



# Continuous liquid fermentation of pretreated waste activated sludge for high rate volatile fatty acids production and online nutrients recovery



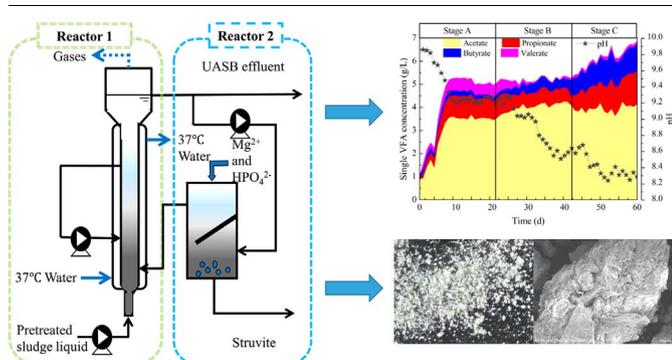
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## GRAPHICAL ABSTRACT



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## ABSTRACT

Raw sludge was pretreated, and the separated sludge liquid was used as substrate in a continuous operated up-flow anaerobic sludge blanket reactor to produce volatile fatty acids (VFAs). The highest VFA productivity of continuous fermentation with sludge liquid at an organic loading rate (OLR) of 10.0 kg COD/m<sup>3</sup>/d was about 5.0-fold and 4.0-fold higher than batch and semi-continuous fermentation with pretreated sludge slurry, respectively. Moreover, the liquid fermentation with an OLR of 10.0 kg COD/m<sup>3</sup>/d consumed the least energy, which was about 10.57% and 12.12% of batch and semi-continuous sludge fermentation, respectively. When combined with online nitrogen and phosphorus recovery, VFA production further increased by 20.67% and struvite recovery efficiency reached 1.98 ± 0.28 g/g PO<sub>4</sub><sup>3-</sup>. The process showed high VFA production, low energy consumption and good nutrients recovery by continuous liquid anaerobic fermentation, significantly increasing the economic potential of VFA production from waste activated sludge.

## 1. Introduction

Excessive waste activated sludge (WAS) produced during biological wastewater treatment contains high levels of proteins, polysaccharides, and lipids (Tyagi and Lo, 2013). Interest in recovering renewable energy from WAS has been driven by a shrinking reserve of fossil fuels,

continuing energy demands, climate change concerns and advancements in renewable energy technologies. Volatile fatty acids (VFAs), one kind of major intermediate from the anaerobic fermentation of sludge, could be generated from the conversion of organic matter in WAS (Lee et al., 2014). It has been proposed that VFAs can be used as feedstocks of biopolymers (Tamis et al., 2015), medium chain fatty

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acids (Grootscholten et al., 2014; Spirito et al., 2014) or external carbon sources to enhance biological removal of nitrogen and phosphorus in wastewater treatment (Ji et al., 2010; Li et al., 2011). Because they have higher values than traditional products such as methane, VFAs may serve as platform products in the carboxylates platform or VFAs platform concept (Cagnetta et al., 2016, 2017). Because of these benefits, more and more studies are focusing on VFA production by anaerobic acidogenic fermentation of WAS.

Previous studies of VFA production from sludge fermentation were primarily conducted by batch or semi-continuous operation using raw sludge as a substrate (Lutpi et al., 2016; Zhang et al., 2015). Since biological hydrolysis is usually identified as the rate-limiting step in WAS anaerobic fermentation, various pretreatments, such as thermal, alkaline, ultrasonic or mechanical disintegration, were introduced prior to fermentation to accelerate the dissolution and hydrolysis of organic matter from WAS, which subsequently improves VFA production (Hadi et al., 2015, Barber, 2016). However, pretreated WAS slurry still contains a large amount of inorganic and inert organic matter, which is difficult or impossible to transform into VFAs during WAS anaerobic fermentation. Moreover, the large amount of inorganic materials will also cause unnecessary energy consumption because of inadequate mixing during sludge fermentation, which will ultimately hinder its application at the industrial scale (Jorgensen et al., 2007).

The high concentration of ammonia released into liquid by pretreatment could also inhibit the growth of microorganisms and further reduce the utilization of organic matter as well as VFAs generation (Yenigün and Demirel, 2013; Jönsson et al., 2013). Indeed, nitrogen and phosphorus could be efficiently recovered from sludge liquid through struvite formation by adding  $Mg^{2+}$ . For example, with  $Mg^{2+}$  addition, the removal of  $PO_4^{3-}$  was higher than 90% and the struvite purity obtained was as high as 95% (Lahav et al., 2013; Bergmans et al., 2014). The recovered ammonia and phosphorus in the form of struvite are valuable fertilizers in agriculture (Yu et al., 2017).

The highly efficient up-flow anaerobic sludge blanket (UASB) reactors have been widely applied in wastewater treatment. Moreover, it has been reported that the energy consumption by sludge slurry mixing accounts for 60% of the total energy in sludge fermentation. Furthermore, we proposed that it would be feasible to conduct continuous liquid fermentation by using separated sludge liquid after pretreatment as a substrate in a UASB to realize high VFA production and energy savings. In that way, traditional sludge fermentation with raw sludge or pretreated sludge slurry can be shifted into highly efficient and continuous liquid fermentation. Moreover, when combined with online phosphorous and ammonia recovery, VFA production could be further enhanced by the removal of ammonia, resulting in the production of struvite, which can be used as agricultural fertilizer.

