



# Effect of initial pH on short chain fatty acid production during the anaerobic fermentation of membrane bioreactor sludge enhanced by alkyl polyglucoside



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## ABSTRACT

Membrane bioreactor (MBR) sludge anaerobic fermentation for short chain fatty acid (SCFA) production has drawn much attention as it can produce value-added product and reduce sludge volume simultaneously. However, SCFA production from sludge is always limited due to the low hydrolysis rate and rapid consumption by methanogens. In this study, an efficient and green strategy was developed by adding biosurfactant alkyl polyglucoside (APG) into MBR sludge to enhance SCFA production. Experimental results showed that the yield of SCFA reached 282.9 mg chemical oxygen demand (COD)/g volatile suspended solids (VSS) with the optimum APG dosage of 0.2 g/g dry sludge (DS), which was 4.8-fold of the blank. Furthermore, the effect of initial pH ranging from 4 to 12 on SCFA production in the presence of APG was also studied. Results showed SCFA productions from MBR sludge in the presence of APG at initial alkaline pH values were more efficient than those at acidic and near-neutral pH values, and the optimum initial pH was 11.

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## 1. Introduction

The membrane bioreactor (MBR) process has attracted growing concerns due to the exceptional separation capabilities (Hwang et al., 2012). The MBR market has been expanding at an average annual growth rate of approximately 10.9%, much faster than other advanced wastewater treatment technologies (Judd, 2008; Mnif et al., 2012). Compared with the conventional activated sludge, the MBR sludge, inevitable by-product during MBR process, is characterized by high biomass concentration and relatively low content of organic matter because MBR process always adopt low food/microorganisms (F/M) operation and long sludge retention time (Yu et al., 2013). It was reported that the treatment and disposal of MBR sludge was more difficult than conventional activated sludge (Wei et al., 2003).

Anaerobic digestion is widely applied in waste activated sludge (WAS) stabilization and biogas production, in which four steps (solubilization, hydrolysis, acidification, and methanogenesis) are generally involved (Jiang et al., 2007; Mu and Chen, 2011). Recently, WAS anaerobic fermentation for short chain fatty acid (SCFA) production has drawn much attention owing to the fact that the produced SCFA is a preferred carbon source for biological nutrient removal (Feng et al., 2009; Yuan et al., 2006; Zheng et al., 2013). However, SCFA production efficiency is limited by the low hydrolysis rate and rapid consumption by methanogens. In order to obtain more SCFA, the hydrolysis rate should be improved, and meanwhile, the consumption of SCFA for methane production needed to be totally prevented.

Microbial cells and organic matters of WAS are generally agglomerated together in a polymeric network formed by microbial extracellular polymeric substance (EPS) (Frølund et al., 1996). Several strategies, such as ultrasonic, microwave, thermal, enzymatic, and alkaline treatments, have been applied to disrupt EPS and bacterial cells of WAS to accelerate the hydrolysis rate (Carrère et al., 2010; Lee et al., 2014; Luste et al., 2011; Park et al., 2013; Oh

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et al., 2014). Previous studies have proposed these meaningful methods to improve the hydrolysis rate of WAS generated from conventional activated sludge process. However, the strategy to improve SCFA production from MBR sludge has been very limited since MBR sludge is less biodegradable (Wei et al., 2003). Yu and his co-workers reported that SCFA yield from MBR sludge was greatly improved by alkaline treatment, and the optimal conditions for SCFA production were pH 11 and fermentation time 4 day (Yu et al., 2013). However, alkaline treatment for MBR sludge are rarely or not practically applied in full-scale waste activated sludge treatment plants, due to the high cost for the alkalinity adjustment. Moreover, alkaline treatment might cause secondary environment pollution. Thus, an efficient, cost-effective, and sustainable method for SCFA production from MBR sludge is required.

