Nutrients removal performance and sludge properties using anaerobic fermentation slurry from food waste as an external carbon source for wastewater treatment

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Abstract: Enhancement of nitrogen and phosphate removal using thermophilic fermentation slurry from food waste (FSFW) as external carbon source was investigated. Based on the batch tests, the soluble and particulate fractions of the FSFW acted as easily and slowly biodegradable carbon sources, respectively, and the fermented slurry showed the combined nutrients removal properties of soluble and solid organics. During the long-term operation of a sequencing batch reactor (SBR) with FSFW for wastewater treatment, the sludge particle size increased obviously, the bacterial metabolic capacity improved significantly, and some functional microorganisms were enriched selectively, which significantly promoted the nitrogen removal efficiency (approximately 90%) by enhancing the anoxic denitrification and simultaneous nitrification and denitrification (SND) processes. Moreover, high phosphate removal efficiency (above 98%) was achieved through the aerobic and anoxic phosphate accumulation processes. Thus, using the

FSFW as supplementary carbon source is a suitable solution for both food waste disposal and wastewater treatment.

Keywords: Nutrients removal; anaerobic fermentation; food waste; carbon source; microbial community

1. Introduction

Nitrogen (N) and phosphorus (P) discharged from agricultural, domestic and industrial processes can result in the eutrophication of surface waters and deterioration of the environment, which has severely impaired the water quality of aquatic ecosystems around the world (Tang et al., 2018; Feng et al., 2017; Lin et al., 2016). Therefore, it is necessary to remove these nutrients from wastewater treatment systems through biological and physical-chemical methods (Tang e al., 2018; Raudkivi et al., 2017; Klein et al., 2017). However, due to the lack of organics in the influent, traditional biological nutrient removal efficiencies are always restricted, and it is difficult to obtain concentrations of N and P in the effluent that meet the discharge standard level (Li et al., 2016; Liu et al., 2016). To improve the nutrient removal efficiencies, many strategies have been suggested and attempted, such as changing the traditional treatment processes to deammonification processes, modifying the treatment systems and optimizing the operational parameters to effectively use the carbon sources (Choi et al., 2018; Rikmann et al., 2018; Zhao et al., 2016; Zekker et al., 2015; Kim et al., 2011). Furthermore, adding external carbon sources is also an efficient method that has been studied widely by researchers in recent years (Choi et al., 2018; Guo et al., 2016; Liu et al. 2016).

Soluble organic compounds such as acetate, alcohol and glucose have been utilized as efficient carbon sources for promoting nutrient removal efficiencies (Tang et al., 2018; Liu et al., 2016).

Aside from the soluble compounds, some particulate biodegradable carbon sources, such as biopolymers, have also been tested (Yang et al., 2018; Guo et al., 2016; Chu and Wang, 2011). However, the high cost has restricted their practical application (Chu and Wang, 2011). Based on the principle of "using waste to treat waste", organic wastes such as excess activated sludge and agricultural wastes have been applied in wastewater treatment systems to optimize the carbon sources and promote nutrient removal efficiencies, which has significantly reduced the operation costs (Jia et al., 2018; Nguyen et al., 2014). In recent years, the fermentation products from organic solid wastes and wastewater have also been successfully utilized as additional carbon sources in wastewater treatment (Tang et al., 2017, 2018; Zheng et al., 2018; Liu et al., 2017). During anaerobic fermentation, organic wastes are hydrolyzed into dissolved organic matter and acidified into organic acids, which allows microorganisms to use solid wastes more easily, thus further enhancing nitrogen and phosphate removal processes. Our previous work has demonstrated that soluble organics (organic acids and carbohydrates) in the fermentation slurry from food waste (FSFW) are easily biodegradable carbon sources that can obviously increase microbial diversity and significantly promote nitrogen removal efficiency in batch tests and long-term practical operation (Tang et al., 2017, 2018; Zhang et al. 2016b, 2016c). However, centrifugation and filtration should be applied to separate the soluble fermentation products from the fermented slurry, which is costly and time-consuming. Thus, it is urgent to provide a highly operable method for the utilization of the FSFW.

Additionally, food waste as a typical organic municipal solid waste (OMSW) has caused serious environmental pollution due to its large amount, high organics content and good biodegradability, which has attracted much attention from governments and researchers (Zhou et

al., 2018; Han et al., 2016). More seriously, due to the more stringent environmental standards in China, traditional food waste disposal methods, such as landfilling, composting, and incineration, are no longer feasible (Zhou et al., 2018). Thus, it is extremely urgent to develop environmentally sustainable and effective solutions for treating the increasing amount of food wastes. Anaerobic fermentation is a suitable method for stabilizing food waste to produce biogas or other intermediates, and it has been regarded as one of the most promising methods (Zhou et al., 2018; Tang et al., 2016). Volatile fatty acids (VFAs), lactic acid, and alcohols, as important components of the fermentation products from food waste, could be utilized effectively as carbon sources to enhance nutrients removal (Tang et al., 2017, 2018; Zhang et al., 2016b). In addition, thermophilic fermentation has come into favor for hydrolysis and shortens the fermentation period compared with mesophilic conditions (Tang et al., 2016). It can also reduce the contagion activity of harmful bacteria/viruses in the food waste, which facilitates more sanitary practical application. Thus, supplying thermophilic fermentation products as external carbon sources to enhance nutrients removal could be a reasonable solution for food waste disposal and wastewater treatment issues.

According to our previous studies, soluble products in FSFW could be effectively utilized as a carbon source for nitrogen removal and stably enhance the denitrification processes (Tang et al., 2017, 2018; Zhang et al., 2016b). However, due to the restriction of solubilization, a portion of the solid organic matter is not transformed into soluble forms. Nevertheless, it has been reported that the particulate organics could be used as slow-release carbon sources during wastewater treatment processes (Akizuki et al., 2016). Thus, using the fermentation slurry directly as a carbon source could fully utilize the solid organic matter and also avoid the separation processes of

soluble fractions from the fermented slurry. Additionally, if the solid fractions of the FSFW could be used by the bacteria in wastewater treatment processes, its negative impact on the environment would be reduced. However, the pollutants removal performance and activated sludge properties with the addition of FSFW in wastewater treatment systems have not been reported. In this study, the FSFW produced by thermophilic anaerobic fermentation was applied to enhance nitrogen and phosphate removal. The pollutant removal efficiencies were firstly observed under different dosages of FSFW, then the characteristics of the activated sludge were investigated to explore the feasibility of using FSFW as a carbon source for wastewater treatment.

2. Materials and Methods

2.1 Preparation of the FSFW

The FSFW was prepared through anaerobic thermophilic fermentation. The food waste, collected from the student canteen in a university campus in Xi'an, China, was mainly composed of rice, vegetables and meat. The pretreatment processes were similar to those in our previous work (Tang et al., 2016, 2017, 2018). Briefly, food wastes were crushed using an electric blender after sorting out animal bones, clamshells and waste tissues. Then, the slurry was sieved (1 mm) and subsequently stored in a refrigerator (4°C) for further use. Prior to the fermentation processes, the total solids (TS) content of the slurry was adjusted to approximately 10% with tap water. Then, 5 L of food waste slurry (TS=10%) was added to a continuous stirring tank reactor (CSTR) and fermented at 55°C without pH adjustment. After 5 days, the pH in the reactor decreased and remained at 3.8±0.2, the concentrations of carbohydrates, proteins and organic acids were almost constant, and the system was regarded as stable (Tang et al., 2017). Each day, 1 L of the fermentation mixture was drained from the reactor and replaced with the same volume of fresh

food waste slurry (TS=10%). Carbohydrate, protein and organic acids were the main soluble components in the FSFW. The COD and SCOD in the FSFW were 90.1±9.5 and 50.7±4.3 g/L, respectively. Other organics in the FSFW are shown in the Supplementary Information.

