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A new activated primary tank developed for recovering carbon source and its application

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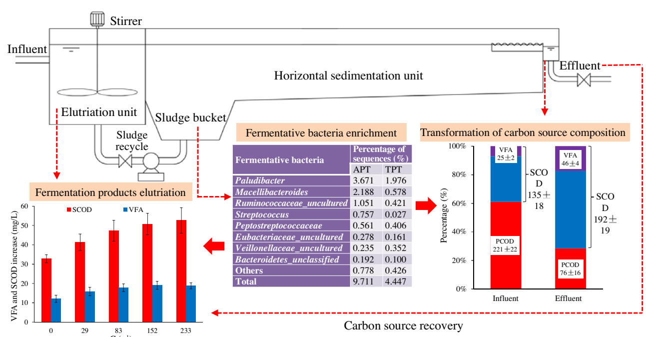
HIGHLIGHTS

- A new activated primary tank (APT) was developed to recover carbon source from sludge.
- APT was beneficial for the breeding of fermentative bacteria and maximised VFAs yield.
- Mechanical elutriation significantly promoted the release of fermentation products.
- APT was applied in a sewage treatment plant and recovered carbon source successfully.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel activated primary tank process (APT) was developed for recovering carbon source by fermentation and elutriation of primary sludge. The effects of solids retention time (SRT), elutriation intensity (G) and return sludge ratio (RSR) on this recovery were evaluated in a pilot scale reactor. Results indicated that SRT significantly influenced carbon source recovery, and mechanical elutriation could promote soluble COD (SCOD) and VFA yields. The optimal conditions of APT were SRT = 5 d, $G = 152\ s^{-1}$ and RSR = 10%, SCOD and VFA production were 57.0 mg/L and 21.7 mg/L. Particulate organic matter in sludge was converted into SCOD and VFAs as fermentative bacteria were significantly enriched in APT. Moreover, the APT process was applied in a wastewater treatment plant to solve the problem of insufficient carbon source. The outcomes demonstrated that influent SCOD of biological tank increased by 31.1%, which improved the efficiency of removing nitrogen and phosphorus.

1. Introduction

The activated sludge biological treatment process has been extensively used in urban wastewater treatment plants (WWTP). In this process, both biological denitrification and phosphorus removal depend on the available biodegradable carbon. For the wastewater treatment plants with sufficient carbon source, the removal of nutrients is strongly affected by the operating conditions of biological system, especially the recycling ratios from aerobic tank to anoxic tank. However, for the wastewater treatment plants with low carbon source, by optimising the recycling ratios, it was difficult to solve the problem of insufficient carbon source, and removal of nitrogen and phosphorus was not ideal. The content of carbon source directly influences the efficiency of denitrification and phosphorus removal, especially in wastewater

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treatment plants with low C/N and C/P ratios (Li et al., 2008; Wang et al., 2010). As the standards for the effluent quality of wastewater treatment plants become more stringent, the insufficient carbon source has become the dominant factor limiting the efficiency of biological denitrification and phosphorus removal in wastewater treatment plants. Thus, the problem of carbon source shortages needs to be solved urgently.

Adding an external carbon source such as acetate, glucose, methanol and ethanol increases the operational costs in wastewater treatment plants (Gao et al., 2011). Therefore, employing an internal carbon source produced by hydrolysis and acidification of primary and secondary sludge, in recent years has been recognised as the main method for solving the problem of insufficient carbon source (Peng et al., 2012; Zhang et al., 2013). Large amount of VFAs can be produced from the hydrolysis and fermentation of sludge, which not only offers carbon source for biological systems but also significantly improves the efficiency of nitrogen and phosphorus removal (Liu et al., 2012a). To improve the efficiency of sludge hydrolysis and fermentation, the effects on sludge fermentation were studied, such as solids retention time (SRT) (Yuan et al., 2009), temperature (Luo et al., 2014) and pH (Wu et al., 2009; Feng et al., 2009). Meanwhile the sludge fermentation conditions were optimised. However, in practice, the application of the method can hardly be accomplished in certain wastewater treatment plants due to limitation of site area. Although it is allowed to have larger site area in some wastewater treatment plants, the cost of setting up and operating fermenters is high. Furthermore, it is not flexible for the fermenters to accommodate the modifications of the flows, retention times (Munch and Koch, 1999). The previous studies have shown that 40% to 60% of the carbon source in the influent consists of particulate organic matter in urban wastewater treatment plants in China (Wang et al., 2007). A large amount of particulate carbon source is lost with suspended solid removal by sedimentation in traditional primary settling tanks, which further exacerbates the shortage of carbon source for biological nitrogen and phosphorus removal. However, withdrawal of the primary sedimentation tank will significantly increase the loading of the biological system and hinder the operation. Therefore, the activated primary tank process is developed comprising: (1) allowing settled raw sludge solids to accumulate in the bottom of the primary settler tanks; (2) partially recycling this sludge to elutriate the fermentation products out of the sludge (Chanona et al., 2006; Bouzas et al., 2007; Ahn and Speece, 2006).

A good sludge fermentation outcome was recorded in the activated primary tank, but the release of fermentation products out of the sludge was incomplete. Some fermentation products were adsorbed by the primary sludge, this portion of the carbon source is difficult to elutriate (Peng et al., 2012), which hinders the release and recovery of carbon sources. In this study, a novel activated primary tank process equipped with a mechanical elutriation function is developed. The mechanical elutriation unit is installed in front of the traditional primary sedimentation tank to elutriate the return sludge and convert fermentation products into water. The aim of the analysis was to suggest a new activated primary tank so that carbon source recovery from primary sludge could be improved. The modification proposed of the primary tank for recovering carbon source could be needed for plants designed for biological nutrients removal (like AAO) probably not for conventional activated sludge plants designed only for carbon removal and nitrification. The new activated primary tank process was operated at the front of the biological treatment system, and it will not interfere with the operation of biological treatment system (especially the recycling ratios). Under the biological system optimal operation conditions, the APT could recover the carbon source from primary sludge and increase the SCOD concentration of sewage. The APT can solve the problem of insufficient carbon source in the wastewater treatment plants, and enhanced the nitrogen and phosphorus removal of biological system. The optimal operating conditions of the system were established, and an examination of the microbial community in the system was conducted by high-throughput pyrosequencing. Additionally, the activated primary tank was utilised in a wastewater treatment plant to verify the technology's feasibility and good outcomes. This study proposed a novel activated primary tank process to do two things: firstly, realise carbon source recovery; and secondly, solve the problem of insufficient carbon source in wastewater treatment plants.

2. Methods

2.1. Activated primary tank pilot scale reactor and its application

The pilot scale experimental device is shown in Fig. 1a. A mechanical elutriation unit was installed in front of a traditional horizontal-flow primary sedimentation tank. A stirrer with adjustable stirring intensity was installed in the elutriation unit. A perforated wall separated the sludge elutriation unit and the sedimentation area to prevent interference from the flow fields of the two units. The volume of the sludge bucket in the primary sedimentation tank and the solid retention time were extended to improve the effects of the sludge hydrolysis and fermentation. A return sludge system was positioned between the sludge bucket and the mechanical elutriation unit so that primary sludge from the sludge bucket could be returned to the mechanical elutriation unit. The mechanical stirring device adequately mixed the sludge with the influent, whereby the fermentation products were elutriated into the influent. The treatment capacity of the activated primary tank was 0.5 m^3/h, the hydraulic retention time (HRT) of the elutriation unit and sedimentation zone was 0.4 h and 1.5 h, respectively.

