) to prepare the suspension for obtaining other types of EPS. The loosely bound EPS (LBEPS) was extracted from the suspension using ultrasound for 2 min, centrifuging at 1000 rpm and 4 °C for 20 min, and then filtering the supernatant through a 0.22-μm filter. Finally, the same extraction process was repeated for the extraction of tightly bound EPS (TBEPS), except that the suspension was heated at 60 °C for 30 min instead of using the ultrasound. The analyses of the extracted SEPS, LBEPS, and TBEPS were conducted for protein using the Lowry method with BSA as the standard reference (Lowry et al. 1951) and for polysaccharides using the phenol-sulfuric acid method with glucose as the standard reference (Herbert et al. 1971).

To determine the fingerprint of the activated sludge bacterial community functional diversity, a Biolog-ECO plate (Biolog, Inc., Hayward, CA, USA) with 31 sole carbon sources was employed. First, the sludge samples were diluted to 1:1000 with a sterilized NaCl solution (0.85 %, w/v), and 150 μL of the diluted mixture was added to each plate well using eight channel pipettes. Then, the inoculated ECO plates were packed into polyethylene bags to reduce desiccation while incubating at 25 °C in darkness. The absorbance (OD$_{590\ nm}$) of the wells was recorded for 240 h using an ELISA plate reader at every 24-h interval. The results obtained at 144 h were used for the diversity index analysis. The bacterial community activity in each ECO plate was expressed as the average well color development (AWCD$_{590}$). The functional diversity index including the McIntosh index and Shannon's index was measured. The calculations for all the above-mentioned parameters followed the documented methods (Magurran 1998; Hu et al. 2015).

Results and discussion

Denitrification performance of fermented food wastes in comparison with sodium acetate

The main carbon components of the fermented food wastes were analyzed as carbohydrates (42.8 %), lactic acid (18.3 %), acetic acid (5.1 %), propionic and butyric acids (1.2 %), and protein (3.3 %). The remaining 29.3 % were unidentifiable SCOD. The organic acids all together took less than 24.6 % of the SCOD. The high content of carbohydrates was an indication of incomplete acidification and acetogenesis in the short-term free anaerobic fermentation. This resulted in a much lower organic acid production than the conventional fermentation process where VFAs could take about 50 % of the SCOD (Jiang et al. 2013; Kim et al. 2008).

In order to evaluate the denitrification performance of fermented food wastes, NUR tests were conducted in comparison with sodium acetate which is believed to be one of the most favorable carbon sources for denitrifying bacteria. As shown in Fig. 1, sodium acetate could be utilized by denitrifying bacteria easily to bring about an almost linear decrease in the first 40 min in NO$_3$-N and TN or NO$_x$-N which is the nitrate utilization curve obtained as NO$_x$-N = NO$_3$-N + 0.6 NO$_2$-N based on the fact that the reduction of 1 g NO$_2$-N to produce 1 g of gaseous N$_2$ requires the same number of electrons as the reduction of 0.6 g NO$_3$-N to produce 0.6 g of gaseous N$_2$ (Henze 1986; Cokgör et al. 1998), together with a gradual accumulation of NO$_2$-N. After t = 50 min, almost all forms of nitrogen reached a very low level (TN or NO$_x$-N concentration about 1 mg/L)