Surfactants can not only improve the solubilization and dispersion of organic matters, but also change the affinity between the microbial cells and the organic matters through increasing cell surface hydrophobicity, thus accelerating the dissolution rate of non-aqueous phase substance (Mayer et al., 1999; Zhang and Miller, 1992; Luo et al., 2013). It was reported that sodium dodecylbenzene sulphonate could effectively accelerate the solubilization of EPS in WAS and subsequently promote the accumulation of SCFA (Jiang et al., 2007). Ewa and Marcin demonstrated that volatile suspended solids (VSS) reduced remarkably in the presence of sodium dodecyl sulfate (Ewa and Marcin, 2006). Whereas, the surfactants used in those works are chemosynthetic and non-biodegradable, thereby causing great negative impact on the environment. Compared with chemical surfactants, biosurfactants are more desirable in environmental application for their biodegradation and low toxicity (Zhang et al., 2009). Biosurfactants such as surfactin, rhamnolipid have been employed to enhance the production of SCFA from WAS (Huang et al., 2015; Zhou et al., 2013). The presence of surfactin or rhamnolipid could result in nearly 3300 mg chemical oxygen demand (COD)/L SCFA production, which was only 24.6% of the total COD (Huang et al., 2015), these results indicate that potential of SCFA production enhanced by surfactin or rhamnolipid is still promising. Alkyl polyglycoside (APG), a mild non-ionic surfactant made from fatty alcohol and glucose derived from recyclable starch, is low-irritant, nontoxic and easily degradable in the environment (Zhang et al., 2011). Recently, APG has been applied to improve the organic matter degradation and composting process of agricultural wastes (Zhang et al., 2011). APG seems to be a promising biosurfactants for SCFA production from WAS. To date, however, the application of APG to enhance SCFA production from MBR sludge anaerobic fermentation has never been reported.

It was also reported that pH played an important role in SCFA production from WAS anaerobic fermentation. The culture pH can affect the activities of specific acidogenic microbial populations (Horiuchi et al., 1999; Zhang et al., 2012) and methanogenic archaea (Ghosh et al., 2000). Yuan et al. (2006) noted that SCFA production from WAS was greatly enhanced under alkaline conditions (such as pH10). Furthermore, some studies also reported the effect of pH/or alkaline addition on SCFA production after pretreatment (such as ultrasonic pretreatment, microwave pretreatment) (Liu et al., 2014; Jang and Ahn, 2013). However, it is difficult to control pH during the entire anaerobic process in a full-scale sludge treatment plant, Kang et al. (2011) investigated the effect of initial pH adjustment on hydrolysis and acidification of sludge by ultrasonic pretreatment. In addition, the physical properties of biosurfactant including the morphology and surface tension reduction were sensitive to ambient pH (Zhang and Miller, 1992). Thus, the effect of initial pH on SCFA production from MBR sludge in the presence of biosurfactant should be further investigated.

The main purpose of this study is to assess the feasibility of biosurfactant APG for enhancing SCFA production from MBR sludge

anaerobic fermentation and investigate the effect of initial pH on SCFA production in the presence of APG. Firstly, effect of APG dosage on SCFA production from MBR sludge anaerobic fermentation was studied. Then, the effect of initial pH on the SCFA production at optimal APG dosage was also investigated. Variation of soluble COD, VSS, protein and carbohydrate as well as methane production,  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  release were analyzed. The activities of hydrolytic and acid-forming enzymes at different initial pHs were also measured to reveal the microbial activities.

## 2. Materials and methods

### 2.1. Source of MBR sludge

The MBR sludge used in this study was collected from the aeration tank of a full-scale submerged MBR for municipal wastewater treatment in Changsha, China. The collected MBR sludge was concentrated by settling at 4 °C for 24 h, and the main characteristics (average data plus standard deviation of three tests) are as follows: pH  $6.8 \pm 0.2$ , total suspended solids (TSS)  $13,030 \pm 315$  mg/L, VSS  $7815 \pm 150$  mg/L, total COD  $12,110 \pm 210$  mg/L, soluble COD  $82 \pm 4$  mg/L, total protein  $3387 \pm 120$  mg COD/L, total carbohydrate  $1390 \pm 105$  mg COD/L,  $\text{NH}_4^+\text{-N}$   $4.2 \pm 0.3$  mg/L,  $\text{PO}_4^{3-}\text{-P}$   $5.5 \pm 0.2$  mg/L.