The activated primary tank process was applied in Xi'an No. 4 Wastewater Treatment Plant (WWTP); the traditional primary sedimentation tank was transformed into an activated primary tank (Fig. 1b). Section 2.4 describes the wastewater treatment plant.

2.2. Operating conditions of the pilot scale reactor

The APT was installed at Xi'an No. 4 wastewater treatment plant. The influent of the reactor is the grit chamber effluent of this plant. The solids retention time (SRT) of the primary sludge was controlled at 1, 3, 5 and 7 d according to the sludge height in the sludge bucket. The stirring blades' rotation speeds in the mechanical elutriation unit varied at 0, 20, 40, 60 and 80 rpm, with the corresponding stirring velocity gradients (G) being 0, 29, 83, 152, and 233 s^{-1}, respectively. The return sludge ratios (the ratio of return sludge flow to influent flow) were 0%, 5%, 10% and 15%, respectively. The reactor was the traditional primary settling tank when the return sludge ratio (RSR) was 0%. Water quality analysis was conducted for 21 days under each operational condition.

2.3. The batch experiments

To analyse the effect of velocity gradient on sludge sizes, the batch fermentation experiments were conducted in two identical reactors with working volume of 2 L. The primary sludge was taken from the primary sedimentation tank of the Xi'an No. 4 Wastewater Treatment Plant. The two reactors were stirred with velocity gradient of 31 s^{-1} and 160 s^{-1}, respectively. The changes of sludge size during the process of sludge fermentation with different velocity gradient were analysed.

The trial experiments were conducted to analyze the function of mechanical elutriation in promoting SCOD release from sludge.

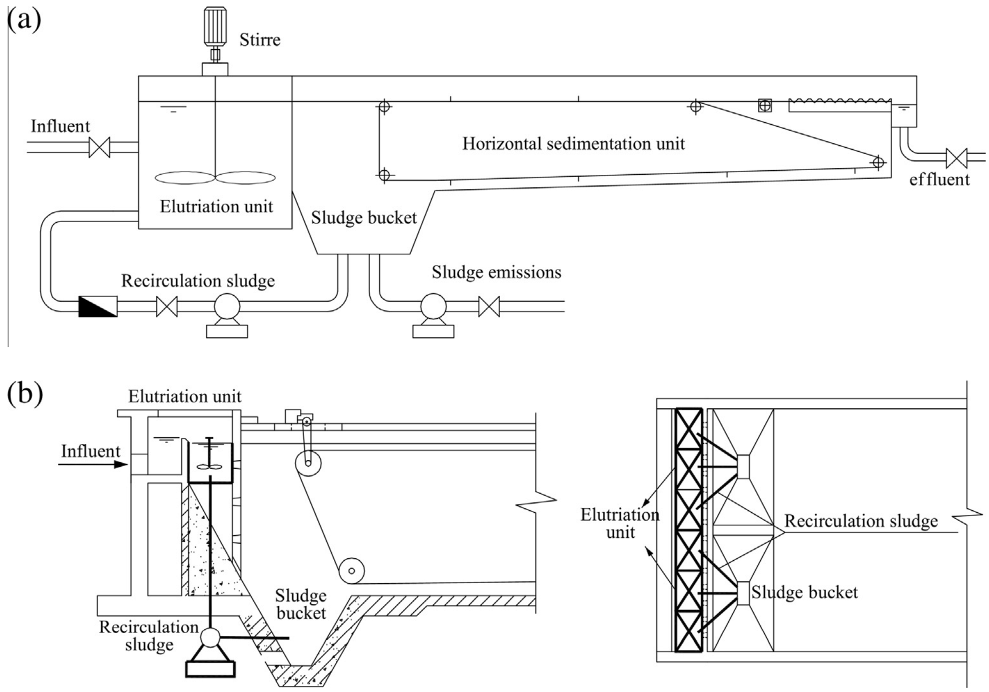

Fig. 1. (a) Schematic diagram of the activated primary tank (APT) and (b) application of the APT in a wastewater treatment plant.

600 mL of sludge was taken from the sludge bucket of APT with SRT = 5 d, RSR = 10% and $G = 152\ s^{-1}$, and was divided into six beakers equally. Because the return sludge of APT was mixed with sewage (1:10) in the elutriation unit when the RSR was 10%. Therefore, 1000 mL of ultrapure water was added to each beaker, and then the sludge was elutriated for 10 minutes with different velocity gradient by MY3000-6N stirrer (MeiYu, China). The SCOD concentration was measured after mechanical elutriation.

2.4. Wastewater treatment plant description and the full scale APT

An AAO process with a treatment capacity of 250,000 m^3/d was employed by Xi'an No. 4 Wastewater Treatment Plant (WWTP), China. Ten parallel traditional horizontal primary sedimentation tanks were used in the WWTP, and the hydraulic retention time was 1.5 h. Our previous studies showed that the C/N and C/P ratios in the influent of the WWTP were low, and 33% COD was lost in the traditional primary sedimentation tank (TPT). The operational effect of the AAO process was seriously compromised by the insufficient carbon source. In fact the TN and TP concentrations in the effluent were 15.6 ± 1.8 mg/L and 0.63 ± 0.23 mg/L, respectively, which cannot meet China's current discharge standards (Table 1).

To solve the problem of insufficient carbon source for the AAO process in Xi'an No. 4 Wastewater Treatment Plant caused by low COD in the influent and carbon source loss in the primary sedimentation tank, the activated primary tank process was applied. The primary sedimentation tank in Xi'an No. 4 Wastewater Treatment Plant was transformed into the activated primary tank (Fig. 1b). The solids retention time of the primary sludge in the sludge bucket rose to 5 d, and a return sludge system with RSR = 5% was positioned between the sludge bucket and the distribution channel. A stirrer with a stirring velocity gradient of $160\ s^{-1}$ was installed in the distribution channel to elutriate the fermentation products derived from sludge.

2.5. Chemical analysis

The total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total phosphorus (TP), total nitrogen (TN), nitrate, phosphate, and suspended solids (SS) were measured according to standard methods (APHA, 1998). The particulate chemical oxygen demand (PCOD) was calculated as the difference between the TCOD and SCOD. The particle size of sludge was measured by LS320/SVM + Laser particle size distribution analyser (Beckman-Coulter, USA).

VFAs composition was determined using an Agilent 6890N GC with a flame ionisation detector and DB-WAXETR column (30 m × 1.0 μm × 0.53 mm). Nitrogen was the carrier gas, and the flux was 20 mL/min. The injection port and detector were maintained at 230 °C and 250 °C, respectively. The oven of the GC was programmed to remain at 100 °C for 2 min, then to increase at a rate of 6 °C/min to 200 °C, and to be maintained at 200 °C for an additional 2 min. The filtrate was collected in a 1.5 mL gas chromatograph (GC) vial and acidified with 3% H_3PO_4 to pH 4.0. The sample injection volume was 1.0 μl. The VFAs concentration was converted into the COD concentration using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic

Table 1
Influent and effluent characteristics of the Xi'an No. 4 wastewater treatment plant. (The data represent the averages and standard deviations, number of samples (n) ⩾ 200.)