Therefore, we developed a novel process that combined high-rate VFA production by pretreated sludge liquid fermentation in UASB and online nitrogen and phosphorous recovery. The objectives of this study were to: (1) compare VFA production, organic conversion and energy consumption between continuous sludge liquid fermentation and sludge slurry fermentation by batch and semi-continuous operation; and (2) enhance VFA productivity and recovery of nitrogen and phosphorus with online nitrogen and phosphorus removal during continuous sludge liquid fermentation. The results of this study will facilitate novel process development of VFA production by anaerobic fermentation and future industrial applications.

## 2. Materials and methods

### 2.1. Waste activated sludge and seeding sludge

Waste activate sludge (WAS) used in fermentation was dewatered sludge obtained from a municipal wastewater treatment plant in Wuxi, China (Shuofang wastewater treatment plant, Wuxi, Jiangsu). The characteristics of the dewatered sludge were as follows: pH

$6.60 \pm 0.08$ , solid content  $15.43 \pm 0.80\%$ , volatile suspended solids (VSS)/total suspended solid (TSS)  $0.61 \pm 0.03$ , total chemical oxygen demand (TCOD)  $1.09 \pm 0.07$  g/g VSS. The collected WAS was stored at 4 °C for subsequent fermentation experiments. All measurements were conducted in triplicate and the averages and standard deviations were reported.

Seeding sludge was obtained from the Shuofang wastewater treatment plant, and an up-flow anaerobic sludge blanket (UASB) reactor with a working volume of 5.0 L was used for acidogenic bacteria acclimation. The WAS was heated at 105 °C for 2 h to kill non-spore-forming methanogens (Nie, et al., 2007). Before being used as sludge inoculum, the heat-treated sludge was added into the UASB for re-activation of acidogenic microorganisms. Glucose was continuously pumped into the UASB to enrich the acidogenic bacteria while maintaining the temperature at  $37 \pm 2$  °C. Seeding sludge was obtained when the effluent pH fell below 4.0. The cultivation period was more than 3 weeks (Wang et al., 2013) and the characteristics of the seeding sludge were: TSS  $54.90 \pm 0.72$  g/L, VSS/TSS  $85.72 \pm 1.23\%$ , protein  $653.20 \pm 10.01$  mg/g TS, carbohydrate  $208.41 \pm 80.21$  mg/g TS, and VFAs  $3.45 \pm 0.23$  g/L. The cultivated seeding sludge was washed three times with deionized water before use in the fermentation reactors.

### 2.2. Preparation and characteristics of pretreated sludge liquid and sludge slurry

The WAS was first diluted to 75 g TSS/L, then pretreated at 105 °C and an initial pH of 12.5 for 4 h. The reactor used for pretreatment was made of stainless steel and had a working volume of 30 L and the sludge was heated by water steam. The sludge was continuously stirred at 100 rpm during the pretreatment period. Following pretreatment, the pretreated sludge slurry, which was rich in dissolved organic materials, was obtained from the bottom outlet of the reactor. The separated pretreated sludge liquid was obtained by centrifuging the sludge slurry at 5000 rpm for 10 min. Both pretreated sludge slurry and separated sludge liquid were cooled to  $37 \pm 1$  °C before fermentation. The characteristics of the sludge are shown in Table 1.

### 2.3. Batch, Semi-continuous and continuous operation of acidogenic fermentation

One 5.0 L up-flow anaerobic sludge blanket (UASB) reactor made of glass was continuously operated for liquid fermentation under different organic loading rates (OLRs). The liquid fermentation was divided into three stages (I, II and III) with the OLR maintained at 4.0 kg COD/m<sup>3</sup>/d, 6.5 kg COD/m<sup>3</sup>/d and 10.0 kg COD/m<sup>3</sup>/d, respectively. The UASB was started by inoculation with 1.0 L seeding sludge and 4.0 L separated sludge liquid. The effluent from the UASB was recirculated from the top outlet to the bottom inlet of the reactor with a flow velocity of 12 L/h. During the liquid fermentation, the UASB was operated at  $37 \pm 1$  °C.

For comparison with liquid fermentation, two anaerobic fermentors with a working volume of 6.0 L were operated simultaneously for VFA production by using the pretreated sludge slurry as substrate. The first fermentor was run in batch operation and the second in semi-

**Table 1**  
Characteristic of pretreated sludge liquid and pretreated sludge slurry.

Items	Pretreated sludge liquid	Pretreated sludge slurry
pH	$9.80 \pm 0.20$	$9.80 \pm 0.20$
TSS (g/L)	$6.50 \pm 0.20$	$74.23 \pm 1.43$
SCOD (mg/L)	$16398.46 \pm 837.47$	$16454.34 \pm 1525.93$
Soluble protein (mg/L)	$4423.41 \pm 241.42$	$4923.75 \pm 135.77$
Soluble polysaccharides (mg/L)	$4338.64 \pm 103.53$	$4318.65 \pm 119.39$
Free amino acid (mg/L)	$3776.80 \pm 58.53$	$3583.23 \pm 58.14$
VFAs (mg/L)	$1025.75 \pm 123.73$	$1193.34 \pm 102.46$