### 2.2. Enhanced SCFA production from MBR sludge in the presence of APG

This batch test was conducted in 5 identical anaerobic reactors with each working volume of 600 mL, and 3000 mL raw MBR sludge was divided equally into 5 reactors. Then different volume of APG stock was added into serum bottles to obtain the designed APG dosage 0, 0.1, 0.2, 0.3, 0.4 g/g dry sludge (DS). All reactors were mixed with a mechanical stirrer at a speed of 120 rpm in a  $35 \pm 1$  °C temperature-controlled room for 15 days. The initial pH was controlled at 11 by adding 2.0 M sodium hydroxide (NaOH) or hydrochloric acid (HCl). After feeding, all reactors were flushed with nitrogen gas to displace oxygen, sealed with rubber stoppers. By analyzing SCFA production in fermentation system, the optimum APG dosage for SCFA accumulation from MBR sludge anaerobic fermentation was determined.

The contribution of APG to SCFA production was evaluated with APG as the fermentation substrate. The APG whose amount was corresponding to the fermentation reactor above were dissolved into 500 mL tap water, and 100 mL WAS was added as inoculum. The operation conditions were the same with the experiments mentioned above. Thus, the SCFA production from APG degradation was determined by analyzing SCFA concentration in the anaerobic fermentation system.

### 2.3. Effect of different initial pH on SCFA production from MBR sludge enhanced by APG addition

9 identical anaerobic reactors (working volume of 600 mL each) were established to investigate the effect of different initial pH on MBR sludge hydrolysis for SCFA accumulation. The initial pH in reactors was respectively adjusted at 4, 5, 6, 8, 9, 10, 11, 12 by 2.0 M NaOH or 2.0 M HCl, and pH was not controlled during anaerobic fermentation. The reactor without pH adjusting was set as the blank (initial pH 6.8). The APG dosage was 0.2 g/g DS according to the preliminary result of this study.

By analyzing soluble COD production, VSS reduction, variations of protein and carbohydrate, SCFA yield,  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  release in fermentation systems, the effect of initial pH on SCFA production from MBR sludge in the presence of APG was determined.

## 2.4. Analytical methods

The analyses of  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ , COD, TSS, VSS were conducted according to standard methods (APHA, 1976). Soluble proteins and carbohydrate were determined by the Lowry-Folin method with bovine serum albumin (BSA) as the standard and the phenol-sulfuric method with glucose as the standard, respectively (Herbert et al., 1971; Lowry et al., 1951). The measurement of SCFA concentration and composition were the same as described in the literatures (Yuan et al., 2006; Zhao et al., 2015b). The activities of key hydrolytic enzymes (protease and  $\alpha$ -amylase) were determined according to the method developed by Goel et al. (1998), and the key acid-forming enzymes, phosphotransacetylase (PTA), acetate kinase (AK), oxaloacetate transcarboxylase (OATC), CoA transferase, were measured according to the method reported in the literatures (Feng et al., 2009; Mu and Chen, 2011; Zhao et al., 2015a).

## 2.5. Statistical analysis

All experiments were performed in triplicate. An analysis of variance was applied to evaluate the significance of results, and  $p < 0.05$  was considered to be statistically significant.

## 3. Results and discussion

### 3.1. Effect of APG dosage on SCFA production from MBR sludge

Fig. 1 shows the SCFA production from MBR sludge at different APG dosage. Compared with the blank, the SCFA production was significantly improved ( $p < 0.05$ ) by APG in the range of 0–0.2 g/g DS, and further increase of APG dosage gave little benefit to SCFA accumulation. The SCFA production with 0.2 g APG/g DS reached 282.9 mg COD/g VSS, which was 4.8-fold of that in blank. In addition, the fermentation time for the maximal SCFA production was also shorten from day12 to day 6 due to the presence of APG. Obviously, the presence of APG not only enhanced the SCFA yield but also accelerated the SCFA production rate, and the optimum APG dosage was 0.2 g/g DS. However, Luo and his co-workers achieved the maximal production of SCFAs 297.3 mg COD/g VSS from conventional WAS with APG 0.3 g/g TSS without pH adjustment (Luo et al., 2015), which was much higher than that in blank (124.3 mg COD/g VSS) of this study. In addition, Chen et al. (2014)

reported 2221.6 mg COD/L was achieved when WAS was treated with 0.2 g APG/g TSS, however, 971.4 mg COD/L (124.3 mg COD/g VSS  $\times$  7.815 g VSS/L = 971.4 mg COD/L) was obtained in this study. These results suggested that MBR sludge was less biodegradable compared with conventional activated sludge.