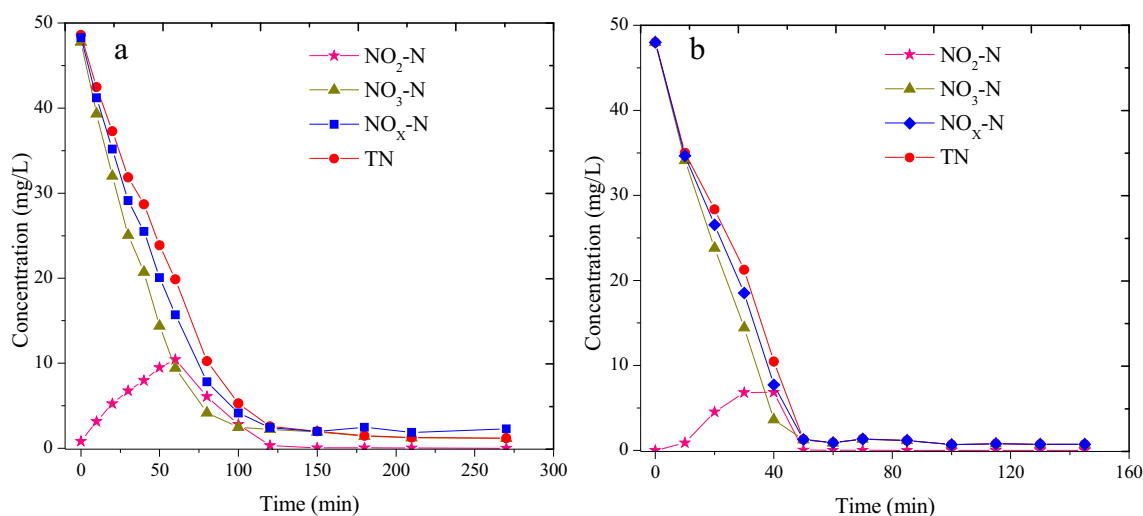**Fig. 1** Variation of NO$_3$-N, NO$_2$-N, TN, and NO$_X$-N (NO$_3$-N + 0.6 × NO$_2$-N) in denitrification processes using fermented food wastes (**a**) and sodium acetate (**b**) as carbon sources

until the end of the NUR test. As for fermented food wastes, the variation in the concentrations of all these nitrogen forms was similar to that for sodium acetate but took apparently longer time for NO_3-N and TN or NO_x-N to reach the minimum concentrations (about 120 min for fermented food wastes vs. 50 min for sodium acetate). Although the final denitrification result was not as better as sodium acetate, the residual TN or NO_x-N concentration was below 3 mg/L.

Two characteristic denitrification parameters, namely denitrification potential (P_{DN}) and denitrification rate (V_{DN}), were calculated based on Fig. 1 following the methods proposed by Sage et al. (2006) for comparing the fermented food wastes with sodium acetate. As a result, $V_{DN} = 12.89$ mg N/g VSS h and $P_{DN} = 0.174$ g N/g COD were calculated for the fermented food wastes, in contrast to $V_{DN} = 15.69$ mg/g VSS h and $P_{DN} = 0.182$ g N/g COD for sodium acetate. Although the P_{DN} and V_{DN} for the fermented food wastes were slightly lower than that for sodium acetate, the fermented food wastes showed sufficiently good denitrification performance.

On the other hand, following the method proposed by Sage et al. (2006) to evaluate the readily biodegradable fraction (S_S) of the fermented food wastes, $S_S = 58.4$ % was obtained, indicating that the fermented food wastes are highly utilizable organics by microorganisms. In fact, large fractions of the SCOD in the fermented food wastes were not organic acids but fermentation intermediates which were either already readily biodegradable or bio-utilizable through longer reaction time as indicated by Fig. 1a.

Comparison of nitrogen removals with fermented food wastes and sodium acetate as supplementary carbon sources in SBR

Overall nitrogen removal performances

Figure 2 compares the performances of nitrogen removal in the two SBRs in continuous operation under identical conditions except for external carbon sources, namely fermented food wastes and sodium acetate, added to adjust the C/N ratios of the wastewater to the same level (about 6.6:1 based on SCOD and TN concentrations). Continuous monitoring of the influent TN concentration (TN_{in}), effluent nitrogen components (NH_4-N_{out}, NO_3-N_{out}, and NO_3-N_{out}), and TN removal started on day 51 after both SBRs reached stable conditions.

As shown in Fig. 2, the fermented food wastes were competitive with or even slightly better than sodium acetate for enhancing nitrogen removal because the average TN removals from day 51 to day 110 were about 75 and 70 % for the fermented food wastes and sodium acetate, respectively. It indicated a significant improvement of nitrogen removal based on the TN removal of about 50 % when no external carbon source was added (C/N ratio about 2.2:1) and about 58 % when either fermented food wastes or sodium acetate was added with half of the prescribed dose (C/N ratio about 4.4:1).