Parameter	BOD_5 (mg/L)	COD (mg/L)	SCOD (mg/L)	TN (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	SS (mg/L)
Influent of WWTP	180 ± 42	342 ± 32	149 ± 24	41.4 ± 2.6	28.7 ± 2.1	3.9 ± 0.5	241 ± 43
Effluent of TPT	172 ± 31	228 ± 24	142 ± 21	37.2 ± 2.3	27.6 ± 1.8	3.6 ± 0.4	152 ± 36
Effluent of WWTP	7.2 ± 1.2	21.2 ± 2.9	19.4 ± 2.4	15.6 ± 1.8	0.52 ± 0.32	0.63 ± 0.23	8.1 ± 1.1
Discharge standard	10	50	–	15	5	0.5	10

acid, 1.82 for n-butyric and iso-butyric acid, and 2.04 for n-valeric and iso-valeric acid.

Readily biodegradable organic matter (S_S) and slowly biodegradable organic matter (X_S) were analysed via respirometric assessment. Biomass (2000 mL) from the aerobic tank of the Xi'an No. 4 wastewater treatment plant was elutriated three times and precipitated to 1 L. The biomass was aerated for 12 h, after which it was in the endogenous respiration stage. The biomass was placed into a hermetic reactor, and an influent or effluent volume of 1000 mL from the APT was added. The sludge was maintained in suspension by a magnetic stirrer and kept at 20 ± 1 °C. ATU reagent was added to inhibit nitrification, and the pH was adjusted to neutral with a phosphate buffer solution. The dissolved oxygen (DO) in the reactor was monitored online with a DO meter (Hamilton Bonaduz AG ARC120, Bonaduz, Switzerland). The reactor was aerated until DO reached 6 mg/L; then, DO change was monitored on-line. The aeration was repeated when the DO in the reactor was reduced by 2 mg/L. This procedure was repeated to monitor the oxygen uptake rate (OUR) of the hermetic reactor. S_S and X_S concentrations were calculated using Matlab software and correlation formulas and inert organic matter (I) was calculated as the difference between TCOD and the S_S and Xs.

The nitrogen and phosphorus removal performance of the carbon source were represented by the maximum specific denitrification rate and phosphorus release rate of the activated sludge when the reactor's effluent served as carbon source. The following method was used: 1000 mL of biomass from the wastewater treatment plant was placed into two beakers (2000 mL each) and maintained at 25 °C. Potassium nitrate was added to one beaker to obtain an initial nitrate concentration of 20 mg/L. An effluent volume of 1000 mL from the reactor was added to each beaker as a carbon source. Eight samples were taken over time to measure the maximum specific denitrification rate, which was based on the consumption of nitrate (linear correlation coefficient $R^2 > 0.97$), and the maximum specific phosphorus release rate. This was based on the increase in the amount of phosphate (linear correlation coefficient $R^2 > 0.95$).

2.6. DNA extraction and PCR amplification

Sludge samples from the traditional primary sedimentation tank (TPT) in the Xi'an No. 4 wastewater treatment plant and the activated primary tank reactor (SRT = 5 d, $G = 152\ s^{-1}$, RSR = 10%) were taken for DNA extraction with a Power Soil DNA Isolation Kit (MO Biomedicals, USA). The V4-V5 regions of the bacterial 16S ribosomal RNA gene were amplified by PCR (95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) using primers 338F (5′-barcode-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GG ACTACHVGGGTWTCTAAT-3′) (Dennis et al., 2013). Here the barcode is an eight-base sequence unique to each sample. PCR reactions were done in triplicate 20 μL mixtures containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

2.7. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.A.) according to the manufacturer's instructions, and quantified using QuantiFluor™-ST (Promega, U.S.A.). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols.

2.8. Processing of sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) with the following criteria: (i) the 250 bp reads were truncated at any site receiving an average quality score <20 over a 10 bp sliding window, discarding the truncated reads that were shorter than 50 bp; (ii) exact barcode matching, 2 nucleotide mismatches in primer matching, and reads containing ambiguous characters were removed; and (iii) only sequences with overlaps longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Operational units (OTUs) were clustered with a 97% similarity cut off using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME.

3. Results and discussion

3.1. Factors that influence carbon source recovery in pilot reactor

3.1.1. Effect of the SRT on carbon source recovery

The solids retention time is an important factor influencing the hydrolysis and fermentation of primary sludge. As shown in Fig. 2, when the stirring velocity gradient (G) and RSR were 233 s^{-1} and 5%, increases of the SCOD and VFA (△SCOD and △VFA) in the reactor's effluent with SRT = 1 d were 11 mg/L and 3 mg/L, respectively. When the SRT was increased to 3 d and 5 d, the △SCOD and △VFA in the effluent of the reactor were significantly increased (Fig. 2). The △SCOD of the reactor effluent increased to 33 mg/L and 53 mg/L, respectively, and the △VFA were 12.3 mg/L and 18.8 mg/L, respectively. By increasing the SRT, the amount of

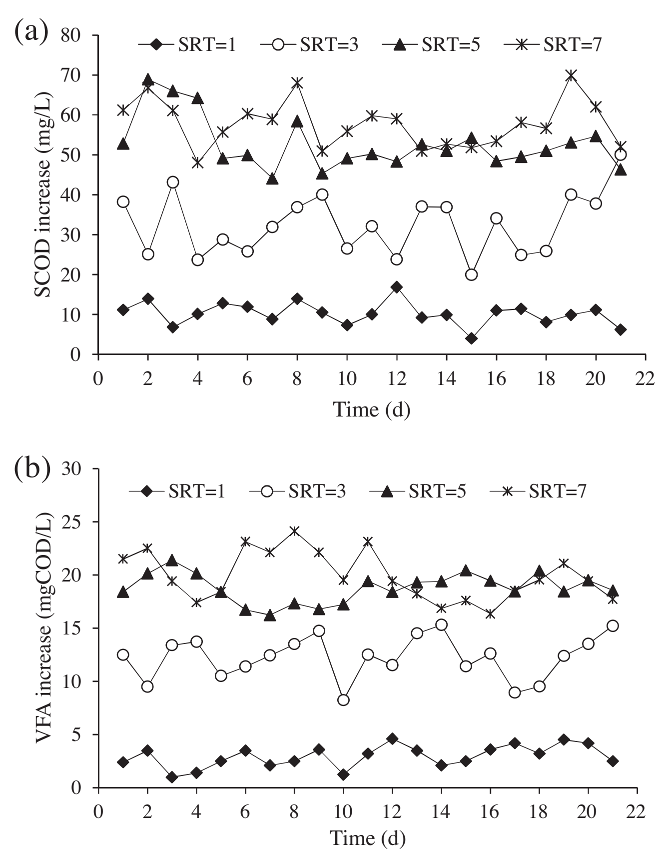

Fig. 2. Effect of SRT on (a) SCOD and (b) VFA production in activated primary tank with RSR = 5% and $G = 233\ s^{-1}$.

carbon source recovery was enhanced significantly, which is accordance with other researches (Bouzas et al., 2007). When the SRT = 1, 3, and 5 d, the organic contents in the sludge (VSS/SS) in the reactor were 0.556, 0.532, and 0.501, respectively. The VSS/SS of the sludge reduction indicated that the particulate organic matter in the primary sludge was gradually hydrolysed into soluble substance. However, when SRT increased to 7 d, the SCOD and VFAs yields slightly increased. The SCOD and VFAs yields were 57.8 mg/L and 19.9 mg/L, which were not significantly higher that at SRT = 5d. Miron et al. (2000) analysed the role of solids retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge. They found that digestion of sludge at 25 °C resulted in methanogenic conditions for SRT over 8 days. In addition, the other studies have also revealed that SCOD and VFAs yields decreased with a longer SRT during the primary sludge fermentation (Wu et al., 2009; Ji et al., 2010). Meanwhile, the longer SRT, the larger volume of sludge bucket is required, which increased the cost of ATP. Therefore, the optimal SRT for the APT is 5 d. Ahn and Speece (2006) obtained identical results in that most of the SCOD (78–84%) was produced within the initial 5 d during the primary sludge fermentation, and the soluble carbon source increased slowly when SRT was extended beyond 5 d.