Fig 1b exhibits the distribution of individual SCFA in the presence of APG. In this study, the detectable SCFA mainly included straight- or branched-chain SCFA from 2 to 5-carbon atoms, i.e., acetic, propionic, iso-butyric, n-butyric, iso-valeric, and n-valeric acids. As shown in Fig. 1b, the top two individual SCFA in all reactors were acetic and propionic acids, with a total percentage of 68–75%. Further study showed that the average order of individual SCFA was in the sequence of acetic > propionic > iso-valeric > n-butyric > iso-butyric > n-valeric.

It is well known that APG, as a kind of biosurfactant, could be degraded in the anaerobic fermentation system. In this study, the variations of APG in each reactor were measured and results were shown in Table S1 (Supporting Information). It was found APG was consumed at the beginning of this fermentation test and completely exhausted at the fermentation time of day 15. In addition, the contribution of APG degradation to SCFA production was listed in Table S1. The maximum SCFA production from APG degradation was only 152 mg/L, which was far less than that from MBR sludge fermentation. So the contribution of APG degradation to SCFA production was very limited. All those results showed that SCFA production efficiency and rate from MBR sludge in the presence of APG were greatly enhanced, and the optimum APG dosage was 0.2 g/g DS.

### 3.2. Effect of the initial pH on MBR sludge hydrolysis at 0.2 g APG/g DS

Sludge hydrolysis rate can be expressed by the variations of soluble COD concentrations (Andreassen et al., 1997). Fig. 2 depicts the variation of soluble COD at different initial pH values. It was found that the soluble COD concentration increased gradually with fermentation time, which implied that particulate organics in the MBR sludge became soluble substrates. It should be highlighted that soluble COD at alkaline pH (10, 11 and 12) was significantly higher than that at near neutral pH (6.0 and 8.0), acidic pH (4.0 and 5.0) or in blank (6.8) ( $p < 0.05$ ). The maximal soluble COD at initial different pH was 3241 (pH4), 2948 (pH5), 2658 (pH6), 2358 (pH8), 3415 (pH9) 4128 (pH10), 4859 (pH11), 5031 (pH12) and 2104

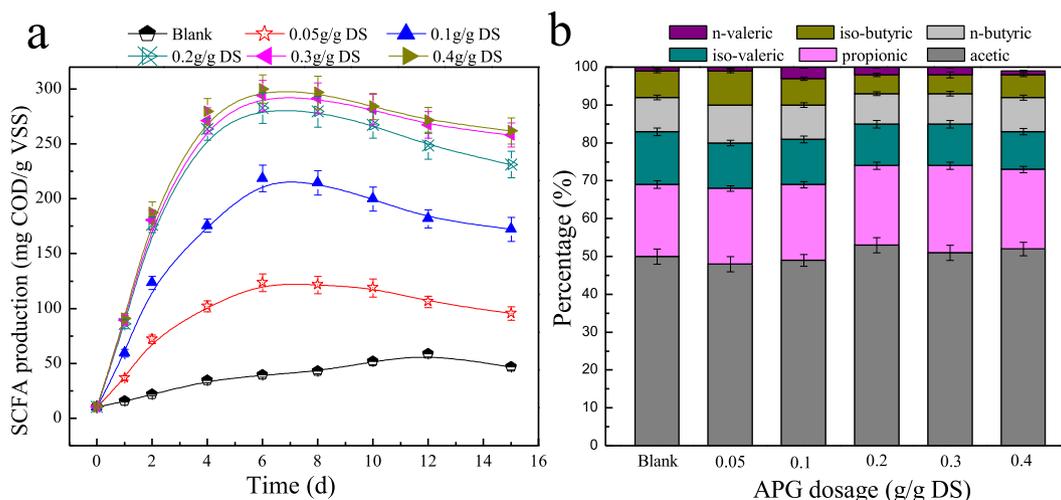


Fig. 1. The total SCFA production from MBR sludge with different APG dosage during anaerobic fermentation (a) and the fraction of individual SCFA under their optimal fermentation time (b). Error bars represent standard deviations of triplicate determinations.