2.2 Continuous operation of the SBR with FSFW as a carbon source

To explore the effects of the FSFW on N and P removal, the characteristics of activated sludge, and its microbial communities, a sequencing batch reactor (SBR; working volume of 5 L) was operated in the laboratory with the FSFW as an external organic carbon source. The schematics and operation sequences of the SBR are described in the Supplementary Information. To utilize the organics for the denitrification process effectively, the influent was intermittently fed into the reactor during the anoxic phases. The inoculated sludge was obtained from the anoxic tank of the pilot-scale membrane bioreactor described in our previous study (Tang et al., 2017). Before adding the FSFW, the SBR had been operated for two months with the low organics content domestic wastewater (Tang et al., 2017), during which time it exhibited a low nitrogen (20-30%) and phosphate (5-10%) removal efficiency. Then, the FSFW, ammonia and phosphate were added into the influent to adjust the COD (300-500 mg/L), NH_4^+-N (20-50 mg/L) and PO_4^{3-}-P (2-6 mg/L), respectively, to explore the effects of FSFW addition at various nitrogen and phosphate loading rates. The hydrolytic retention time (HRT) was 10 hours, and the sludge retention time (SRT) of the SBR was maintained at approximately 30 days by discharging the excess sludge every day. The mixed-liquid suspended solids (MLSS) fluctuated between 3 and 5 g/L, and the ratio of mixed-liquid volatile suspended solids (MLVSS) to MLSS was approximately 0.7-0.8.

2.3 Nitrogen uptake rate (NUR) tests

To elucidate the denitrification properties of the FSFW, a series of NUR tests were conducted

following the methods presented in the previous studies (Tang et al., 2017, 2018; Zhang et al., 2016b; Sage et al., 2006). Firstly, activated sludge was transferred from the SBR into three flasks and washed with deionized water three times to remove the residual nitrogen compounds and organic matter from the sludge. The activated sludge in each flask was then diluted with tap water to make up a volume of 2 L and produce a MLVSS of approximately 3 g/L. Oxygen in the mixed liquor was flushed with nitrogen gas (N_2). Following this, sodium nitrate ($NaNO_3$) was added to each flask to generate a final NO_3^--N content of approximately 50 mg/L. The soluble (filtrate using the 0.45-μm filter) and solid fractions (residuals on the 0.45-μm filter) of the fermentation slurry and FSFW were then added in the flask to generate a COD content of approximately 300 mg/L. A control test was also conducted with 50 mg/L of NO_3^--N, but no additional carbon source was supplied. The flasks were then sealed and the liquor was mixed with stirrers. Periodically, 10 mL of the mixed liquor was sampled from each flask to analyze the NO_3^--N, NO_2^--N and COD contents. The specific denitrification rate (SDNR), denitrification potential (P_{DN}) and anoxic sludge yield were calculated according to Sage et al., 2006 and the SDNR was obtained as follows:

$$\mathrm{SDNR(mg/g\ VSS \cdot h)} = \frac{\mathrm{NO_{x,initial}\text{-}NO_{x,t}}}{\mathrm{MLVSS \cdot t}}$$

where t is the time when the content of the nitrogen compounds were not changed (h); $NO_{x,initial}$ and $NO_{x,t}$ are the initial NO_x-N concentration and that at t, respectively (mg/L); NO_x-N was calculated with NO_3^--N+0.6NO_2^--N, respectively.

2.4 Phosphate release and accumulation test

2 L activated sludge was taken from the SBR in the last aerobic phase and washed three times using deionized water. Then the sludge was added to two identical sealed reactors (working

volume: 1.5 L) and mixed with deionized water, where the MLVSS was 3±0.2 g/L. Oxygen in the mixed liquor was replaced with nitrogen gas. FSFW was then added to generate an initial COD concentration around 200 mg/L. After that, the reactors were stirred, and mixed liquor samples were periodically obtained to analyze the P release rate. After anaerobic stir, one reactor (A-A reactor) was added with $NaNO_3$ to obtain an initial NO_3^--N concentration of 30 mg/L for detecting anoxic P uptake processes, the other reactor (A-O reactor) was aerated (DO was 3.0-4.0 mg/L) to explore the oxic P uptake performance. The P release and accumulation rates were calculated by the simulation results of the phosphate content in the test.

2.5 Analysis methods

2.5.1. Extraction and measurement of EPS

Extracellular polymeric substances (EPS) in the activated sludge during the operation were investigated. Both soluble EPS (SEPS) and bound EPS (BEPS) were extracted from activated sludge using a thermal treatment method according to Hu et al., 2013. The contents of EPS samples were analyzed in terms of proteins and polysaccharides. Polysaccharides were detected using the anthrone method with glucose as a standard (Frølund et al., 1996), and proteins were quantified using a modified Lowry method using bovine serum albumin (BSA) as the standard reference (Lowry et al., 1951).

2.5.2. Particle size distribution (PSD) analysis

PSD of the sludge samples in the SBR during the four phases was measured using a laser granularity distribution analyzer (LS 230/SVM+, Beckman Coulter Corporation, USA), which had a detection range of 0.4-2000 μm and showed a good accuracy and reproducibility. In this study, the typical PSD curves were reported.

2.5.3. Biolog plate analysis

The metabolic characteristics of microorganisms in activated sludge before and after adding the FSFW were assessed using the Biolog-ECO plates (Biolog, Inc., Hayward, CA, USA) as it was described in the previous studies (Tang et al., 2018, Zhang et al., 2016c; Kong et al., 2013). Firstly, the activated sludge samples from the SBR were diluted to 1:1000 with sterilized NaCl solution (0.9 %, w/v) and shaken four times for 15 s. The resulting suspension of 1 mL was diluted with the saline solution to control the optical density (OD) close to 0.05 at 600 nm, which ensured that sample solutions contained approximately the same biomass concentration. Then 150 μL of the diluted mixture was added to the Biolog plate well using eight channel pipettes and the plates were inoculated at 25°C in darkness. The absorbance (OD 590 nm) of the wells was recorded for 168 h using an ELISA plate reader at every 24-h interval. The data analysis processes were according to the previous studies (Tang et al., 2017, 2018).

2.5.4. Microbial communities analysis

Activated sludge samples were sent to Sangong, Inc. (Shanghai, China) for DNA extraction and next-generation sequencing processes. The extracted DNA was amplified by PCR using the primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG-3') for the V1-V3 region of the 16S rRNA genes (Luo et al., 2017; Tang et al., 2018). Pyrosequencing was conducted using an Illumina MiSeq platform. The homologous or ambiguous sequences or those with a length shorter than 200 bp were trimmed to obtain high-quality sequences with an average length larger than 500 bp for the taxonomic classification.