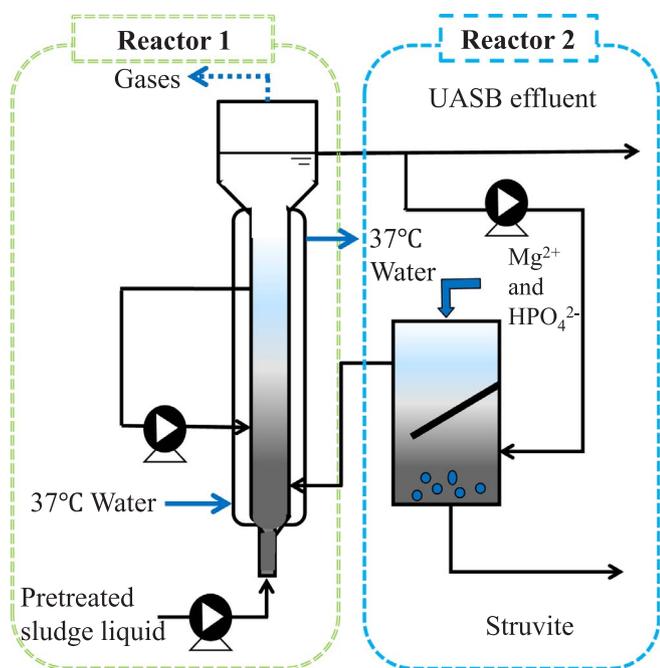


Fig. 1. The process diagram of the combined UASB and online nutrients recovery reactor.

continuous operation. For batch operation, 1.0 L seed sludge and 4.0 L pretreated sludge slurry were pumped into the fermenter with the agitation speed controlled at 100 rpm. For semi-continuous operation, the same volume of feeding sludge and pretreated sludge slurry were pumped into the fermenter in the start-up period. During the operational period, 1.56 L fresh pretreated sludge slurry was used as the feed and the same volume of fermented sludge slurry was pumped out every two days to keep the OLR at about 2.5 kg COD/m<sup>3</sup>/d. The pH value and temperature of the two fermentors were controlled at 10.0 ± 0.2 and 37 ± 1 °C, respectively.

#### 2.4. Continuous liquid fermentation with online nitrogen and phosphorus recovery

The continuous liquid fermentation was divided into three stages (A, B and C). During stage A, the OLR of the UASB was maintained at 8.0 kg COD/m<sup>3</sup>/d, while stage B started after the VFAs concentration was stabilized. In stage B and C, an additional reactor (Reactor 2) was coupled with the UASB (Reactor 1) to remove and recover the ammonia and phosphate in the form of struvite online with the addition of MgCl<sub>2</sub> solution (Fig. 1). Reactor 2 was airtight, constructed of Plexiglas and had a working volume of 5.0 L. This reactor also contained a baffle to improve the mixing effect of the MgCl<sub>2</sub> solution with the effluent of UASB. The MgCl<sub>2</sub> solution was pumped into Reactor 2 and mixed for 4 h, after which the formed struvite was manually removed. In stage B, 0.8 M MgCl<sub>2</sub> solution was continuously pumped into Reactor 2 daily, but in stage C, the mixture solution of 0.8 M MgCl<sub>2</sub> and 0.4 M K<sub>2</sub>HPO<sub>4</sub> was continuously pumped into Reactor 2 daily. The effluent velocity from the UASB into Reactor 2 was controlled at 12 L/h in three stages. The temperature was 37 ± 1 °C and pH was not controlled.

#### 2.5. Analytical methods

For chemical measurement, 20.0 mL of the sample was collected and measured immediately after collection. The concentrations of TSS, VSS, soluble chemical oxygen demand (SCOD), total COD (TCOD), total phosphorus (TP) and NH<sub>4</sub><sup>+</sup>-N were measured according to the Standard Methods (APHA, 1998). The concentrations of proteins and polysaccharides were measured using the Lowry–Folin (Lowry et al., 1951)

and phenol–sulfuric (Herbert et al., 1971) methods. To measure SCOD, soluble protein, soluble polysaccharides and soluble free amino acids, samples were first centrifuged at 10,200 rpm for 10 min, after which they were filtered with 0.45 μm syringe filters.

The VFAs concentrations in the filtrate samples were detected using a gas chromatograph (GC-2010, Japan) equipped with a flame ionization detector (FID) and a fused-silica capillary column (PEG-20 M, 30 m × 0.32 mm × 0.5 mm, China). The column temperature was maintained at 80 °C initially, then held at 210 °C for 2 min. The running temperature was maintained at 80 °C during detection. Both the injection port and detector temperatures were 250 °C. Before GC measurement, 4-methyl-valeric acid (internal standard), 3 M phosphoric acid (acidifier) and filtrate sample were mixed at 1:1:1 (V:V:V). The method was the same as that used for previous studies (Ma et al., 2016).