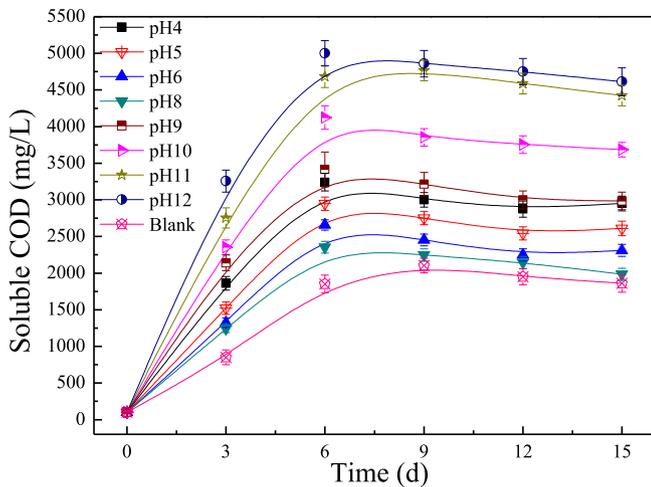


Fig. 2. Variations of soluble COD with time at different initial pH values in the presence of APG (0.2 g/g DS). Error bars represent standard deviations of triplicate determinations.

(blank) mg/L. These indicated that initial alkaline (pH 10, 11 and 12) adjustment was more beneficial for sludge hydrolysis in the presence of APG than other initial pH. In addition, acid pH is beneficial for sludge hydrolysis than neutral blank since more soluble COD was detected under acid pH condition than that in blank. The results were coincident with the reports that alkaline condition could accelerate WAS hydrolysis rate in the literatures (Chen et al., 2007; Kang et al., 2011; Liu et al., 2014).

Protein and carbohydrate are the main substrates in MBR sludge, thus the change of soluble protein and carbohydrate are applied to express the sludge hydrolysis rate. Moreover, sludge reduction also reflects the degree of sludge hydrolysis. Table 1 lists the variations of protein, carbohydrate and VSS reduction at different initial pH after 3 days. Obviously, soluble protein, soluble carbohydrate generation and VSS reduction at initial alkaline pH were much higher than those at the initial near neutral or acidic pH, which were consistent with the change of soluble COD (Fig. 2). In addition, it was found that the concentration of soluble protein (carbohydrate) under acid pH was higher than that in blank. Herein, the hydrolysis of MBR sludge in the presence of APG at alkaline pH was more efficient than that at acidic and near-neutral pH.

### 3.3. Effect of the initial pH on SCFA production and composition

Fig. 3 illustrates the effect of initial pH on total SCFA production. Generally, the SCFA production divided into two stages. From fermentation beginning to day 6, the SCFA production increased with fermentation time. Then the SCFA production reached its peak on day 6 and declined slightly with fermentation time. SCFA production at initial pH 11 was significantly higher than that at other initial pH ( $p < 0.05$ ), suggesting pH11 was the optimum initial pH for SCFA production from MBR sludge in the presence of APG (0.2 g/

g DS). Meanwhile, it was noticeable that SCFA yield was higher under acid pH than neutral blanks. The similar phenomenon also appeared in mesophilic hydrolysis and acidification of swine manure (Lin et al., 2013).

The SCFA composition at different initial pH within optimal fermentation time was shown in Fig. 3b. It was observed that acetic acid was the most prevalent individual SCFA and propionic acid was the second individual SCFA at all initial pHs, and their maximum concentration at initial pH11 was 1169 and 463 mg COD/L, respectively. It is noteworthy that the individual SCFA percentage at different initial pHs shows little difference.

### 3.4. Enzymes activity analysis

It has been demonstrated that several key enzymes were involved in the metabolic pathway for SCFA formation during anaerobic fermentation process, and their activities were directly related with the yield of SCFA (Feng et al., 2009). It should be noted that substantial enzymes took part in the production of different SCFA, whereas, only some key enzymes were measured to investigate the effect of initial pH on their activities in the presence of APG in this study. As for hydrolases, protease and  $\alpha$ -glucosidase are extensively studied since they are responsible for protein and carbohydrate hydrolysis, respectively (Goel et al., 1998). Phosphotransacetylase (PTA) and acetate kinase (AK) are the key enzymes responsible for the transformation of acetyl-CoA to acetic acid (Feng et al., 2009). In addition, oxaloacetate transcarboxylase (OAATC) and CoA transferase are directly responsible for propionic acid formation (Feng et al., 2009; Wang et al., 2013). The data in Table 2 clearly show that these key enzymes relating with SCFA production exhibited higher activity at initial alkaline pH (9, 10 and 11), which was nicely in agreement with the results of SCFA production (Fig. 3). In addition, enzymes activities under acid pH (4 and 5) were higher than those in blank, which is in correspondence with more SCFA generation compared with blank. The reason of the key enzymes enhancement at alkaline pH and acid pH might be as follows: the key enzymes originally embedded in the pellet fraction of EPS matrix were released due to the improved solubilization led by APG and alkaline condition (Yu et al., 2008).