3.1.2. Effect of mechanical elutriation on carbon source recovery

Fig. 3 shows the effect of mechanical elutriation on the reactor' carbon source recovery when the RSR = 5% and SRT = 5 d. The △SCOD and △VFA in the reactor's effluent were 32 mg/L and 12.1 mg/L, respectively, when only the sludge return did not undergo mechanical elutriation ($G = 0\ s^{-1}$), indicating that a small amount of carbon source can be recovered. At a mechanical elutriation intensity of $29\ s^{-1}$, the △SCOD and △VFA in the effluent of the reactor were 42 mg/L and 15.9 mg/L, respectively, which were 30% higher than those of the reactor without mechanical elutriation. When the mechanical elutriation intensity rose to $83\ s^{-1}$, $152\ s^{-1}$ and $233\ s^{-1}$, the △SCOD of the reactor effluent were 47 mg/L, 51 mg/L and 53 mg/L, respectively (Fig. 3a). Compared to the effluent of the reactor without mechanical elutriation, the △VFA of the reactor effluent was increased by 47.1%, 57.9% and 55.4%, respectively.

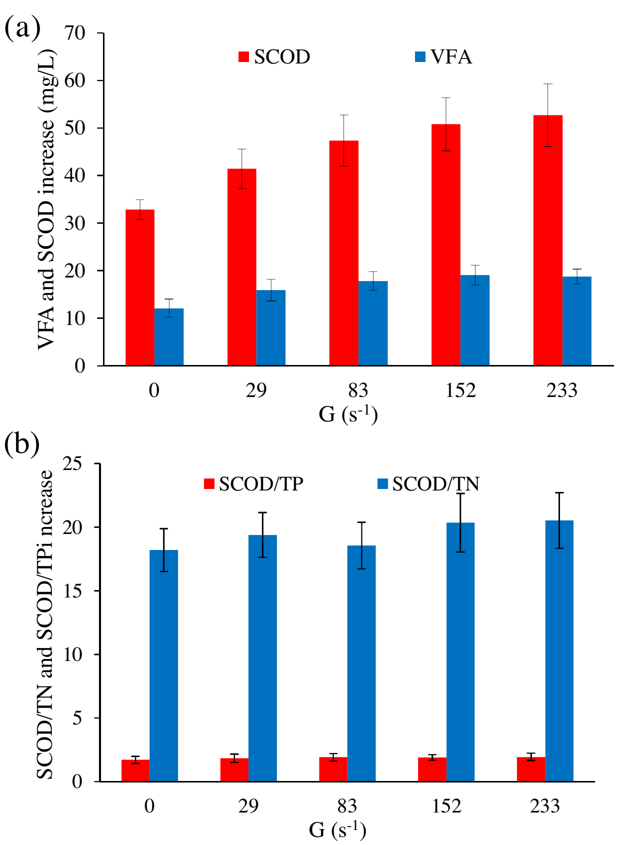

Fig. 3. Effect of the elutriation velocity gradient on (a) SCOD and VFA and (b) SCOD/TN and SCOD/TP increase in the activated primary tank with RSR = 5% and SRT = 5 d. (The data represent the average and standard deviations, number of samples (n) = 21.)

Mechanical elutriation significantly improved the activated primary tank's carbon source recovery. However, there is a limit to which the carbon source recovery through mechanical elutriation can be done, i.e., at a velocity gradient of $152\ s^{-1}$, above which the carbon source recovery from the reactor is difficult to improve significantly. Therefore, the optimal elutriation intensity for ATP was $152\ s^{-1}$. The power of the stirrer could be calculated by the velocity gradient, and it was about 4.5×10^{-3} KW. The treatment capacity of the APT was $0.5\ m^3/h$, so the additional energy of elutriation unit need for the operation of the APT was about $9 \times 10^{-3}\ KW\ h/m^3$.

The functional mechanisms of mechanical elutriation in improving carbon source recovery can be analysed with reference to three issues. First, the sludge sizes of the APT were analysed, the results showed that particle size of sludge in the APT with $G = 0\ s^{-1}$ and $G = 152\ s^{-1}$ were 55.2 ± 2.3 μm and 47.0 ± 2.5 μm. The particle size of sludge in the APT with $G = 152\ s^{-1}$ was smaller than that with $G = 0\ s^{-1}$, which indicated that some large-particle organic matter were broken into small particles through mechanical stirring. Table 2a showed the changes of sludge size during the process of primary sludge fermentation in the batch reactors with different velocity gradient ($G = 31\ s^{-1}$ and $G = 160\ s^{-1}$). It can be seen, the sludge size in the reactor with $G = 160\ s^{-1}$ decreased significantly during the process of fermentation, but decreased slightly in the reactor with $G = 31\ s^{-1}$. This also elucidates the function that higher velocity gradient facilitates the decomposition of particles. The small particle size benefitted the hydrolysis and fermentation of the particulate organic matter. Second, some hydrolysis and fermentation products were adsorbed by the sludge during fermentation (Peng et al., 2012). The shear force of the mechanical elutriation can enhance the release of fermentation products into water. In order to analyze the function of mechanical elutriation in promoting SCOD release from sludge, the trial experiments were conducted to verify the assumption. The sludge taken from APT was diluted and elutriated with different velocity gradient, the SCOD concentration was shown in Table 2b. When the velocity gradient increased from $28.2\ s^{-1}$ to $152.7\ s^{-1}$, the SCOD concentration increased from 71.3 mg/L to 77.9 mg/L, which indicated that the mechanical elutriation can enhance the SCOD release into water. When the velocity gradient was greater than $182.8\ s^{-1}$, the SCOD concentration slightly increased. This indicated that it was difficult to improve the SCOD release by increase the velocity gradient, when the velocity gradient was above $182.8\ s^{-1}$. Third, according to the studies of Leslie Grady et al. (1999) and Bouzas et al. (2007), the acetogenesis step can only take place at low hydrogen partial pressures and when the hydrogen partial pressure is high, this conversion will not proceed. Therefore, fermentation might be further enhanced with the decrease of the SCOD, VFA and hydrogen partial pressure on the surface of the sludge.