2.6 Additional chemical analysis methods

The components in the fermented slurry was analyzed according to our previous study (Tang et al.,

2016). Influent, effluent and mixed liquor were periodically sampled from the SBR. The nitrogen compounds (NH_4^+-N, NO_3^--N, NO_2^--N and total nitrogen (TN)), chemical oxygen demand (COD), PO_4^{3-}-P, MLSS, MLVSS, biomass yield and sludge volume index (SVI) were measured according to standard methods (APHA, 2012). All tests were performed in triplicate, and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1 Performance of nitrogen and phosphate removal with fermentation products

3.1.1 Nitrogen removal performance with FSFW

Using the soluble fermentation products as carbon sources, it was noticed that NO_3^--N content decreased from 52.1 mg/L to 1.2 mg/L in only 90 min (Fig. 1a), showing a denitrification rate of 10.4 mg-NO_x^--N/g-VSS·h (Fig. 1c). The organic acids, carbohydrates and protein in the soluble fractions of the FSFW could be easily utilized by microorganisms and provided energy to the denitrification processes (Tang et al., 2018; Zhang et al., 2016b), which significantly promoted the denitrification rate. However, the NO_3^--N content in the reactor with solid fractions decreased slowly, exhibiting a denitrification rate of 1.8 mg-NO_x^--N/g-VSS·h, which indicated that even though the denitrification rate was relatively lower, the particulate organics in the FSFW could also be utilized as carbon sources for nitrogen removal. This occurred because the solid organics must first be hydrolyzed into soluble forms prior to being utilized by microorganisms, which restricted the denitrification processes because the hydrolysis rate was very slow (Akizuki et al., 2016).

The FSFW exhibited the dual properties of soluble and solid organics during the denitrification

processes (Fig. 1). In the first 15 min, the NO_3^--N content showed the same decreasing tendency as the soluble fractions, which was mainly due to the fact that the easily biodegradable organics in the FSFW were firstly utilized in this phase. Thereafter, the denitrification rate decreased because most of the easily biodegradable organics were consumed and the bacteria could only use the particulate fractions or macromolecular organic matter to realize denitrification. However, the overall nitrogen removal rate with the fermented mixture was much faster than that with the solid fractions, and the residual NO_3^--N content was lower (0.5 mg/L), which indicated that a complete nitrogen removal was achieved and that the FSFW could be utilized effectively as a carbon source for enhancing denitrification.

In the reactor with soluble fractions (Fig. 1b), NO_2^--N increased to 11.7 mg/L in 60 min and then sharply decreased to 0.3 mg/L, which could be explained by the competition for electrons between the nitrite reductase and nitrate reductase (Tang et al., 2018; Zhang et al., 2016b; Ge et al., 2012). The NO_2^--N content in the reactor with solid fractions remained within 2-3 mg/L, showing a slight accumulation of NO_2^--N during the experiment. However, with the FSFW as a carbon source, the NO_2^--N content increased to approximately 8.4 mg/L in only 15 min, which further explained the sharp decline of NO_3^--N in this period (Fig. 1a). The NO_2^--N remained stable until NO_3^--N was completely transformed and decreased to 0.5 mg/L, which further proved that the FSFW exhibited the combined properties of soluble and particulate organics during the nitrogen removal processes.

The denitrification performance with different carbon sources was compared (Table 1). The denitrification rate of FSFW was a bit lower than that of the fermentation liquid from food wastes used in our previous study (Zhang et al., 2016b), but it was much higher than that of hydrolysis

and acidogenic liquid from activated sludge, which might be a result of the different components in the carbon sources. Additionally, more complex organics coexisted in the FSFW, which might cause synergistic effects of these organics on denitrification (Kim et al., 2016).

Fig. 1.
Table 1.

3.1.2 Nutrients removal with FSFW during the long-term operation

Nitrogen and phosphate removal with FSFW as a carbon source during the long-term operation of the SBR was investigated (Fig. 2). Irrespective of its increase in content from 300 to 500 mg/L, the COD of the effluent remained at a low level (20-30 mg/L) (Fig. 2a), indicating that the FSFW could be consumed completely by the microorganisms. In addition, although the NH_4^+-N in the influent increased from 20 to 50 mg/L, its content in the effluent retained stable and was lower than 1 mg/L (Fig. 2b), showing a removal efficiency of approximately 97-99%, which further proved that the FSFW slightly influenced the nitrifying bacteria, consistent with the results of our previous study (Tang et al., 2017). However, the nitrate content in the effluent was obviously affected by the NH_4^+-N and COD in the influent. In phase I, NH_4^+-N in the influent was approximately 20 mg/L, but the NO_3^--N content in the effluent was 1-2 mg/L, so the reactor showed a nitrogen removal rate (NRR) of 48 mg/L·d and a high nitrogen removal efficiency (approximately 90.1%). With an increase in NH_4^+-N to 30 mg/L in phase II, the NO_3^--N concentration in the effluent increased slightly (4-5 mg/L), indicating that the organics in the influent were effectively utilized for nitrogen removal. However, in phase III, NH_4^+-N was increased to 40-50 mg/L, and NO_3^--N in the effluent increased gradually from 10.1 mg/L to the highest value of 25.7 mg/L, showing a nitrogen removal efficiency of 40-60%. Interestingly, after 50 days of operation, NO_3^--N in the effluent decreased gradually to 11.7 mg/L, which might be

attributable to the acclimation of microorganisms to the organics, especially the particulate organics in the FSFW, and allowed them to exhibit a high utility capacity for the organics (Tang et al., 2017). In phase IV, with more COD supplied (approximately 500 mg/L) in the influent, denitrification processes were enhanced, and the nitrogen removal efficiency increased and then stabilized at 91.1±5.1% and a nitrogen removal rate of 108 mg/L·d was obtained, which further verified the feasibility of FSFW as a carbon source for nitrogen removal. In our previous studies using soluble acidogenic fermentation products from food waste as carbon sources, the nitrogen removal rate could remain at 75% at a lower C/N ratio (6.6:1) (Zhang et al., 2016c). In our latest work with lactic acid fermentation products from food waste as carbon sources, a TN removal efficiency of 80-90% was obtained at a C/N ratio of 7.5. In this study, with a higher C/N ratio (approximately 10), a similar nitrogen removal efficiency was observed, which was mainly due to the existence of some solid organics in the FSFW and more organics being lost due to the longer metabolic pathway (Tang et al., 2017).

Phosphate removal was also significantly influenced by the dosage of fermented slurry in the influent (Fig. 2c). In phase I, the PO_4^{3-}-P content in the influent was increased gradually from 1.2 mg/L to 6.0 mg/L, but it was approximately 0.02 mg/L in the effluent, showing a complete removal of PO_4^{3-}-P (above 99%), which indicated that the fermented slurry could be utilized effectively as a carbon source for PO_4^{3-}-P removal. However, in phase II, PO_4^{3-}-P concentrations in the effluent increased to 2.2 mg/L, and it was deduced that with the increase of nitrogen content in the influent, most of the organics were consumed by denitrifying bacteria and fewer carbon sources were utilizable for phosphate release in anaerobic conditions (Zhang et al., 2016a), which further affected the phosphate accumulation in the aerobic phase. This phenomenon could be

obviously detected with higher NH_4^+-N content in the influent in phase III, PO_4^{3-}-P in the effluent increased to approximately 4-5 mg/L, or even higher than that in the influent. Although more activated sludge was discharged in this phase, the phosphate removal rate remained at a low level (approximately 14.0%). However, with the addition of more FSFW in phase IV, phosphate in the effluent decreased sharply to approximately 0.05 mg/L, showing a stable removal efficiency of more than 90%. Both stable nitrogen and stable phosphate removal efficiencies were obtained during the long-term operation, further demonstrating that FSFW can be effectively utilized as a carbon source for nutrients removal in wastewater treatment systems.