From the viewpoint of biological nitrogen removal (BNR), the organic carbon in terms of COD in the wastewater may include readily biodegradable, slowly biodegradable, inert soluble, and inert particulate fractions. The total fraction of the readily and slowly biodegradable COD together determines the nitrogen removal potential in a BNR process
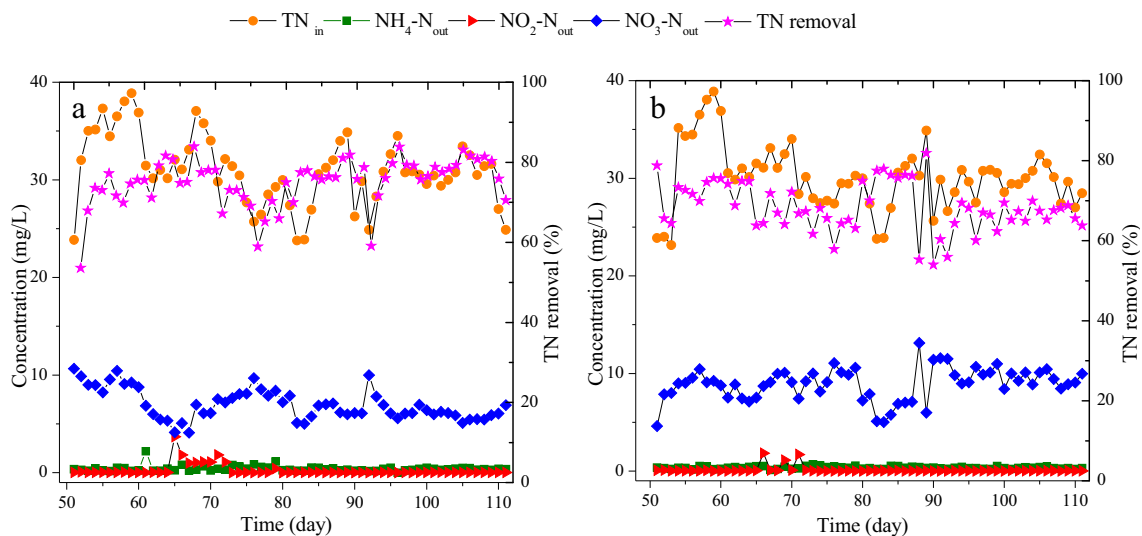

Fig. 2 TN removals and nitrogen components in the treated effluents when fermented food wastes (**a**) and sodium acetate (**b**) were used as carbon sources in long-term operation of SBRs

(Kruhne et al. 2003), and an effective BNR would require a biodegradable COD fraction as high as 96 % of the SCOD (Ekama et al. 1986; Wu et al. 2014). Sodium acetate utilized as the external carbon source is no doubt completely biodegradable. However, competitive BNR was also achieved when fermented food wastes were added; it can thus be concluded that most of the SCOD contained in the fermented food wastes was practically biodegradable though the fraction of organic acids was not sufficiently high.

For a complete denitrification of 1 g of nitrogen, 4–15 g of SCOD would be practically required (Kujawa and Klapwijk 1999; Nyberg et al. 1992). Therefore, the minimum C/N ratio required for complete denitrification should be no less than 4:1 in terms of SCOD. The original C/N ratio of the wastewater in this study (about 2.2:1) was apparently a poor condition for BNR, and if more than 70 % of nitrogen removal was targeted, it was required to adjust the C/N ratio to higher than 6:1 according to the experimental results.

Organic acids are known as effective carbon source for BNR (Elefsiniotis and Li 2006; Sage et al. 2006). However, the large fractions of carbohydrates and other organic constituents in the fermented food wastes may also belong to biodegradable organic carbon for BNR. On the other hand, comparing with sodium acetate, the fermented food wastes may contain a variety of readily or slowly biodegradable organic constituents which can be utilized by more heterogeneous and metabolically versatile microflora (Lee and Welander 1996). This will be further discussed later in "Metabolic characteristics of microorganism" section.