#### 2.6. Calculation of energy consumption

The stirring energy was related to the effective reactor volume, impeller diameter, liquid density, and liquid viscosity. The following equations were used to calculate the stirring energy (Paul et al., 2004):

$$P = N_p n^3 d^5 \rho \quad (1)$$

$$N_p = K (Re)^x (Fr)^y \quad (2)$$

$$Fr = nd^2/g \quad (3)$$

$$Re = nd^2\rho/\mu \quad (4)$$

In the equations,  $P$  is the shaft power of a single impeller, kW;  $N_p$  is the power number, here for the Rushton impeller, the value was 3.9 according to the reactor geometry and the agitation conditions;  $n$  was rotating speed, r/s;  $d$  was the diameter of the impellers, here  $d = 0.08$  m;  $\rho$  was the liquid density, kg/m<sup>3</sup>, it was 1065 kg/m<sup>3</sup> and 1008 kg/m<sup>3</sup> of the pretreated sludge slurry and liquid, respectively;  $K$  related to impeller shape and size, under our experimental conditions,  $K = 71$ ;  $Re$  was the Reynolds number;  $Fr$  was the Froude number;  $x$  and  $y$  were the exponential constants, since the liquid is in a turbulent state,  $x = 1$ , and with full baffle conditions,  $y = 0$ ;  $g$  was the acceleration of gravity,  $g = 9.8$  m/s<sup>2</sup>;  $\mu$  was the liquid viscosity, Pa·s.

Considering the power loss during the process of energy conversion, the total power of the agitation system was described by Eq. (5):

$$P_m = P(0.4 + 0.6m)/\eta \quad (5)$$

In the equations,  $P_m$  was the total power of the multi-impeller agitation system, kW;  $m$  was the number of impellers;  $\eta$  was the work efficiency, which was 0.8 in this study.

The heating preservation of the reactor was calculated according to the Newton cooling formula (6) as follows:

$$P_p = hA(t_l - t_w) \quad (6)$$

where  $P_p$  was the heat preservation power, W;  $h$  was the heat transfer coefficient of the insulation layer, W/(m<sup>2</sup>·K);  $A$  was the inner surface area of the reactor, m<sup>2</sup>;  $t_l$  was the liquid temperature in the reactor, °C; and  $t_w$  was the environmental temperature, which was set to 20 °C.

The lifting power of the liquid referring to the process of continuous anaerobic fermentation in which the liquid material continuously moved from the reactor bottom to the top in the process was determined by formula (7):

$$P_g = q\rho gH \quad (7)$$

where  $P_g$  was the lifting power of the liquid, W;  $q$  was the flow rate of the liquid in the reactor, m<sup>3</sup>/h;  $\rho$  was the liquid density, kg/m<sup>3</sup>;  $g$  was the acceleration of gravity,  $g = 9.8$  m/s<sup>2</sup>; and  $H$  was the height of the liquid lift, m.

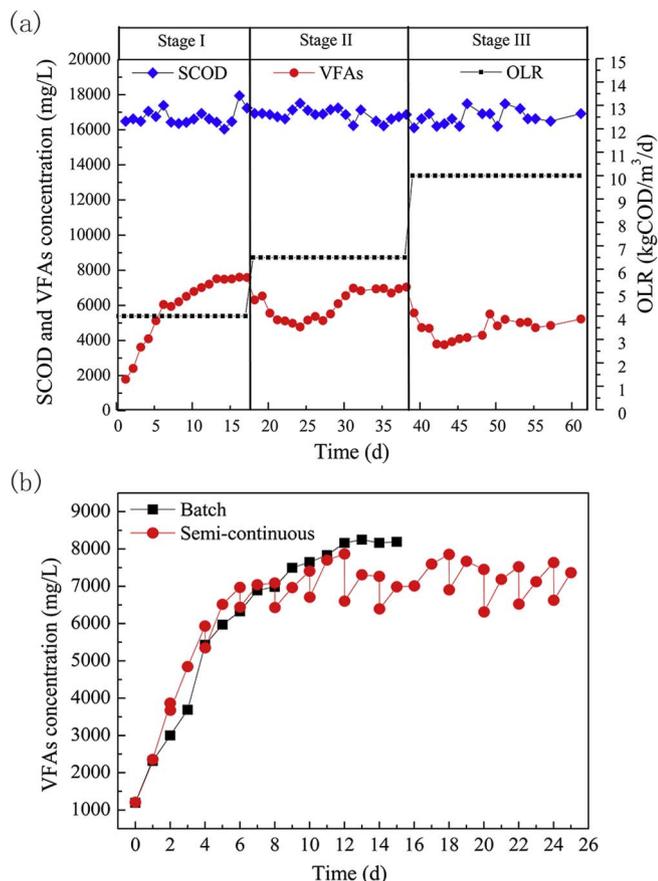


Fig. 2. Daily changes of VFAs concentration in continuous fermentation (a) and semi-continuous and batch fermentation (b).

### 3. Results and discussion

#### 3.1. Continuous liquid fermentation of sludge liquid

Because of the large energy consumption by inorganic components in the pretreated sludge residues and the low content of organic matter in the sludge residues after pretreatment during anaerobic fermentation (Ma et al., 2016), the pretreated sludge slurry was centrifuged and the separated sludge liquid was used as substrate for acidogenic fermentation by continuous liquid fermentation in the UASB reactor. The daily changes in VFAs concentration in the UASB reactor, pretreated sludge slurry fermentation by semi-continuous and batch operation in anaerobic fermentors are shown in Fig. 2a and b, respectively.