Fig. 2.

3.1.3 Cyclic study of the SBR

Cyclic studies were conducted to explore the nitrogen and phosphate removal processes in the reactor (Fig. 3). With the addition of wastewater, ammonia content sharply increased to 15.1 mg/L and remained constant until the end of the first anaerobic phase (Fig. 3a), while nitrate content in the reactor decreased gradually from 6.0 to 2.1 mg/L, exhibiting a specific denitrification rate (SDNR) of 3.9 mg NO_3^--N/g-VSS·h (Table 2).

With air supply in the aerobic phase, NH_4^+-N was transformed into nitrate by nitrifying bacteria and gradually decreased to 1.9 mg/L at 105 min, accompanied by an increase of NO_3^--N from 2.1 mg/L to 10.9 mg/L, showing a specific nitrification rate (SNR) of 3.5 mg/g-VSS·h, which further indicated that the activities of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in the reactor were not negatively influenced by the addition of FSFW. Additionally, due to the existence of simultaneous nitrification and denitrification (SND), the TN content in this phase decreased from 17.2 mg/L to 12.8 mg/L, showing a denitrification rate of 1.5 mg/g-VSS·h. From 105 to 135 min, aeration was paused, the NH_4^+-N content remained constant, while

denitrifying bacteria utilized the residual organics in the reactor to realize denitrification, and the NO_3^--N content decreased gradually to 7.6 mg/L. At 135 min, wastewater was added to the reactor to supply carbon sources and nutrients, and the NH_4^+-N content increased to 10.3 mg/L and remained almost unchangeable thereafter. However, with enough organics for denitrification processes, the NO_3^--N sharply decreased to 1.2 mg/L in only 15 min, exhibiting a high SDNR (2.8 mg/g-VSS·h), which further proved that organics in the FSFW could be effectively utilized as carbon sources for nitrogen removal. At 195 min, the reactor was aerated and NH_4^+-N decreased gradually to 0.5 mg/L. Meanwhile, NO_3^--N increased to 8.7 mg/L and then slightly decreased to 6.4 mg/L at 255 min. The calculated nitrification and denitrification rates were 3.1 and 1.8 mg/g-VSS·h, respectively. In the next 40 min, with the addition of organics from the influent, NO_3^--N decreased gradually to 0.7 mg/L, exhibiting a denitrification rate of 2.8 mg/g-VSS·h. During the last oxic phase (295-325 min), NH_4^+-N decreased to 1.1 mg/L and NO_3^--N increased to 4.2 mg/L, indicating that the NH_4^+-N was completely transformed by nitrifiers into NO_3^--N with a high SNR (2.4 mg/g-VSS·h). Similarly, SND processes were observed in this period, showing a SDNR of 1.3 mg/g-VSS·h.

The concentration of phosphate increased from 0.08 mg/L to 16.9 mg/L in the anaerobic phase (Fig. 3b), demonstrating a high phosphate release rate of 3.4 mg/g-VSS·h (Table 2). Attributable to the phosphate accumulation processes in the aerobic condition, PO_4^{3-}-P gradually decreased to 0.3 mg/L, which demonstrated that the activated sludge had a very high P accumulation rate (3.9 mg/g-VSS·h). Interestingly, although the aeration was paused at 105 min, the PO_4^{3-}-P content decreased unceasingly to 0.01 mg/L, which might be attributable to two causes: (1) the phosphate accumulating organisms (PAOs) absorbed the PO_4^{3-}-P using the oxygen in the microenvironment

and (2) denitrifying phosphate accumulating organisms (DPAOs) were enriched in the reactor and absorbed the PO_4^{3-}-P during denitrification processes. The denitrifying phosphate accumulation processes could be verified further by the PO_4^{3-}-P decreasing tendency in other anoxic phases (142-165 min and 270-295 min). With the addition of wastewater at 135 min, PO_4^{3-}-P increased to 4.0 mg/L and then finally decreased to 0.13 mg/L in the oxic phase. Similarly, in the last oxic phase, the P content decreased gradually to 0.05 mg/L, which ensured a low content (0.04 mg/L) in the effluent.

Variations of DO and ORP in the reactor further verified the nitrogen and phosphate removal processes (Fig. 3c). The negative ORP in the anoxic phases provided suitable conditions for denitrification and phosphate release processes. In addition, the reductive condition was beneficial for the transformation of particulate organics into soluble form by anaerobic bacteria, which provided easily biodegradable carbon sources (e.g., VFAs) for denitrifying bacteria and phosphate removal organisms (PAOs and DPAOs). With air supply, the ORP and DO increased gradually, which was suitable for nitrifying bacteria to transform NH_4^+-N into NO_3^--N. Additionally, the relatively low DO content (< 1.5 mg/L) in the supernatant was suitable for the SND processes, further explaining the TN removal in the aerobic phases (Fig. 3a).

Fig. 3.

Table 2.

3.1.4 Performance of phosphate removal with the fermentation products

To further explain the phosphate removal with the addition of FSFW, a series of batch tests were conducted (Fig. 4a). With a decrease of COD content from 200 mg/L to 50 mg/L (Supplementary Information), PO_4^{3-}-P increased gradually from 5.8 mg/L to 78.5 mg/L, indicating

a phosphate release rate of approximately 15.4 mg/g-VSS·h (Fig. 4b). In the reactor with aeration, PAOs started to absorb PO_4^{3-}-P, and its content decreased gradually to 0.5 mg/L, showing a PO_4^{3-}-P accumulation rate of 4.4 mg/g-VSS·h.

Interestingly, the activated sludge also showed a high P accumulation in the anoxic reactor with NO_3^--N addition (Fig. 4c and d). It has been reported that DPAOs can utilize NO_3^--N as a substrate and realize phosphate removal (Zhang et al., 2016a; Wang et al., 2015). Compared with that under the aerobic conditions, PO_4^{3-}-P content in the anoxic condition decreased more slowly (2.4 mg/g-VSS·h) and reached a final concentration of 28.8 mg/L. Meanwhile, the NO_3^--N content also decreased from 32.2 mg/L to 11.1 mg/L, further testifying to the existence of DPAOs in this process. From the above analysis, it was clearly determined that the P removal was attributable to two mechanisms. The denitrifying phosphate accumulation processes further explained the variations of PO_4^{3-}-P concentration in the cyclic study (Fig. 3b). During the first anaerobic phase, low NO_3^--N existed in the supernatant and obvious phosphate release was observed, whereas in the next anoxic phases, with high NO_3^--N content, PO_4^{3-}-P content did not increase obviously, which might have been due to two reasons: (1) the denitrifiers took up most of the organics for nitrogen removal and less carbon sources were utilizable for phosphate release and (2) due to the existence of denitrifying phosphate accumulation processes, the released phosphate was removed by the DPAOs (Fig. 3b).

Based on the above analysis, it was determined that the FSFW could be utilized effectively as a carbon source for nitrogen and phosphate removal. Nitrogen removal was realized through anoxic denitrification and SND, while phosphate was removed through aerobic phosphate accumulation and anoxic denitrifying phosphate accumulation processes. These characteristics might be related

to the properties of the activated sludge and the microbial communities in the reactor, which will be discussed in section 3.2.