With the increase of the mechanical elutriation intensity, the removal efficiency of the TN and TP in the reactor decreased. When the velocity gradient increased from $0\ s^{-1}$ to $233\ s^{-1}$, the TN removal efficiency in the APT decreased from 13% to 10%, and TP removal efficiency decreased from 15% to 10%. This occurred because some ammonia nitrogen and phosphate were released

Table 2
The changes of sludge size with different velocity gradient, and the effect of velocity gradient on SCOD release.

Time	0 d	1 d	2 d	3 d	4 d	5 d	6 d
a. The changes of sludge size with different velocity gradient							
Sludge size (μm) $G = 31\ s^{-1}$	53.4 ± 1.1	53.8 ± 1.2	52.9 ± 0.9	53.1 ± 0.8	52.6 ± 0.9	53.1 ± 1.1	52.5 ± 0.8
Sludge size $G = 160\ s^{-1}$	53.4 ± 1.3	49.2 ± 0.7	47.7 ± 1.0	46.9 ± 1.1	45.7 ± 0.7	45.2 ± 0.8	44.6 ± 1.1
b. The effect of velocity gradient on SCOD release							
Velocity gradient (s^{-1})	28.3	72.0	124.2	182.8	246.8	315.5	
SCOD (mg/L)	71.3 ± 6.4	73.9 ± 8.3	77.5 ± 5.5	78.2 ± 7.8	79.1 ± 6.3	78.5 ± 8.2	

during the sludge fermentation, which is inevitable during the process of sludge fermentation. Meanwhile, according to the particle size changes of sludge in APT with $G = 0\ s^{-1}$ and $G = 152\ s^{-1}$, it can be seen that the large suspended solids were broken into smaller particles by the mechanical stirrer, and proved to be more difficult to remove by precipitation. Some smaller suspended nutrients were discharged from the reactor, which decreased the nutrient removal efficiencies. Although the TN and TP removal efficiencies decreased in the APT with the increase of mechanical intensity, the TN and TP concentration in the effluent of APT increased slightly. When the APT was operated with SRT = 5 d and $G = 152\ s^{-1}$, the release of TN and TP in APT was less than 2 mg/L and 0.3 mg/L, which would consume about 10 mg/L SCOD and 3 mg/L VFAs in the biological systems. However, the SCOD and VFAs yields of the APT were 51 mg/L and 19 mg/L, which were much higher than that needed for removing the TN and TP released from sludge. Fig. 3b illustrates the increase of the SCOD/TN and SCOD/TP (△SCOD/TN and △SCOD/TP) in the reactor's effluent compared to the influent at different velocity gradient. It can be observed in Fig. 3b, although the removal efficiencies of TN and TP decreased, the △SCOD/TN and △SCOD/TP in the effluent of the reactor increased by 1.7–2.0 and 18–21, respectively.

3.1.3. Effect of the return sludge ratio on the carbon source recovery

Fig. 4 shows the SCOD, VFA, SCOD/TN and SCOD/TP in the influent and effluent of the reactor with different return sludge ratios (RSR = 5%, 10%, and 15%) at a mechanical elutriation intensity of $152\ s^{-1}$ and SRT of 5 d. When the return sludge ratio (RSR) increased from 5% to 10%, the △SCOD of the reactor increased from 50.8 mg/L to 57.0 mg/L, and the △VFA increased from 19.1 mg/L to 21.7 mg/L. With the increase of the RSR, the SCOD and VFAs yields of APT were improved. By increasing the return sludge ratio, the soluble organic matter and VFAs produced by the sludge fermentation could be released into the water instantly through elutriation. Moreover, with the increase of return sludge ratio, the fermentation bacteria at the bottom of the reactor and the particulate organic matter in the influent were well mixed. Consequently, inoculating fermentation was formed, which improved the fermentation.

However, when the return sludge ratio increased from 10% to 15%, the SCOD and VFAs yields were 57.4 mg/L and 22.3 mg/L, the improvement of the SCOD and VFAs yields in the reactor effluent was negligible. When the return sludge ratio increased from 10% to 15%, the sludge concentration at the bottom of the APT decreased from 36,400 ± 3200 mg/L to 29,600 ± 4300 mg/L. This indicted that an excessive return sludge ratio resulted in the lower sludge concentration at the sludge bucket, which might compromise the SCOD and VFAs yields. Meanwhile, the study by Chanona et al. (2006) showed that an excessive return sludge flow rate could lead to lower effluent VFAs concentration due to the lower solids thickening in the bottom of the settler at high return sludge flow rate. Furthermore, with the increase of RSR, although there was a fluctuant of △SCOD/TN and △SCOD/TP in the effluent, no significant increase was observed (Fig. 4b). Because a high RSR increases the reactor's energy consumption, so the appropriate RSR of the reactor was controlled within a range of 5–10%.

In order to analyze the actual effect of SCOD and VFAs production, the SCOD and VFAs concentration in the sludge recycle line of APT with SRT = 5 d, RSR = 10% and $G = 152\ s^{-1}$ were measured. The results showed that the SCOD and VFAs concentration in the sludge recycle line were 742 ± 82 mg/L and 252 ± 33 mg/L, which indicated that lots of soluble organic matters were produced by the primary sludge fermentation in the sludge bucket. The SCOD and VFAs concentration in influent of APT were 134.7 ± 18.5 mg/L and 24.5 ± 2.2 mg/L, and the return sludge and the sewage were mixed (1:10) in the elutriation unit. According to the theoretical calculation, the SCOD and VFAs concentration in sewage could increase 55.2 mg/L and 20.7 mg/L, which were basically similar with the actual SCOD and VFAs yields (57.0 mg/L and 21.7 mg/L) in APT with SRT = 5 d, RSR = 10% and $G = 152\ s^{-1}$.

3.2. Transformation of carbon source composition in pilot reactor

The composition of the carbon source is an important factor that affects biological nitrogen and phosphorus removal. Therefore, it is necessary to analyse the composition of the carbon source in the influent and effluent of the reactor. Fig. 5 depicts the composition of the carbon source in the reactor's influent and effluent with the SRT = 5 d, $G = 152\ s^{-1}$, and RSR = 10%. The carbon source in the reactor influent existed mainly in the particle state, and the particulate COD constituted 61.1% of the total COD. Analysis of the carbon source's biodegradability indicated that the slowly biodegradable organic matter (X_S) formed the majority (56.0%) of the organic matter in the reactor influent. The readily biodegradable organic matter (S_S) accounted for only 12.1% of the total COD; the remainder was inert organic matter (I). However, most of the organic matter in the APT effluent existed in dissolved state, of which the SCOD content formed 71.6% of the total COD, and the content of the readily biodegradable organic matter increased to 34.5%. The above finding demonstrates that the type of carbon source in sewage can be significantly improved by the hydrolysis and fermentation in the reactor. The particulate organic matter was hydrolysed into dissolved matter, and the slowly biodegradable organic matter was converted into readily biodegradable organic matter under microbial fermentation.