As shown in Fig. 2a, the liquid acidogenic fermentation was divided into three stages based on different OLR values of 4.0, 6.5 and 10.0 kg COD/m<sup>3</sup>/d. The SCOD during the three fermentation stages were maintained between 16357.60 and 17484.92 mg/L, illustrating that the process of hydrolysis of pretreated sludge liquid in continuous fermentation was stable. Because the SCOD in influent was about 16398.46 ± 837.47 mg/L, very close to the effluent, few organic materials were converted into methane or carbon dioxide and little carbon was lost during continuous acidogenic fermentation.

In stage I, with the OLR at 4.0 kg COD/m<sup>3</sup>/d, the VFAs concentration in effluent increased from 1125.75 mg/L to 7604.71 mg/L and became stable after 10 days of operation. When the OLR increased to 6.5 kg COD/m<sup>3</sup>/d in stage II, the VFAs concentration first decreased to 4774.50 mg/L in the following 6 days, then recovered gradually to 7042.06 mg/L. In Stage III, when the OLR increased to 10.0 kg COD/m<sup>3</sup>/d on day 39, the VFAs declined to 3756.74 mg/L and were then maintained at about 5226.20 mg/L. These findings indicate that an OLR over 4.0 kg COD/m<sup>3</sup>/d destroyed the steady fermentation state and

caused reduced VFA production. Using the sludge slurry as substrate in a semi-continuous fermentation system, Lim et al. (2008) found that the VFAs concentrations decreased from 0.34–0.37 g/L to 0.29–0.30 g/L as the OLR increased from 5 g/L-d to 13 g/L-d. The sudden increase in OLR might break the balance between substrates and the microbial community, turning the acidogenic fermentation from a steady-state to an unsteady state; however, this was soon recovered with fermentation (Goux et al., 2015). According to the VFAs data in Fig. 2a, the VFAs concentration was observed as stage I > stage II > stage III with increasing OLR, indicating that the acidogenic microorganisms cannot tolerate the OLR in stage II and III. Accordingly, an appropriate substrate supply or hydraulic retention time is crucial for continuous VFA production in UASB reactors.

The VFAs concentrations in batch and semi-continuous fermentation with pretreated sludge slurry as substrate are shown in Fig. 2b. In batch fermentation, the VFAs concentration increased gradually until reaching 8248.68 mg/L in 12 days. During semi-continuous fermentation, the VFAs concentrations were similar to those in batch operation, increasing to about 7806.72 mg/L and then becoming steady in 16 days. When compared with continuous fermentation of pretreated sludge liquid, batch operation showed VFAs concentrations that were about 1.09-fold, 1.21-fold and 1.61-fold those obtained during continuous operation with OLRs of 4.0, 6.5 and 10.0 kg COD/m<sup>3</sup>/d, respectively.

#### 3.2. Comparison of three types of acidogenic fermentation

The VFA productivity represented daily VFA production in the reactor, and the acidification rate represented the extent of transformation from organic substrates into VFAs. The balance of offered OLR, VFA productivity and acidification rate is important for fermentation; thus, it is necessary to analyze the relationship between these three factors. Fig. 3 shows the mathematical relationships between OLRs, VFA productivity and acidification rates.

According to the data shown in Fig. 3, the OLR was in exponential fitted with acidification rate with the R<sup>2</sup> at 0.989. These findings indicate that the acidification rate was negatively correlated with OLR, and this effect was increasingly obvious when the OLR value was low. The relationship between OLR and acidification rate also demonstrated that the higher OLR in this study was not in favor of the transformation from organic substrate into VFAs. Differently, the relationship of OLR and VFA productivity was fitted by a quadratic function with an R<sup>2</sup> of 0.997, indicating that the high OLR would enhance VFA productivity when the OLR increased from 0.0 to 10.0 kg COD/m<sup>3</sup>/d. However, the VFA productivity would decrease if the OLR exceeded 10.0 kg COD/m<sup>3</sup>/d. The different trends in acidification rate and VFA productivity

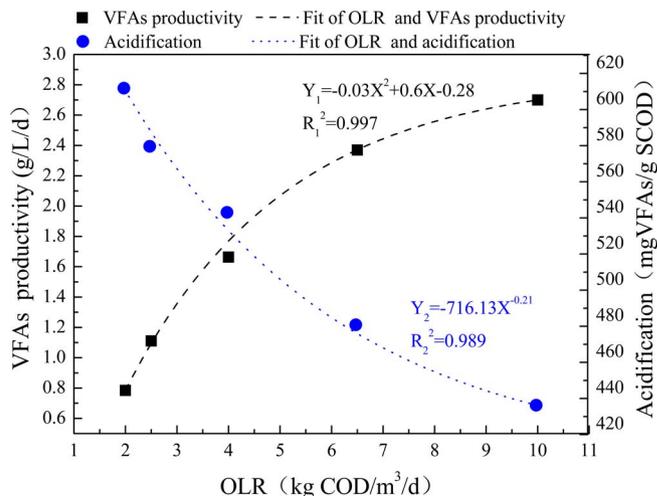


Fig. 3. Mathematical relationships of OLR with VFAs productivity and acidification rate.