Nitrogen transformation and removal in a single operation cycle without and with external carbon source addition

In order to gain knowledge on nitrogen transformation and removal in a single operation cycle of the SBRs, the variations of various nitrogen forms were monitored along with the multi-stage operation sequence as shown in Fig. 3. Under the condition when no external carbon source was added to the wastewater (Fig. 3a), moderate decrease of NH_4-N was firstly observed in the 2-h anaerobic stage (An) with slow increase of NO_2-N while the NO_3-N concentration was almost unchanged. This is an indication of the initial nitrification and denitrification using the biodegradable SCOD contained in the wastewater which resulted in about 20 % TN removal. When the operation was switched to the first aerobic stage (OI), a rapid decrease of NH_4-N was observed with a rapid increase of NO_3-N, which is an evidence of nitrification to convert most of the NH_4-N into NO_3-N. Denitrification also occurred to certain extent as evidenced by the decrease of NO_2-N to the minimum, which resulted in further TN removal for about 20 %. However, much change was not seen in the following anoxic and aerobic stages (AI, OII, AII, and OIII) together with a slight TN removal (less than 10 %). Insufficient biodegradable carbon in the wastewater should be the reason for such phenomena.

As fermented food wastes or sodium acetate was added to the wastewater, Fig. 3b is obtained to show the effects of external carbon source addition. In comparison with Fig. 3a, the tendency of variation in the various forms of nitrogen did

Fig. 3 Effects of carbon source addition on the variation of NH_4-N, NO_3-N, NO_2-N, and TN in an operation cycle of SBR (**a** no supplementary carbon source, **b** with fermented food wastes or sodium acetate (F) as supplementary carbon source (A)). *An* anaerobic stage, *OI* the first oxic stage, *AI* the first anoxic stage, *OII* the second oxic state, *AII* the second anoxic stage, *OIII* the third oxic stage, *Settling* settling stage
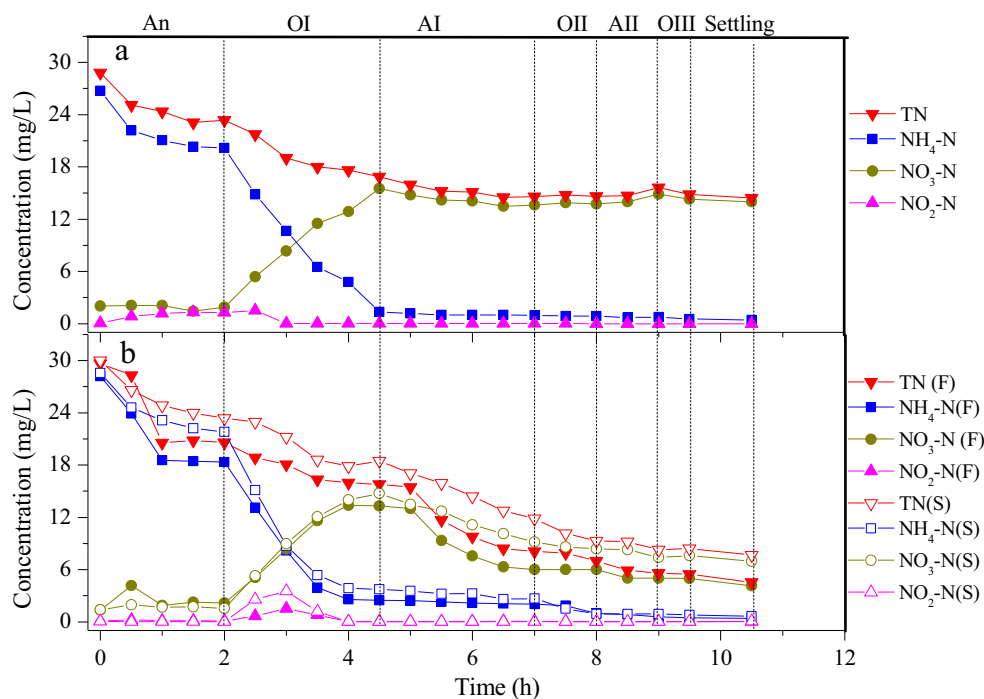
not differ much in the first two stages, namely An and OI, but TN decreased continuously in the later stages till the end of the operation cycle. As the operation condition turned to anoxic (AI), the NO_3-N accumulated in the former OI stage began to decrease under the assistance of the added carbon sources, but better denitrification could be achieved by the fermented food wastes than sodium acetate because of their different denitrification rates in this stage (V_{DN} = 1.12 and 0.64 mg N/g VSS h for fermented food wastes and sodium acetate, respectively). Although not as significant as the former stages, slight reduction of NH_4-N was further observed in the following OII and OIII stages while NO_3-N concentrations were further reduced in the AII stage and even during settling. The difference in the performance of the fermented food wastes and sodium acetate for denitrification in the AI stage greatly influenced the final TN removals which were evaluated from Fig. 3b as 71 and 82 %, respectively.