It has been reported that the content and composition of VFAs in wastewater affects the biological phosphorus removal (Li et al., 2008). It is agreed that acetic acid is the best carbon source for the biological removal of phosphorus, followed by propionic acid (Oehmen et al., 2004). The VFA compositions in the APT effluent were analysed, and results showed that the compositions of VFAs in the APT effluent under different operation conditions are basically similar. Acetic acid was the major component of VFAs, in the 75–85% range, followed by propionic acid at 10–15%, and C4–C5 acids at approximately 10%. These results are basically consistent with the studies of Bouzas et al. (2007). The high content of acetic acid in the system effluent is conducive to enhancing biological phosphorus removal. At a return sludge ratio of 0%, the reactor functioned as a traditional primary

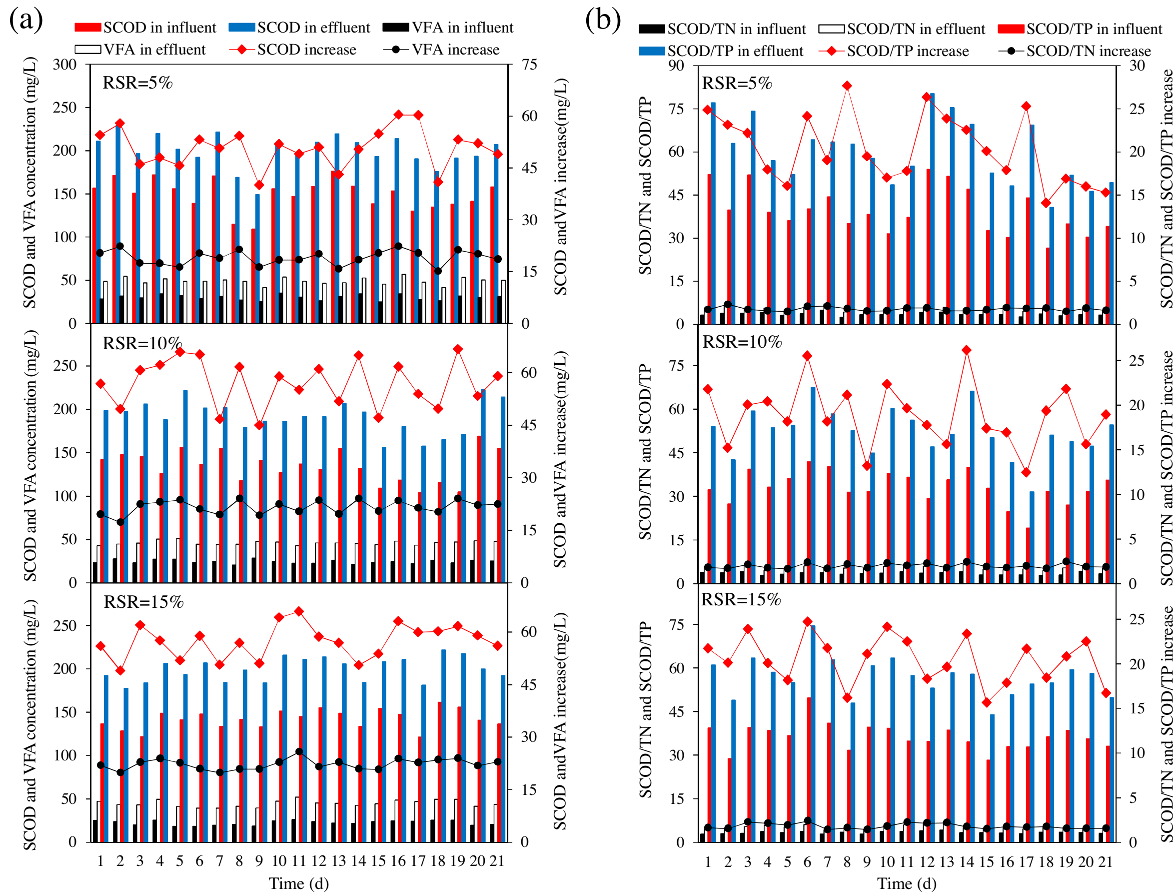

Fig. 4. Effect of the RSR on (a) SCOD and VFAs yields and (b) SCOD/TN and SCOD/TP increase in the activated primary tank with $G = 152\ s^{-1}$ and SRT = 5 d.

sedimentation tank. The maximum specific denitrification and phosphorus release rates were 6.12 ± 0.54 mgNO$_3^-$-N/gVSS·h and 2.21 ± 0.23 mgPO$_4^{3-}$-P/gVSS·h when using the effluent of the traditional primary sedimentation tank as the carbon source. When the effluent of the APT (RSR = 10%, SRT = 5 d and $G = 152\ s^{-1}$) was utilised as carbon source, the maximum specific denitrification and phosphorus release rates were 8.62 ± 0.78 mgNO$_3^-$-N/gVSS·h and 2.81 ± 0.27 mgPO$_4^{3-}$-P/gVSS·h. The rates were increased by 41% and 27%, respectively. This finding demonstrated that the removal of biological nitrogen and phosphorus was significantly improved, due to transformation of the carbon source types in APT.

3.3. Enhancement of fermentative bacteria by the APT

Pyrosequencing of the samples from the traditional primary sedimentation tank (TPT) and the activated primary tank (APT) yielded 26,114 and 25,499 effective sequence reads, respectively. For the sequences determined from pyrosequencing, 1246 and 1326 operational taxonomic units (OTUs) were identified at the 3% cutoff for the TPT and APT, respectively. Fig. 6 displays the relative abundance of bacterial community at the phylum level for the TPT and APT. The phyla with relative abundance higher than 1% are presented. *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* were the three most abundant phyla in the two systems. However, the relative abundance of the three phyla and other phyla were quite different. *Proteobacteria*, which plays a crucial role in the hydrolysis and acetogenesis stages (Jaenicke et al., 2011), was the most abundant phyla in the APT, and its relative abundance (34.2%) was significantly higher than that in the TPT (26.3%). The relative abundances of *Firmicutes* and *Bacteroidetes* in the APT were higher than those in the TPT, and these bacteria may convert the proteins and carbohydrates into propionate and acetate (Ueki et al., 2006; Yu et al., 2010). This outcome indicates two things: firstly, that the overall distribution of microbial communities was significantly changed by the activated primary tank process; and secondly, the change in the bacterial community was beneficial for the hydrolysis and fermentation of primary sludge.

Table 3 summarises the fermentative bacteria communities in the APT and TPT. The relative abundance of the fermentative bacteria in the APT was 9.519%, which was significantly higher than that in the TPT (4.347%). *Paludibacter*, *Macellibacteroides* and *Ruminococcaceae* were the most abundant fermentative bacteria in the two systems. The relative abundances of the three bacteria in the APT were 3.671%, 2.188% and 1.051%, respectively, and only 1.976%, 0.578% and 0.421%, respectively, in the TPT. *Streptococcus*, with a relative abundance of 0.757%, was another important fermentative bacteria in the ATP. However, the relative abundance of *Streptococcus* was extremely low at only 0.027% in the TPT. This finding indicated that there were significant differences in the fermentative bacteria communities in the two systems. The solid retention time (SRT) of primary sludge in traditional primary sedimentation tank is only several hours. It is not conducive to the breeding and enrichment of the fermentative bacteria in sludge bucket with short SRT and small amounts of sludge. However, the volume of the sludge bucket was extended and the SRT increased to 5 d in the activated primary tank, lots of sludge was accumulation in the sludge bucket for a long time, which provides the sufficient nutrition medium and time for the growth and reproduction of the fermentative bacteria. Therefore, compared to the traditional primary sedimentation tank, the APT was beneficial