Fig. 4.

3.2. Characteristics of the activated sludge

3.2.1 PSD

The particle size of the activated sludge flocs increased with the addition of fermentation products (Supplementary Information). The mean size of the sludge flocs in phase Ⅰ was approximately 136.2 μm, and it increased to 155.4 μm in phase Ⅱ. The larger sludge flocs were mainly caused by the addition of particulate organic matter from the fermented slurry (Tang et al., 2017). The particulate organics may have behaved as a core for gathering bacteria and contributed to increasing the floc size. Larger sludge floc size was beneficial for the separation of activated sludge from the treated water (Table 3). In phase Ⅰ, the SVI of the sludge were approximately 125.0 mL/g-VSS, respectively. However, with larger floc size (155.4 μm) in phase Ⅱ, the SVI decreased to 107.3 mL/g-VSS. In phase Ⅲ and Ⅳ, the particle size increased further to 247.4 and 424.9 μm, respectively, and the SVI decreased to approximately 65-80 mL/g-VSS. Moreover, the larger sludge flocs could provide suitable microenvironments for the denitrifying bacteria, which was beneficial for enhancing the SND processes, and this further explained the nitrogen removal during the aerobic phase shown in Fig. 3.

Table 3.

3.2.2 EPS

The EPS in sludge cultured with FSFW were analyzed (Table 4). It was found that the content of SEPS was much lower than that of BEPS and that protein was the main component in EPS, which is consistent with the results of the previous studies (Tang et al., 2017, 2018). In phase Ⅰ,

polysaccharide was 1.3 mg/g-VSS, and protein was only 4.1 mg/g-VSS, showing a low P/C ratio of 3.3. In phase Ⅱ and Ⅲ, the concentration of polysaccharide increased slightly to 1.4 mg/g-VSS, but the protein content increased sharply to 7.5 mg/g-VSS and 8.9 mg/g-VSS, respectively, which may have been due to the fact that, with the addition of FSFW, a portion of protein from the fermentation products was not completely utilized by the bacteria and was released into the supernatant (Tang et al., 2018). In phase Ⅳ, protein remained stable (9.2 mg/g-VSS) and the polysaccharide content increased to 1.9 mg/g-VSS, which might have been due to the microorganisms becoming acclimated to digest the carbohydrates and proteins in the sludge or to the microbial communities changing during the long-term cultivation. This topic will be further analyzed in future studies.

BEPS was obviously lower in phase Ⅰ than in other phases. In phase Ⅱ, protein increased to 54.2 mg/g-VSS, possibly because protein from FSFW was accumulated in the sludge flocs. In phase Ⅲ, with more FSFW added, polysaccharides increased to 19.1 mg/g-VSS and protein increased slightly to 65.0 mg/g-VSS, which could be explained by the addition of FSFW and some of the protein and carbohydrate being adsorbed onto the sludge. However, in phase Ⅳ, the polysaccharides and proteins decreased slightly to 15.9 and 54.2 mg/g-VSS, respectively, which might be attributable to the bacteria in the activated sludge being able to digest the residual carbon sources from the FSFW and the microbial metabolites through the long-term cultivation (Tang et al., 2017).

Table 4.

3.2.3 Microbial metabolic properties

It has been reported that the carbon sources influence both microbial metabolism and also

bacterial community structures, which in reverse affects the removal of the pollutants. To further reveal the effect of FSFW addition on microbial communities, the characteristics of bacterial metabolism and microbial diversity before and after the FSFW addition were investigated.

To characterize the metabolic activity of the microorganisms in the activated sludge, the variations of average well-color development (AWCD) during the incubation were investigated using the Biolog ECO-plate. As shown in Fig. 5a, during the first 24 h, the AWCD value of the raw (before) and cultured (after) sludge increased from approximately 0.01 cm^{-1} to 0.29 and 0.43 cm^{-1}, respectively, indicating high carbon source metabolic activity (Tang et al., 2017, 2018). Following a linear increase, the AWCD values approached constant values after 96 h, which demonstrated that most of the carbon sources in the plate were consumed by bacteria in the activated sludge. By simulating the AWCD data in the first 72 h, the increasing rate of AWCD was determined to be 0.011 and 0.014 cm^{-1}/h, respectively, which indicated that bacterial metabolic activity was promoted by the addition of FSFW. Additionally, the higher AWCD value of the sludge cultured with FSFW further verified its more rapid bacterial growth and higher microbial metabolic activity (Tang et al., 2017), which might be attributable to the higher bacterial diversity in the activated sludge (Tang et al., 2018; Zhang et al., 2016c). In the FSFW, particulate and soluble organics (e.g., soluble carbohydrate, protein and organic acid) provided more types of carbon sources for the microorganisms; thus, a higher microbial diversity existed in the activated sludge to utilize the substrates (Tang et al., 2017, 2018).

Additionally, substrates in the ECO-plate can be assigned into the groups of alcohols, amines, amino acids, carbohydrates, carboxylic acids, esters and polymers. Qualitative measures of substrate utilization by each group can be calculated using the average absorbency fraction at 120

h in the same way as for the whole plate (Fig. 5b). The microorganisms in the activated sludge exhibited similar utilization capacity for these carbon sources, which indicated that the addition of FSFW did not change the bacterial metabolism processes and further demonstrated that the FSFW had no obvious impact on the carbon sources digestion processes. This might be due to the fact that the components in the FSFW are similar to those in the wastewater; thus, the bacteria could easily accommodate the fermented products (Tang et al., 2017). It was noted that the activated sludge after the addition of FSFW exhibited higher utility capacity on polymers, amino acids and carboxylic acids than that before the addition of FSFW, which was mainly due to the fact that high contents of carbohydrate, protein and organic acids existed in the FSFW and bacteria adjusted their metabolism to digest these organics. These results are consistent with those of previous studies (Tang et al., 2017; Zhang et al., 2016c).

To further compare the utilization capacities on individual carbon sources by the microorganisms in the two sludge samples, the AWCD value of each organic in the plate at 120 h was analyzed (Fig. 5c). It was found that the activated sludge before addition of FSFW showed higher utilization capacity on carboxylic acids, amino acids and amines, but lower ability on polymers and alcohols, which might be due to the fact that protein existed in the FSFW and microorganisms became acclimated to use these carbon sources. Moreover, the cultured sludge showed higher utilization rates on organics in amino acids (B4, C4, D4 and E4) and carboxylic acids (B3, C3, D3 and F3), which further proved that the bacteria exhibited higher utilization rates for complex organics after the addition of FSFW.

Using the McIntosh index (U) to evaluate the diversity of metabolism in multi-dimensional space and the Shannon's diversity (H) to assess the richness (Zhang et al., 2016c), Fig. 5d was

obtained for further comparison of the two sludge samples. The activated sludge cultured with FSFW showed a higher U value of 6.1 than that before the addition of FSFW (5.2), indicating that the bacteria with FSFW addition could digest more complex carbon sources, which further verified the higher AWCD value in Fig. 5a. It was deduced that organics in the FSFW could be utilized as carbon sources by the microorganisms or that more types of bacteria were accumulated in the sludge to utilize the organics collectively. The Shannon's diversity (H) is positively related to the number of utilizable carbon sources (Zhang et al., 2016b). No evident difference was found in Fig. 5d, which indicated that the carbon sources in the plate were almost equally utilizable by both sludge samples.