Regarding the performance of various external carbon sources for denitrification, the difference between single chemical such as acetic acid and mixtures such as fermentation products from food wastes, activated sludge, or other wastes was noticed by other studies (Lemos et al. 2006; Tong and Chen 2007; Zheng et al. 2009, 2010; Huang et al. 2015). Similar result was also reported by Zheng et al. (2010) who compared the fermentation liquid from waste activated sludge with acetic acid. It was reported that VFAs could be easily transported into the bacteria cells and utilized as a carbon source for polyhydroxyalkanoate (PHA) formation which could be degraded rapidly to provide energy for microorganism growth (Zheng et al. 2010), while PHA formation would not be very significant when fermentation mixture was used as external carbon source rather than acetic acid (Lemos et al. 2006; Tong and Chen 2007). The fast utilization of PHA may preferentially occur for the growth of many aerobic heterotrophic bacteria rather than denitrifying bacteria. This may help

us to explain the reason for the higher V_{DN} of the fermented food wastes than sodium acetate, as well as its better denitrification result shown in Fig. 3b.

Comparison of the physicochemical properties of the activated sludge with fermented food wastes and sodium acetate as external carbon sources

EPS and PSD

As shown in Fig. 4a, SEPS, LBEPS, and TBEPS were measured using sludge samples from the two SBRs with fermented food wastes and sodium acetate as external carbon sources. It is obvious that lower EPS concentration was detected when fermented food wastes were used, regarding either the total EPS (TEPS) or each EPS type. Such a result seemed to disagree with the common knowledge that the sludge fed with slowly biodegradable substrates may produce more EPSs than that fed with readily biodegradable organic matter (Yang and Li 2009). However, in other studies (Zhang and Bishop 2003; Sheng et al. 2010), it is also reported that, in a bioreactor, there may exist certain bacteria species which could produce enzymes to degrade polymeric organic substances and make them utilizable by other bacteria. It can thus be suggested that when the fermented food wastes were used as external carbon source, the chemical condition in the SBR may have become more suitable for the growth of the EPS degrading bacteria. Microbiological studies may need to be conducted for further investigation of these phenomena.