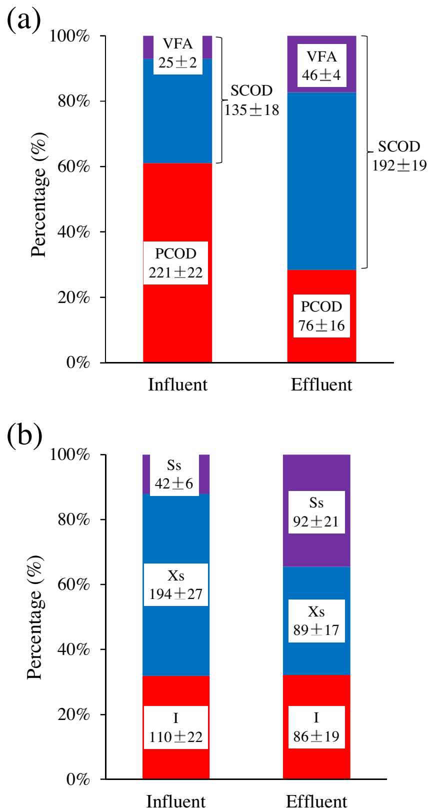

Fig. 5. (a) VFA, SCOD and PCOD and (b) S_S, X_S and I in influent and effluent of activated primary tank with RSR = 10%, $G = 152\ s^{-1}$, SRT = 5 d. (The figure labels indicate the average concentration ± standard deviation from at least $n = 7$ measurements, mg/L.)

for the breeding of fermentative bacteria. More fermentative bacteria were enrichment in the APT. Consequently, the particulate carbon source can be converted into soluble organic matter and volatile fatty acids by fermentative bacteria to recover the carbon source successfully from primary sludge.

3.4. The application of the APT in the full scale WWTP

The influent and effluent characteristics of the full scale activated primary tank and No. 4 wastewater treatment plant after transformation are shown in Table 4. The table shows that the efficiency in removing the SS in the full scale activated primary tank was approximately 28.7%, which indicated that the activated primary tank proved to be effective in removing the SS. Compared to the traditional primary sedimentation tank before transformation, the carbon source loss in the activated primary tank was less obvious; approximately 19.4% of the TCOD in the influent was removed by the activated primary tank. The SCOD concentration in the activated primary tank effluent was significantly increased (approximately 31.1%), and the △SCOD in the full scale activated primary tank was 47 mg/L. The SCOD/TN and SCOD/TP of the activated primary tank effluent rose by 37.6% and 38.4%, respectively. Due to the soluble carbon source increase and carbon source type transformation in the effluent of the full scale activated primary tank, the ability to denitrify and remove phosphorus in the AAO process was enhanced. The TN and TP in the effluent of the WWTP decreased to 13.2 ± 1.2 mg/L and 0.41 ± 0.14 mg/L, respectively, which could meet the discharge standards.

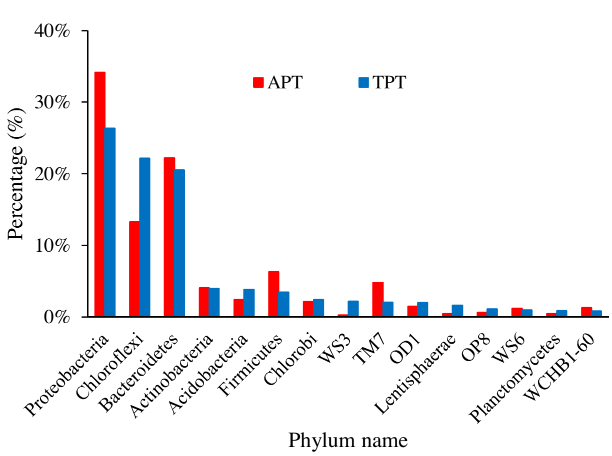

Fig. 6. Bacterial community composition at phylum level revealed by pyrosequencing.

4. Conclusions

A novel activated primary tank with mechanical elutriation function was developed to recover carbon source from primary sludge. Compared to the TPT, the APT was beneficial for the breeding of fermentative bacteria and lots of fermentative bacteria were

Table 3
Fermentative bacterial community composition at genus level by pyrosequencing.

Fermentative bacteria	References	APT		TPT	
		Reads	%	Reads	%
Paludibacter	Maspolim et al. (2015)	936	3.671	516	1.976
Macellibacteroides	Jabari et al. (2012)	558	2.188	151	0.578
Ruminococcaceae_uncultured	Xing (2006)	268	1.051	110	0.421
Streptococcus	Flythe and Andries (2009)	193	0.757	7	0.027
Peptostreptococcaceae	Flythe and Andries (2009)	143	0.561	106	0.406
Eubacteriaceae_uncultured	Xing (2006)	71	0.278	42	0.161
Veillonellaceae_uncultured	Liu et al. (2012b)	60	0.235	92	0.352
Bacteroides	Xing (2006)	35	0.137	5	0.019
Clostridium	Xing (2006)	32	0.125	46	0.176
Parabacteroides	Maspolim et al. (2015)	28	0.110	0	0
Zymomonas	Yamashita et al. (2008)	27	0.106	9	0.034
Smithella	Schink (1997)	19	0.075	12	0.046
Lachnospiraceae	Xing (2006)	9	0.035	1	0.004
Lachnospiraceae_unclassified	Xing (2006)	8	0.031	1	0.004
Petrimonas	Maspolim et al. (2015)	7	0.027	6	0.023
Lachnospiraceae_uncultured	Xing (2006)	6	0.024	2	0.008
Acetobacterium	Drake et al. (2002)	5	0.020	2	0.008
Veillonella	Xing (2006)	5	0.020	0	0
Syntrophomonas	Xing (2006)	4	0.016	2	0.008
Proteiniphilum	Maspolim et al. (2015)	4	0.016	2	0.008
Treponema	Wang, 2013	3	0.012	5	0.019
Enterobacter	Lu et al. (2011)	3	0.012	0	0
Caldisericum	Maspolim et al. (2015)	3	0.012	10	0.038
Levilinea	Maspolim et al. (2015)	0	0	8	0.031
Total		2427	9.519	1135	4.347

Table 4
Influent and effluent characteristics of full scale activated primary tank (APT) and wastewater treatment plant after transformation. (The data represent the averages and standard deviations, number of samples (n) ⩾ 90.)

Parameter	COD (mg/L)	SCOD (mg/L)	TN (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	SS (mg/L)
Influent of WWTP	351 ± 27	151 ± 21	40.1 ± 2.1	27.2 ± 2.4	3.8 ± 0.8	254 ± 37
Effluent of APT	283 ± 24	198 ± 18	38.2 ± 1.9	26.4 ± 2.1	3.6 ± 0.5	181 ± 31
Effluent of WWTP	22.5 ± 1.6	18.4 ± 1.4	13.2 ± 1.2	0.68 ± 0.29	0.41 ± 0.14	7.9 ± 1.4

enrichment in the APT to convert the primary sludge into soluble COD. Mechanical elutriation significantly promoted the recovery of carbon source by releasing fermentation products out of the sludge. The APT recovered the carbon source from primary sludge and significantly transformed the composition of carbon source in the sewage, which enhanced the performance of biological nitrogen and phosphorus removal.