**Table 2**  
Characteristics of three kinds of fermentation.

Type of fermentation	Batch fermentation <sup>1*</sup>	Semi-Continuous fermentation	Continuous fermentation		
			4.0 kg COD/m <sup>3</sup> /d	6.5 kg COD/m <sup>3</sup> /d	10.0 kg COD/m <sup>3</sup> /d
VFAs concentration (mg/L)	8248.68 ± 162.32	7653.04 ± 129.93	7555.20 ± 49.52	6826.24 ± 120.56	5108.56 ± 92.54
VFAs yield (mg/g SCOD)	589.64 ± 21.34	533.11 ± 36.21	470.82 ± 21.43	447.92 ± 31.54	436.36 ± 31.47
VFAs productivity (kg/m <sup>3</sup> /d)	1.04 ± 0.02	1.21 ± 0.02	1.89 ± 0.04	2.78 ± 0.05	3.19 ± 0.06
Sludge consumption capacity <sup>2*</sup> (kg/m <sup>3</sup> /d)	40.61 ± 5.34	50.45 ± 5.93	81.25 ± 6.45	131.95 ± 6.23	203.13 ± 8.91

1\* The period of batch fermentation is generally about 7–10 d, and we define the fermentation period as 8 d.

2\* The sludge was generally dewatered sludge with its solid content about 20%.

indicated that an appropriate OLR is important for the balance of VFA productivity and acidification rate. To acquire the most suitable OLR, the equations of acidification rate and VFA productivity were united and the multiplication of acidification and VFA productivity was calculated. The highest multiplication of acidification and VFA productivity value represented the highest VFA production efficiency. In this study, the most suitable OLR was about 8.33 kg COD/m<sup>3</sup>/d.

Table 2 shows the VFAs concentration, VFAs yield, VFA productivity and sludge consuming capacity in batch, semi-continuous and continuous operations, respectively, when fermentation was steady. The VFAs concentrations and yields ranked from high to low with the sequence of batch fermentation > semi-continuous fermentation > continuous fermentation. Moreover, the VFAs concentrations and yields with the OLR at 4.0 kg COD/m<sup>3</sup>/d were higher than with the OLR at 6.5 kg and 10.0 kg COD/m<sup>3</sup>/d under continuous fermentation. However, the VFA productivity showed an opposite trend to VFAs concentration. Specifically, the highest VFA productivity (3.19 ± 0.06 kg/m<sup>3</sup>/d) was observed with an OLR of 10.0 kg COD/m<sup>3</sup>/d during continuous fermentation, while the lowest (1.06 ± 0.02 kg/m<sup>3</sup>/d) appeared in batch fermentation with the OLR at 10.0 kg COD/m<sup>3</sup>/d. Moreover, the lowest VFA productivity was only 0.33-fold of that observed during continuous fermentation. Similarly, the sludge consumption during continuous operation with the OLR at 10.0 kg COD/m<sup>3</sup>/d was highest (203.125 ± 8.91 kg/m<sup>3</sup>/d), being about 5.0-fold and 4.0-fold higher than that observed during batch and semi-continuous fermentation, respectively. In summary, batch fermentation benefited VFAs accumulation and organic transformation, but the continuous fermentation showed better sludge consumption capacity and achieved higher VFA production rate. In addition, the VFAs composition between the three kinds of fermentation did not differ (data not shown). The above results and analysis are very important for the parameters selection in sludge fermentation. When the objective is focused on higher VFAs concentration or yield, batch fermentation is a better choice, while if the VFA productivity or sludge consumption capacity is the prior target, continuous operation is the preferred fermentation mode for sludge treatment.

### 3.3. Energy consumption of three types of acidogenic fermentation

During acidogenic fermentation, the energy consumption includes stirring energy, heat preservation energy and liquid lifting energy

**Table 3**  
Energy consumption of three kinds of fermentation.

Type of energy consumption (kW h/kg VFAs)	Batch fermentation	Semi-continuous fermentation	Continuous fermentation		
			4.0 kg COD/m <sup>3</sup> /d	6.5 kg COD/m <sup>3</sup> /d	10.0 kg COD/m <sup>3</sup> /d
Agitation	81.13 ± 0.34	70.78 ± 0.43	0.00 <sup>*</sup>	0.00	0.00
Liquid lifting	0.00	0.00	9.91 ± 0.21	6.72 ± 0.31	5.86 ± 0.21
Heat preservation	12.54 ± 0.35	10.94 ± 0.32	6.83 ± 0.34	4.63 ± 0.26	4.04 ± 0.37
Total	93.66 ± 0.69	81.72 ± 0.75	16.74 ± 0.55	11.35 ± 0.57	9.90 ± 0.58

\* There is no stirring or liquid lifting operation in the process, so the energy consumption is regarded as zero.

(Alzate and Toro, 2006). The energy consumption throughout the acidogenic fermentation is shown in Table 3.

As shown in the table, the majority of energy is consumed by stirring in batch and semi-continuous fermentation, when 81.13 ± 0.34 and 70.78 ± 0.43 kW·h/kg VFAs were consumed, accounting for 86.67% and 86.66% of the entire fermentation process, respectively. For example, when compared with the energy consumption in large scale high solid sludge fermentations with or without stirring, 60% of the energy input was saved by removing the mixing apparatus (Liu et al., 2015). However, the energy consumption originating from the stirring was zero because of the absence of stirring in continuous liquid fermentation. It is well known that mixing of the substrate is very important for the performance of fermentation because it improved the substrate mass transfer, toxicant dispersion and microbial metabolic activities in the bioreactor. Unfortunately, mixing also consumes a large amount of energy, especially when the sludge slurry is used as the substrate in the scenarios of batch fermentation and semi-continuous fermentation (Kaku et al., 2006). In such cases, continuous fermentation with pretreated sludge liquid as substrate showed a significant advantage for energy consumption because it waived the mixing during the liquid fermentation.