Figure 4b compares the PSDs of the sludge flocs from the two SBRs. When the fermented food wastes were used as external carbon source, particle sizes moved to the larger size range and the distribution narrowed much comparing with that when sodium acetate was used. This is an indication of better bioflocculation condition and better settleability of sludge
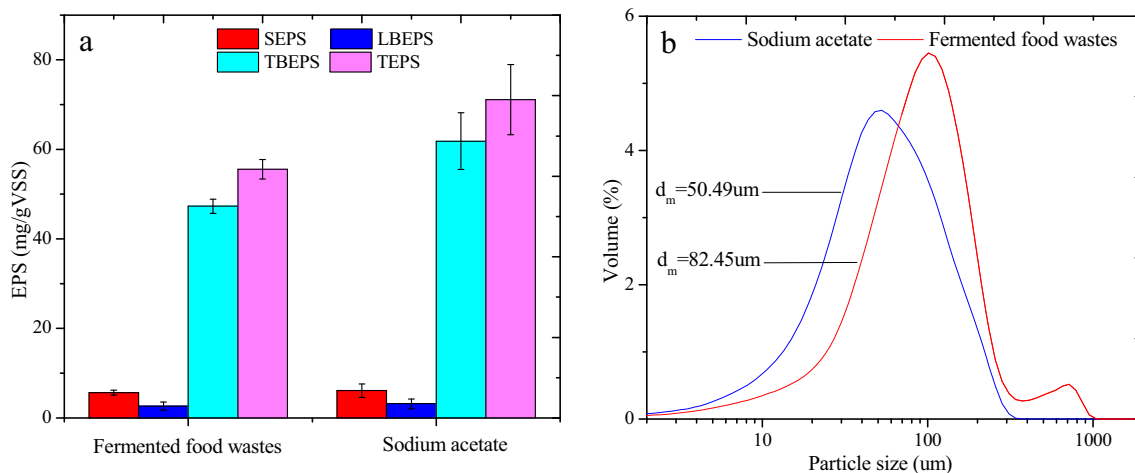

Fig. 4 Comparison of EPS (**a**) and particle size distributions (**b**) of activated sludge flocs

particles in the SBR fed with the fermented food wastes than sodium acetate. A difference was also found in the sludge volume indexes (SVIs) between the two SBRs as 153 and 275 ml/g, respectively, for the fermented food wastes and sodium acetate. The bioflocculation condition may have also been influenced by EPS production, because EPS (especially LBEPS) molecules may strongly affect the surface charge and hydrophobicity of bacteria cells (Ye et al. 2011). Excessive LBEPS may also weaken the cell attachment and floc structure and eventually result in poor bioflocculation and retarded sludge-water separation (Li and Yang 2007).

Metabolic characteristics of microorganism

As can be seen from Fig. 5a, the $AWCD_{590}$ curves, which show the bacterial community activity of the activated sludge sampled from the two SBRs added with two external carbon sources, varied in similar trends during the 240-h Biolog-ECO test. There was no apparent lag in the first 24 h, and then, the $AWCD_{590}$ increased with time till 168 h before a slight decrease was observed. This

indicated that the sludge samples from both SBRs were capable of metabolizing the organic substrates in the Biolog-ECO plate (Yang et al. 2011). However, the sludge sample from the SBR added with the fermented food wastes showed a slightly higher metabolic rate and higher maximum $AWCD_{590}$ value than that from the SBR added with sodium acetate.

By dividing the carbon sources in the Biolog-ECO plate into seven biochemical categories (Wang et al. 2009) and using the results obtained at 144 h, the $AWCD_{590}$ value of each biochemical category could be normalized as absorbance ratios shown in Fig. 5b. With the addition of fermented food wastes, apparently higher absorbance ratios were obtained for amino acids and carbohydrates, while with the addition of sodium acetate, the absorbance ratio for esters was higher. For the other categories, the differences between the two sludge samples were not very significant. However, as reported in other studies (Yang et al. 2011), carboxylic acids were found to be with relatively high catabolic activity for both samples.