Fig. 5.

3.2.4. Microbial community analysis

Microbial community structures in the activated sludge before and after adding the FSFW were investigated using high-throughput sequencing technology. The rarefaction curves of the samples drawn from each of the units reached a plateau, indicating that the bacterial communities were completely characterized in this study. The number of OTUs, Good's coverage, the Shannon, Chao1, and ACE indices, as well as the Simpson index at a cutoff level of 3% were calculated (Table 5). The number of OTUs in the two samples was 1901 and 1598, respectively. Good's coverage of the two samples was 98%, which demonstrated that the sequence libraries constructed in this study covered the diversity of the microbial community. The greater microbial richness in the sludge before adding the FSFW than that in the activated sludge cultured with FSFW, which might have been caused by the selective enrichment of microorganisms in the reactor, was evidenced by the Chao 1 estimator of total OTU numbers and validated by the respective ACE indices. Similar to the Simpson index, the Shannon index values were 5.6 and 5.3, showing the

differences of microbial diversity in the two samples (Table 5).

Table 5.

Further classifying the OTUs, Proteobacteria and Bacteroidetes were the main phyla in both samples (Fig. 6a). Proteobacteria, accounting for a relatively high relative abundance of 50.5%, was the main phylum in the activated sludge (Tang et al., 2017, 2018). Bacteroidetes and Firmicutes, with a relative abundance of 15.3% and 15.7%, respectively, were the second main microorganisms, followed by Planctomycetes (6.2%) and Chloroflexi (2.8%). Other phyla such as Acidobacteria (1.2%), Verrucomicrobia (1.4%), Gemmatimonadetes (1.2%) and Actinobacteria (0.5%) were also detected with relatively lower proportion. Nitrospirae, a phylum of nitrifying bacteria, accounted for a relative abundance of 2.0%, indicating that the incubated sludge had a higher nitrification capacity and activity.

With the addition of FSFW, microbial communities in the activated sludge changed. The relative abundance of Proteobacteria decreased to 46.6%. However, the Bacteroidetes (26.8%) and Chloroflexi (12.8%) increased significantly. It has been reported that Bacteroidetes can digest proteins and are able to ferment amino acids to acetate (Rivière et al., 2009). The increase of Bacteroidetes, which might be attributable to the existence of protein in the FSFW, was beneficial for N and P removal. In addition, Chloroflexi is responsible for the degradation of soluble microbial products (SMP) and other complex substrates (Miura et al., 2007). Thus, the obvious increase in these phyla provided an advantage in digesting the complex organics (such as particulate organics and soluble carbohydrates) in the fermentation products. Interestingly, the relative abundance of Firmicutes decreased from 15.7% to 0.7%, and Planctomycetes also showed a slight decrease with the addition of FSFW. Actinobacteria, responsible for the hydrolysis and fermentation of various types of organic matter (Zhou et al., 2015), increased from 0.5% to 3.5%

after adding the FSFW, which is beneficial for the digestion of particulate organics in the FSFW. Although Nitrospirae decreased from 2.0% to 0.7%, this did not influence the nitrification processes.

At the family level (Fig. 6b), Hydrogenophilaceae and Xanthomonadaceae, belonging to the Proteobacteria, increased from 0.8% to 4.4% and 4.3% to 7.1%, respectively. It has been reported that the genera in the Hydrogenophilaceae and Xanthomonadaceae can digest the complex organic matter and contribute to denitrification processes (Tang et al., 2018). Thus, the obvious accumulation of these families in the reactor further enhanced the nitrogen removal. Rhodocyclaceae, a phosphate accumulating organism that uses either oxygen or nitrate as a final electron acceptor (Guadie et al., 2014), was detected in both sludge samples, which further verified the denitrifying phosphate removal shown in Fig. 3 and Fig. 4. Moreover, the high metabolic flexibility of Comamonadaceae in the activated sludge has benefits for promoting the organics utilization efficiency and nutrients removal rate. In the Bacteroidetes, Flavobacteriaceae and Saprospiraceae were the main families and were obviously enriched during the operation. It has been documented that the families of Flavobacteriaceae and Saprospiraceae can use proteins and are favorable for nitrogen and phosphate removal, which further explains the high nutrient removal efficiencies (Adrados et al., 2014; Guadie et al., 2014). Roseiflexaceae, with a relative abundance of 8.3%, was the main family of Chloroflexi detected in the activated sludge, and it can digest more complex carbon sources, which is important for utilizing the residual organics and ensuring low COD content in the effluent (Tang et al., 2017). Anaerolineaceae, a family for organics removal, increased from 2.6% to 3.3%, further contributing to the high effluent quality. However, Christensenellaceae, a family in Firmicutes, decreased from 15.0% to 0.2% with the

addition of FSFW, which might have been due to the change of the wastewater biodegradability. Phylum Firmicutes was essentially eliminated from the reactor, which will be investigated in the future. Thus, by adding the FSFW, functional microorganisms were selectively enriched in the reactor, which obviously improved the nutrient removal efficiencies.

Fig. 6

Conclusions

FSFW exhibited combined nutrient removal properties of both easily and slowly biodegradable organics and could obviously improve the nitrogen (90%) and phosphate (98%) removal efficiencies in wastewater treatment during the long-term operation. The nitrogen removal was achieved through anoxic denitrification and SND processes, whereas the phosphate was eliminated by PAOs and DPAOs. With the addition of FSFW, the sludge floc size increased significantly, EPS accumulated slightly, microbial metabolic capacity and diversity were enhanced obviously and the microbial communities were enriched selectively. Overall, using thermophilic fermentation to produce external carbon sources is a suitable way to recycle food waste.

E-supplementary data for this work can be found in e-version of this paper online.

Acknowledgments

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Table 1. Nitrogen removal performance with different external carbon sources

Carbon source	C/N	SDNR (mg-N/g-VSS·h)	P_{DN} (g-N/g-COD)	Y_{HD} (g-COD/g-COD)	Reference
Hydrolysis liquid from activated sludge	7	2.8	0.17	0.52	Guo et al., 2017
Acidogenic liquid from activated sludge	7	3.2	0.29	0.16	Guo et al., 2017
Fermentation liquid from food wastes	5.5	12.9	0.17	0.50	Zhang et al., 2016
Soluble products of FSFW	6	10.4	0.19	0.51	This study
FSFW	6	3.7	-	-	
Solid fraction of FSFW	6	1.8	-	-	

- represents no detection as the COD content could not be measured in the mixed liquor.

Table 2. The net nitrogen and phosphate removal rate during the cyclic study

Unit: mg/g-VSS·h

	Anaerobic (0-45 min)	Aerobic (45-105 min)	Anoxic (105-195 min)	Aerobic (195-255 min)	Anoxic (255-295 min)	Aerobic (295-325 min)
Nitrification rate	-	3.5	-	3.1	-	2.4
Denitrification rate	3.9	1.5	2.8	1.8	2.8	1.3
P release rate	3.4	-	0.5	-	-	-
P accumulation rate	-	3.9	0.5	1.7	0.5	1.0

Note: The nitrogen and phosphate removal rate was calculated with the data in the cyclic study; - represented no detection.