The energy consumption from liquid lifting and heat preservation was related to the length of the fermentation period. For batch and semi-continuous fermentation, the liquid lifting energy was zero because of the substrate state, while in continuous fermentation, it is a considerable energy input. As OLR increased, the energy input declined gradually from 9.91 ± 0.21 to 5.86 ± 0.21 kW·h/kg VFAs because of the shorter hydraulic retention time under higher OLR conditions. The levels of heat preservation in batch and semi-continuous fermentation, which were higher than in continuous liquid fermentation, were 12.54 ± 0.35 and 10.94 ± 0.32 kW·h/kg VFAs, respectively. However, for the liquid fermentation, the sludge supernatant should be separated from the pretreated sludge slurry before the liquid fermentation and this process will consume energy. At the same time, since the sludge slurry fermentation in batch or semi-continuous fermentation need dewatering operation in the post fermentation process that will also consume energy. The dewatering operation is similar to the separation process of sludge supernatant in continuous liquid fermentation. Therefore, the energy consumption from the two processes can be offset and ignored during the total energy comparison.

According to the total energy consumption in the three kinds of

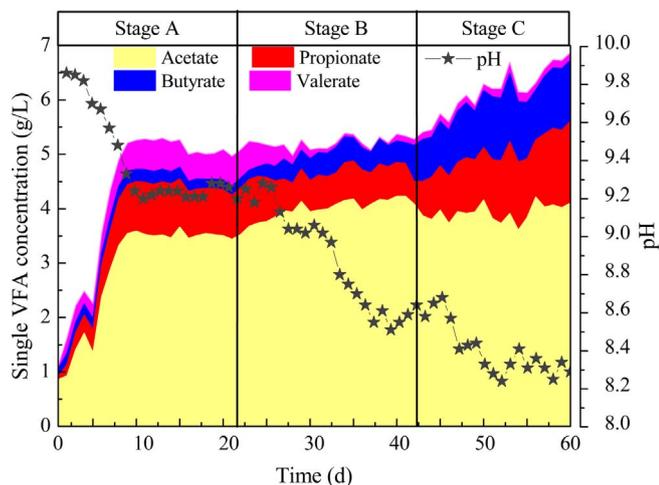


Fig. 4. Daily changes of single VFA concentration and pH during the continuous liquid fermentation with online nitrogen and phosphorus recovery.

sludge fermentation, the continuous liquid fermentation consumed much less energy ( $9.90 \pm 0.58$ – $16.74 \pm 0.55$  kW·h/kg VFAs) than the batch ( $81.13 \pm 0.69$  kW·h/kg VFAs) or semi-continuous fermentation ( $70.78 \pm 0.75$  kW·h/kg VFAs). This demonstrates that the continuous liquid fermentation of separated sludge liquid was much better than the sludge slurry fermentation in batch and semi-continuous fermentation in terms of energy consumption.

### 3.4. VFAs enhancement coupled with online nitrogen and phosphorus recovery

The changes in VFAs concentrations and pH in pretreated sludge liquid fermentation with online nitrogen and phosphorus recovery in the three fermentation stages are shown in Fig. 4. The concentration of VFAs in stage A increased significantly before 10 days, but stabilized after 10 days, and the average concentration of VFAs reached  $5.42 \pm 0.07$  g/L at steady state. The concentration of VFAs in stage B did not change significantly and were maintained at  $5.45 \pm 0.04$  g/L. The concentration of VFAs in stage C increased gradually to  $6.54 \pm 0.04$  g/L, which was about 20.67% higher than that of stage A and B. The enhancement of VFAs concentration in stage C can be attributed to the removal of high strength  $\text{NH}_4^+$ -N by struvite generation, which is usually an inhibitor of acidogenic microorganisms (Yenigün and Demirel, 2013). The pH value decline to 8.1 also explains the VFAs enhancement. Our previous study reported that neutral pH benefited the growth of most acidogenic bacteria, then improved the VFA production with sludge liquid as the substrate (Ma et al., 2016).

Evaluation of the distribution of different individual VFAs revealed that, when compared with stage A, the acetate and butyrate concentration in stage B were increased obviously from  $3.89 \pm 0.01$  g/L and  $0.18 \pm 0.02$  g/L to  $4.30 \pm 0.08$  g/L and  $0.52 \pm 0.14$  g/L, respectively. However, the propionate and valerate decreased from  $0.86 \pm 0.08$  g/L and  $0.53 \pm 0.02$  g/L to  $0.60 \pm 0.03$  g/L and  $0.11 \pm 0.01$  g/L, respectively. Moreover, the propionate and butyrate concentration were further increased obviously to  $1.37 \pm 0.03$  g/L and  $1.12 \pm 0.02$  g/L in Stage C compared with stage B.