Fig. 5 Characteristic of carbon source utilization when fermented food wastes and sodium acetate were used (a $AWCD_{590}$ curves, b absorbance ratio, c functional diversity indexes)
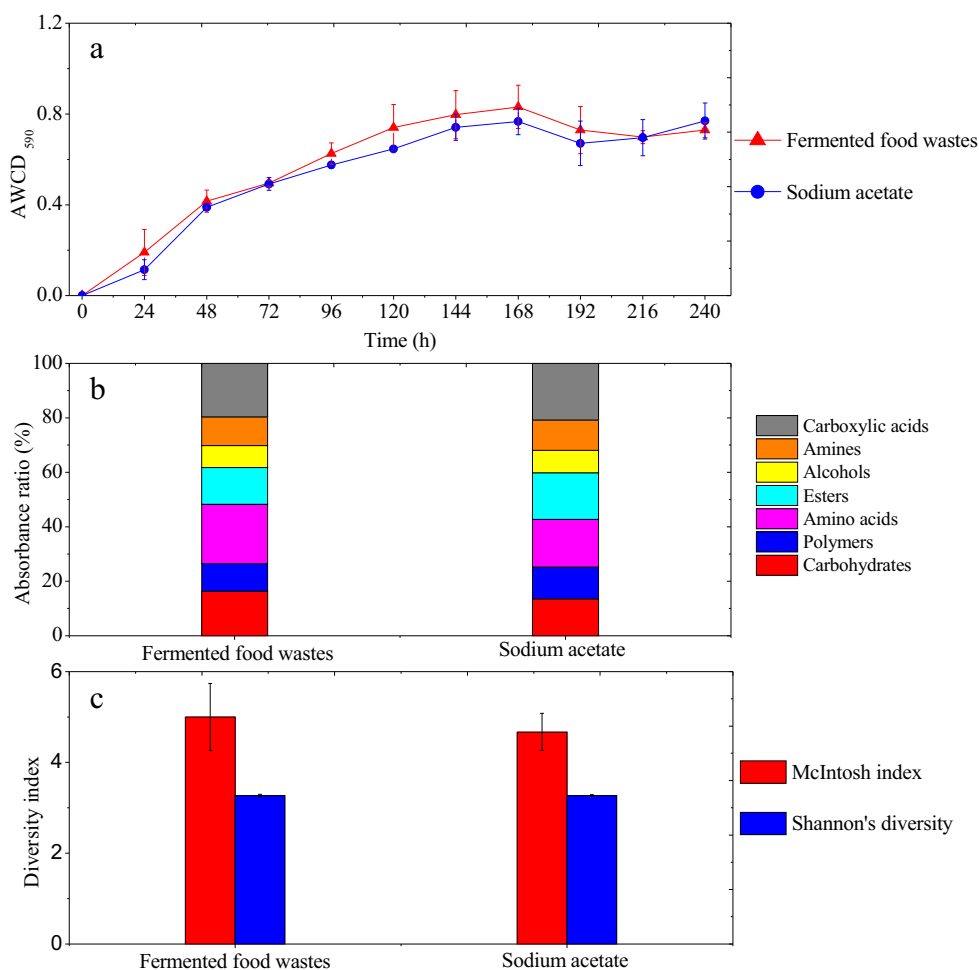
By using the McIntosh index to evaluate the biodiversity of community species in multi-dimensional space and the Shannon's diversity to evaluate the species richness (Magurran 1998), Fig. 5c is obtained for further comparison of the two sludge samples. Regarding Shannon's diversity, no appreciable difference was found between the two samples. This is an indication that the carbon sources were almost equally utilizable for both the fermented food wastes and sodium acetate samples because the Shannon's diversity is positively related to the number of utilizable carbon sources (Yang et al. 2011). Nevertheless, the McIntosh index was larger for the fermented food waste sample than the sodium acetate sample, indicating that microbial community was more abundant in the sludge due to the more diverse carbonaceous materials from the fermented food wastes.

Conclusions

By short-term free anaerobic fermentation of food wastes, external carbon source was produced and its effect on the enhancement of nitrogen removal was examined in this study. Although the fractions of organic acids were much lower than the hydrolyzed carbohydrates and other unidentifiable soluble organics, the fermented food wastes displayed favorable denitrification performance ($V_{DN} = 12.89$ mg N/g VSS h and $P_{DN} = 0.174$ g N/g COD) which was competitive with sodium acetate. The addition of the fermented food wastes even showed better improvement of nitrogen removal than sodium acetate for the treatment of wastewater with low C/N ratio. Many of the soluble organics other than organic acids in the fermented food wastes are believed to be intermediates of anaerobic fermentation which may not be readily biodegradable but can be utilized by bacteria in the bioreactor. Nevertheless, by Biolog-ECO tests, these intermediates were identified to be beneficial to microbial community abundance in the activated sludge, as well as their physicochemical properties as shown by the EPS content and sludge particle size. It can thus be concluded that as a multi-organic mixture, fermented food wastes are superior over mono-organic chemicals as alternative external carbon source for enhancing denitrification in wastewater treatment.

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