Table 3. Properties of the activated sludge during the operation

Phase	MLSS (g/L)	MLVSS/MLSS	SVI (mL/g-VSS)	Particle size (μm)
I	3.5±0.4	0.78±0.2	125.0±20.2	136.2
II	4.7±0.3	0.80±0.1	107.3±12.3	155.4
III	4.4±0.4	0.81±0.4	78.6±11.6	247.4
IV	5.2±0.9	0.85±0.1	77.4±14.9	424.9

Table 4. Variations of EPS in the activated sludge during the operation

Phase	SEPS (mg/g-VSS)			BEPS (mg/g-VSS)		
	Polysaccharide	Protein	P/C	Polysaccharide	Protein	P/C
I	1.26±0.7	4.13±0.6	3.28	7.47±1.2	18.43±2.5	2.47
II	1.36±0.2	7.49±1.2	5.51	16.82±2.2	54.21±7.3	3.22
III	1.44±0.4	8.88±1.5	6.17	19.12±2.4	64.96±5.6	3.40
IV	1.89±0.6	9.15±1.1	4.84	15.91±1.9	54.19±8.9	3.41

Table 5. Parameters of the microbial communities in both activated sludge samples

Sample	Sequence number	OUT number	Shannon index	ACE index	Chao1 index	Coverage	Simpson
before	23420	1901	5.7	2191. 4	2068.1	0.98	0.03
after	28115	1598	5.3	2074.8	1920.1	0.98	0.02

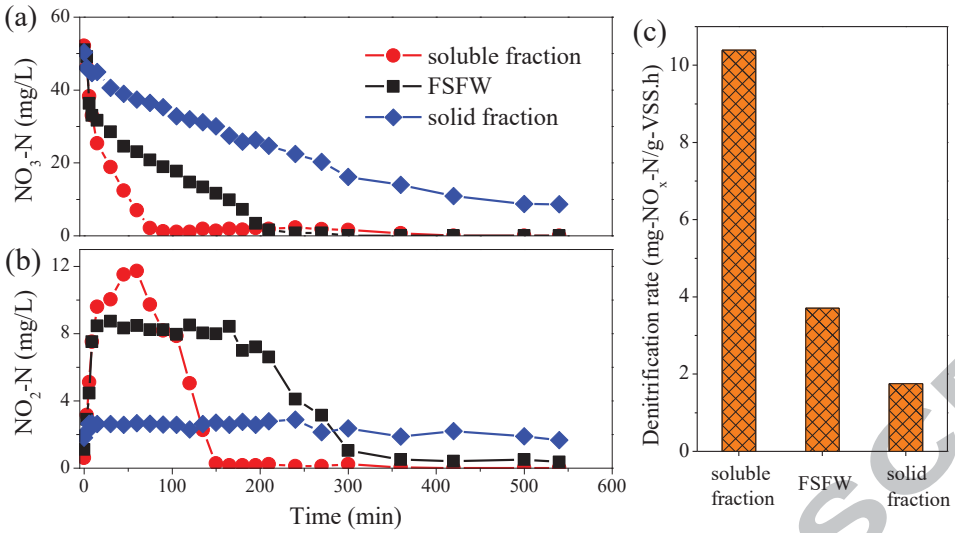

Fig. 1. Nitrogen removal performance with different fractions of the FSFW.