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References

Ahn, Y.H., Speece, R.E., 2006. Elutriated acid fermentation of municipal primary sludge. Water Res. 40, 2210–2220.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.
Bouzas, A., Ribes, J., Ferrer, J., Seco, A., 2007. Fermentation and elutriation of primary sludge: effect of SRT on process performance. Water Res. 41, 747–756.
Chanona, J., Ribes, J., Seco, A., Ferrer, J., 2006. Optimum design and operation of primary sludge fermentation schemes for volatile fatty acids production. Water Res. 40, 53–60.
Dennis, K.L., Wang, Y., Blatner, N.R., 2013. Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells. Cancer Res. 73, 5905–5913.
Drake, H.L., Küsel, K., Matthies, C., 2002. Ecological consequences of the phylogenetic and physiological diversities of acetogens. Antonie Van Leeuwenhoek 81, 203–213.
Feng, L.Y., Chen, Y.G., Zheng, X., 2009. Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH. Environ. Sci. Technol. 43, 4373–4380.
Flythe, M.D., Andries, K., 2009. The effects of monensin on amino acid catabolizing bacteria isolated from the Boer goat rumen. Small Ruminant Res. 81, 178–181.
Gao, Y.Q., Peng, Y.Z., Zhang, J.Y., Wang, S.Y., Guo, J.H., Ye, L., 2011. Biological sludge reduction and enhanced nutrient removal in a pilot-scale system with 2-step sludge alkaline fermentation and A2O process. Bioresour. Technol. 102, 4091–4097.
Jabari, L., Gannoun, H., Cayol, J.L., Hedi, A., Sakamoto, M., Falsen, E., Ohkuma, M., Hamdi, M., Fauque, G., Ollivier, B., Fardeau, M.L., 2012. *Macellibacteroides fermentans* gen. nov., sp. nov., a member of the family *Porphyromonadaceae* isolated from an upflow anaerobic filter treating abattoir wastewaters. Int. J. Syst. Evol. Microbiol. 62, 2522–2527.
Jaenicke, S., Ander, C., Bekel, T., Bisdorf, R., Droge, M., Gartemann, K.H., Junemann, S., Kaiser, O., Krause, L., Tille, F., Zakrzewski, M., Puhler, A., Schluter, A., Goesmann, A., 2011. Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing. PLoS One 6, 1–5.
Ji, Z.Y., Chen, G.L., Chen, Y.G., 2010. Effects of waste activated sludge and surfactant addition on primary sludge hydrolysis and short-chain fatty acids accumulation. Bioresour. Technol. 101, 3457–3462.
Leslie Grady, C.P., Daigger, G.T., Lim, H.C., 1999. Biological Wastewater Treatment, second ed. Marcel Dekker Inc., New York, NY.
Li, H.J., Chen, Y.G., Gu, G.W., 2008. The effect of propionic to acetic acid ratio on anaerobic-aerobic (low dissolved oxygen) biological phosphorus and nitrogen removal. Bioresour. Technol. 99, 4400–4407.
Liu, H.B., Zhao, F., Mao, B.Y., Wen, X.H., 2012a. Enhanced nitrogen removal in a wastewater treatment process characterized by carbon source manipulation with biological adsorption and sludge hydrolysis. Bioresour. Technol. 114, 62–68.
Liu, H., Wang, J., Liu, X.L., Fu, B., Chen, J., Yu, H.Q., 2012b. Acidogenic fermentation of proteinaceous sewage sludge: effect of pH. Water Res. 46, 799–807.
Lu, Y., Zhang, C., Lai, Q.H., Zhao, H.X., Xing, X.H., 2011. Improved hydrogen production under microaerophilic conditions by overexpression of polyphosphate kinase in Enterobacter aerogenes. Enzyme Microb. Technol. 48, 187–192.
Luo, J.Y., Feng, L.Y., Zhang, W., Li, X., Chen, H., Wang, D.B., Chen, Y.G., 2014. Improved production of short-chain fatty acids from waste activated sludge driven by carbohydrate addition in continuous-flow reactors: influence of SRT and temperature. Appl. Energy 113, 51–58.
Maspolim, Y., Zhou, Y., Guo, C.H., Xiao, K.K., Ng, W.J., 2015. The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation. Bioresour. Technol. 190, 289–298.
Miron, Y., Zeeman, G., Van Lier, J.B., Lettinga, G., 2000. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. Water Res. 34, 1705–1713.
Munch, V.E., Koch, F.A., 1999. A survey of prefermenter design, operation and performance in Australia and Canada. Water Sci. Technol. 39, 105–112.
Oehmen, A., Yuan, Z., Blackall, L.L., Keller, J., 2004. Short-term effects of carbon source on the competition of PAO and GAO. Water Sci. Technol. 50, 139–144.
Peng, Y.Z., Zhang, L., Zhang, S.J., Gan, Y.P., Wu, C.C., 2012. Enhanced nitrogen removal from sludge dewatering liquor by simultaneous primary sludge fermentation and nitrate reduction in batch and continuous reactors. Bioresour. Technol. 104, 144–149.
Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol. Rev. 61, 262–280.
Ueki, A., Akasaka, H., Suzuki, D., Ueki, K., 2006. *Paludibacter propionicigenes* gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. Int. J. Syst. Evol. Microbiol. 56, 39–44.
Wang, J., 2013. Research of Microbial Community Ecology and the Trophic Link During Acidogenic Fermentation. Jiangnan University, Wuxi (In Chinese).
Wang, X.C., Jin, P.K., Zhao, H.M., Meng, L.B., 2007. Classification of contaminants and treatability evaluation of domestic wastewater. Front. Environ. Sci. Eng. China 1, 57–62.
Wang, Y.Y., Jiang, F., Zhang, Z.X., Xing, M.Y., Lu, Z.B., Wu, M., Yang, J., Peng, Y.Z., 2010. The long-term effect of carbon source on the competition between polyphosphorus accumulating organisms and glycogen accumulating organism in a continuous plug-flow anaerobic/aerobic (A/O) process. Bioresour. Technol. 101, 98–104.
Wu, H.Y., Yang, D.H., Zhou, Q., Song, Z.B., 2009. The effect of pH on anaerobic fermentation of primary sludge at room temperature. J. Hazard. Mater. 172, 196–201.
Xing, G.F., 2006. Structure and Function of Hydrogen Producing-Ethanologens Community. Harbin Institute of Technology, Harbin (In Chinese).
Yamashita, Y.Y., Kurosumi, A., Sasaki, C., Nakamura, Y., 2008. Ethanol production from paper sludge by immobilized Zymomonas mobilis. Biochem. Eng. J. 42, 314–319.
Yu, Z., Morrison, M., Schanbacher, F.L., 2010. Production and utilization of methane biogas as renewable fuel. In: Biomass to Biofuels: Strategies for Global Industries. Wiley, Chichester, UK, pp. 403–413.
Yuan, Q., Sparling, R., Oleszkiewicz, J.A., 2009. Waste activated sludge fermentation: effect of solids retention time and biomass concentration. Water Res. 43, 5180–5186.
Zhang, L., Zhang, S.J., Wang, S.Y., Wu, C.C., Chen, Y.G., Wang, Y.Y., Peng, Y.Z., 2013. Enhanced biological nutrient removal in a simultaneous fermentation, denitrification and phosphate removal reactor using primary sludge as internal carbon source. Chemosphere 91, 635–640.