The pH value decreased consistently from 9.86 in stage A to 8.79 in stage B and finally to 8.31 in stage C. Generally, the pH reflects the  $\text{H}^+$  concentration in fermentation liquid and indicates ion buffer capacity. The pH value or the  $\text{H}^+$  concentration is closely related to the VFAs concentration in fermentation liquid (Yuan et al., 2015). However, the change in pH values was not matched by the change in VFAs concentration or single VFA concentration in stage B and C. We deduced that the change in pH was influenced significantly by the alkalinity concentrations in the liquid, such as  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$

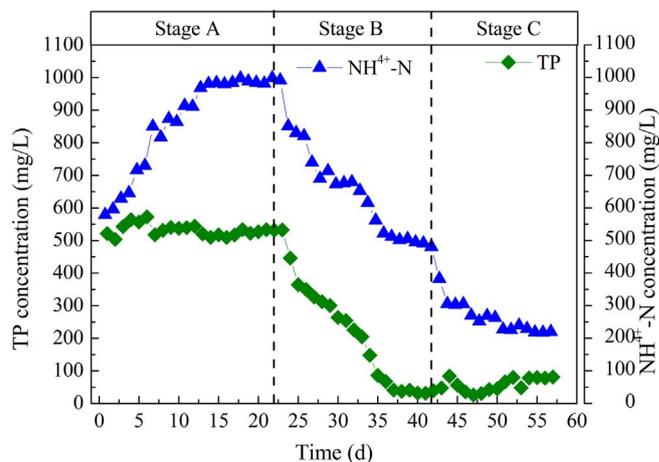


Fig. 5. Daily changes of ammonia and TP concentrations during continuous liquid fermentation with online nitrogen and phosphorus recovery.

concentrations, etc. With the removal of  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$  through struvite formation, the alkalinity decreased significantly and caused the pH to decline accordingly.

### 3.5. Recovery of nitrogen and phosphorus

Phosphorus is an essential, yet limited resource in agriculture that cannot be replaced by any other elements (Cornel et al., 2009). Moreover, the high concentration of ammonia in the fermentation liquid would inhibit microbial activity, thereby causing low VFAs generation. This explains the increasing efforts to recycle phosphorus and nitrogen during sludge anaerobic fermentation.

As shown in Fig. 5, the  $\text{NH}_4^+$ -N increased gradually to  $995.08 \pm 3.36$  mg/L at stage A because of the hydrolysis of proteins in pretreated sludge liquid. The TP concentration remained relatively stable at  $518.50 \pm 14.79$  mg/L during stage A. With the addition of  $\text{Mg}^{2+}$  at stage B, the TP and  $\text{NH}_4^+$ -N concentration decreased obviously to  $31.86 \pm 0.40$  mg/L and  $501.71 \pm 10.11$  mg/L, respectively, because of the formation of struvite. The  $\text{NH}_4^+$ -N further decreased to  $223.52 \pm 5.70$  mg/L during stage C, but the TP concentration was relatively stable. Finally, the TP and  $\text{NH}_4^+$ -N in stage C was reduced by 93.86% and 77.54%, respectively.

Struvite is an effective nitrogen and phosphorus fertilizer with a slow-release effect in agriculture that mainly consists of  $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ,  $\text{MgO}$ , and  $\text{Mg}_3(\text{PO}_4)_2$  (Doyle et al., 2002). The solid was recovered from the bottom of reactor 2 (Fig. 1), which was regarded as synthetic struvite sediment. The recovered solid was light brown after it was washed and dried, similar to the results observed in a previous study (Martí et al., 2017; Rahman et al., 2014). As the effective constituent of struvite,  $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$  solid was white, but the color of recovered struvite was deeper than that of  $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$  solid. Conversely, the recovered solid was a mixture of  $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$  and small sludge particles.

The recovered solid was mainly granular with some rhombus shaped crystals on the surface of the solid. These granular solids might be a combination of small sludge particles and struvite. The crystals of the recovered struvite were mainly of rhombus shape. Previous studies reported that the crystal shape of struvite was closely related to pH, and when the pH value was between 8.50 and 9.80, the struvite crystal was of rhombus shape, while it gradually changed to a needle shape when the pH was higher than 10.00 (Le Corre et al., 2009).

The actual solid recovery in stage B was  $1.82 \pm 0.30$  g solids/g  $\text{PO}_4^{3-}$ , and the actual solid recovery of stage C reached  $1.98 \pm 0.28$  g solid/g  $\text{PO}_4^{3-}$ , which were higher than the theoretical value ( $1.45$  g  $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$ /g  $\text{PO}_4^{3-}$ ). The reason for the higher actual solid recovery than the theoretical one may be adhesion to small sludge

particles and other inorganic minerals on the surface of struvite, which increased the weight of recovered solids. Based on calculations, the struvite could be recovered at the highest level of  $13.11 \pm 0.10$  g/g TS from sludge at stage C (data not shown).

#### 4. Conclusions

The continuous fermentation of sludge liquid could realize high-rate VFA production. Moreover, the continuous fermentation helped reduce energy consumption when compared with batch and semi-continuous operation. The combination of continuous sludge liquid fermentation and online nitrogen and phosphorus recovery provide a novel and promising process to realize high-rate continuous VFA production with lower energy demand and nutrient recovery, which will facilitate further industrial applications.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2017.10.103>.

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