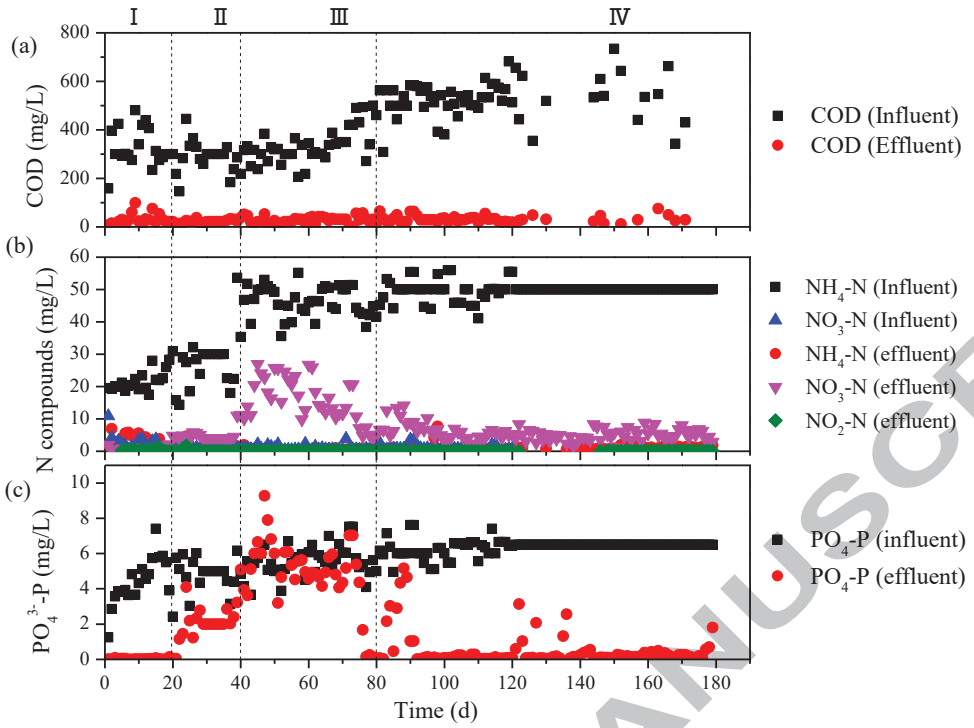

Fig. 2. Nitrogen and phosphate removal properties with the FSFW in the SBR

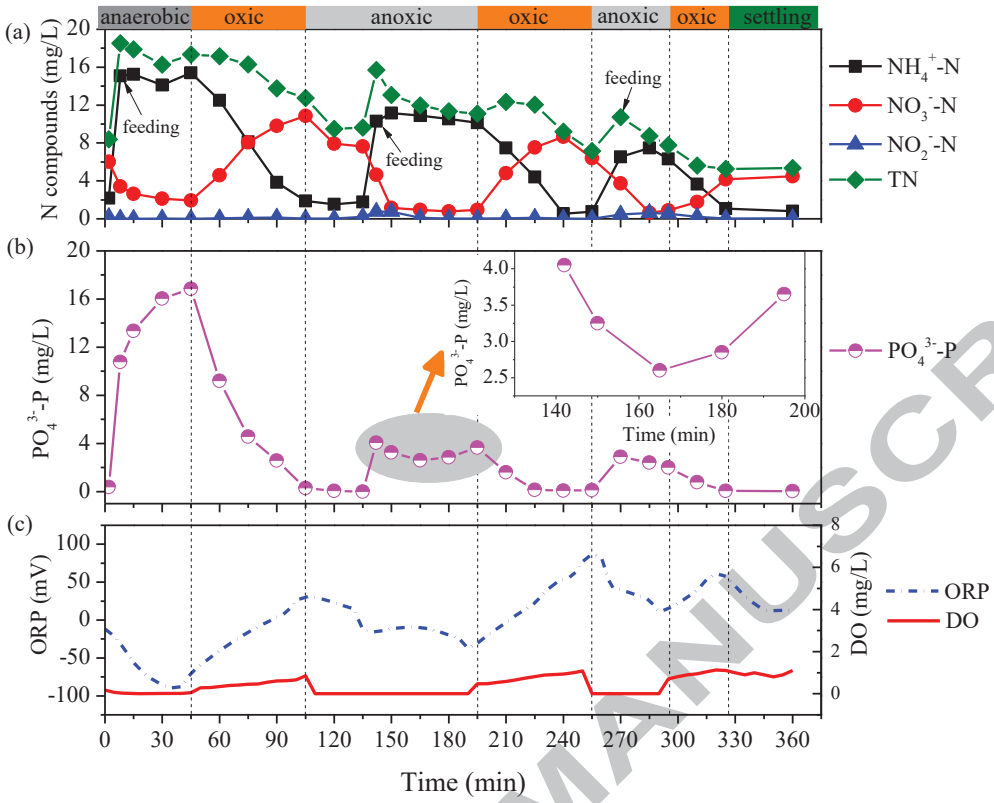

Fig. 3. Variations of nitrogen and phosphate in the cyclic study

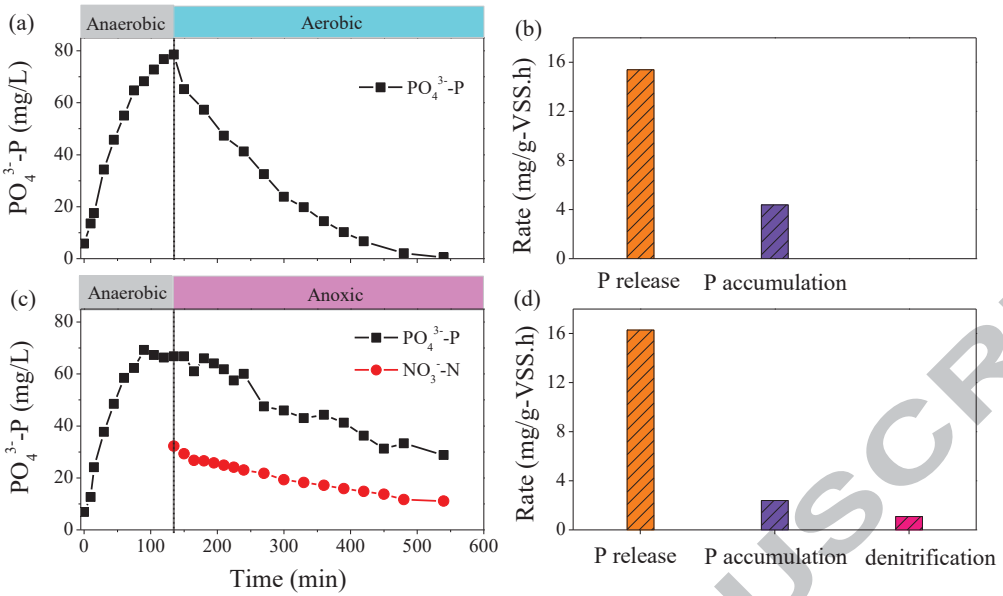

Fig. 4. Phosphate removal performance with the FSFW in the batch tests. (a) A-O reactor for detecting oxic phosphate accumulation processes, (b) A-A reactor for detecting denitrifying phosphate accumulation processes.

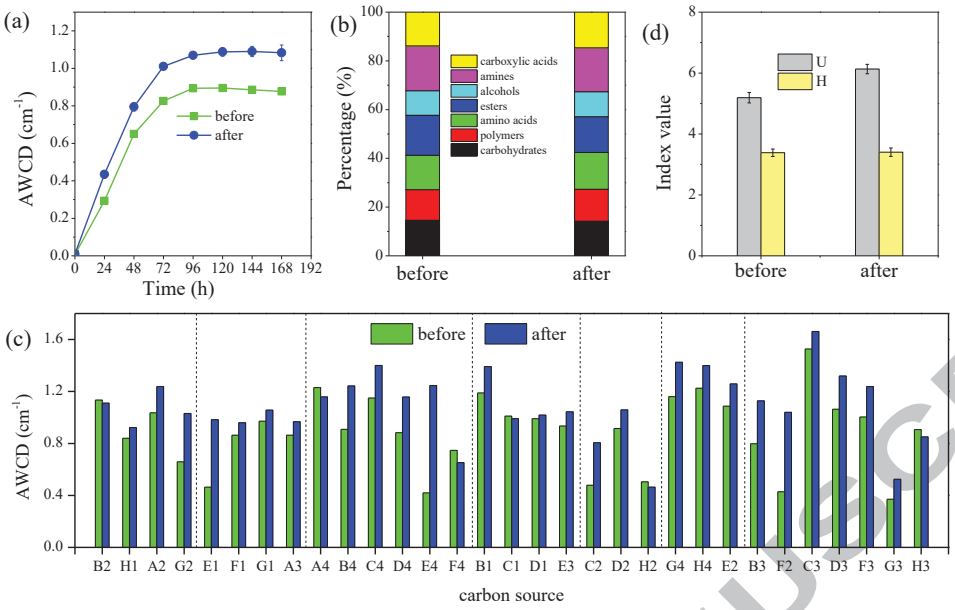

Fig. 5. Microbial metabolic activities of the activated sludge samples before and after adding the FSFW

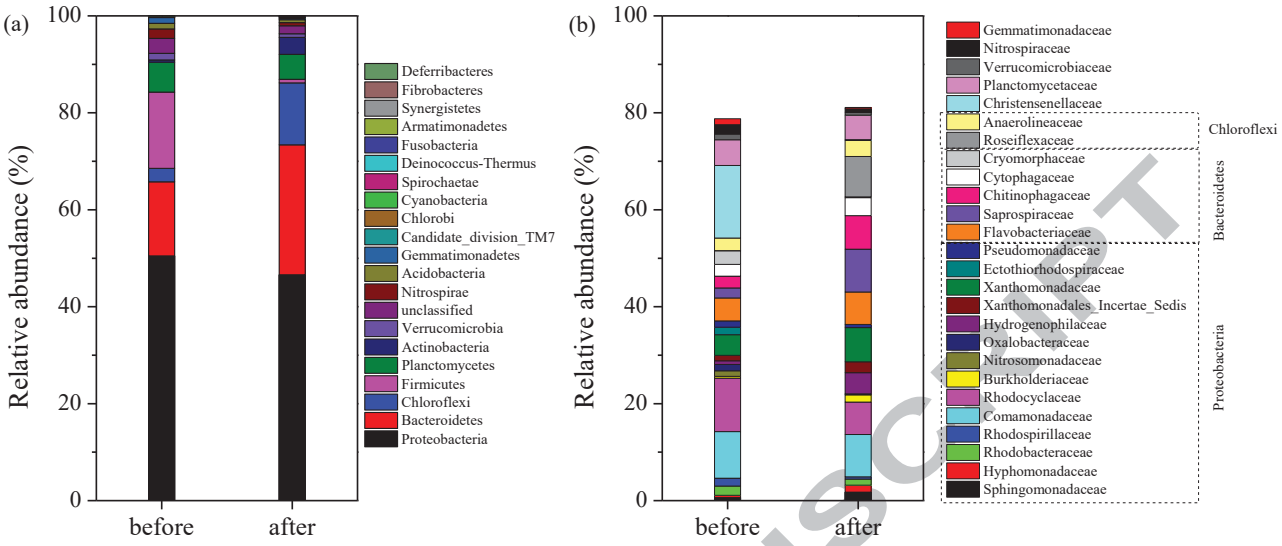

Fig. 6. Microbial communities in the activated sludge samples before and after adding the FSFW. (a) at phylum level and (b) at family level