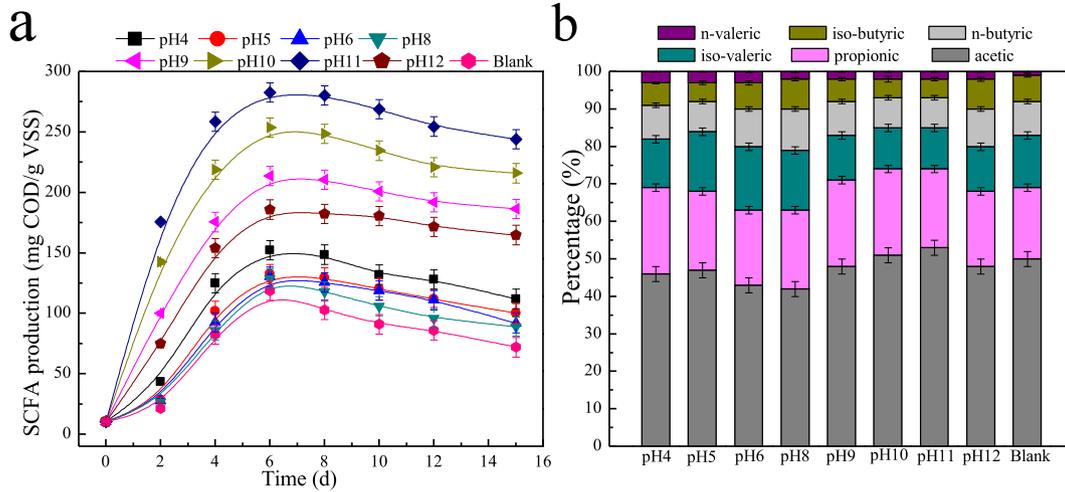
From Fig. 2, it can be seen that soluble COD increased with the initial pH increasing from 9 to 12, which implies that the higher initial pH level applied, the more substrates will be solubilized and provided for subsequent SCFA production. Nevertheless, we found that SCFA accumulation was declined when the initial pH increased from 11 to 12 (Fig. 3). Although initial pH12 resulted in higher sludge hydrolysis than initial pH11, no significant increase of soluble COD, soluble protein and soluble carbohydrate were observed on day 3 ( $p > 0.05$ , Table 3). In addition, the activities of key enzymes relevant to hydrolytic and acid-forming microbes at the initial pH12 were lower than those at the initial pH11 (Table 2). Therefore, the initial pH12 caused lower SCFA production might be due to the toxic effect of extreme alkaline conditions to acidogenic bacteria (Wu et al., 2010; Liu et al., 2014).

Table 1

Effect of the initial pH on solubilization of particulate organic matters in MBR sludge in the presence of APG (0.2 g/g DS) with fermentation time of day 3.<sup>a</sup>

Items	Initial pH values								
	pH4	pH5	pH6	pH8	pH9	pH10	pH11	pH12	Blank
Protein	1115 ± 53	912 ± 38	785 ± 24	748 ± 26	1284 ± 50	1416 ± 54	1682 ± 62	1712 ± 38	510 ± 24
Carbohydrate	231 ± 12	182 ± 11	158 ± 9	150 ± 8	256 ± 10	282 ± 12	330 ± 15	335 ± 16	102 ± 6
VSS reduction	8.6 ± 0.8%	7.6 ± 0.8%	6.4 ± 0.5%	6.0 ± 0.4%	10.6 ± 0.6%	14.2 ± 0.7%	15.9 ± 0.8%	16.5 ± 0.9%	5.8 ± 0.3%

<sup>a</sup> The units of soluble protein and carbohydrate are mg COD/L. The data are the averages and their standard deviations in triplicate tests.



**Fig. 3.** The total SCFA production from MBR sludge at different initial pH with 0.2 g APG/g DS (a) and the fraction of individual SCFA under their optimal fermentation time (b). Error bars represent standard deviations of triplicate determinations.

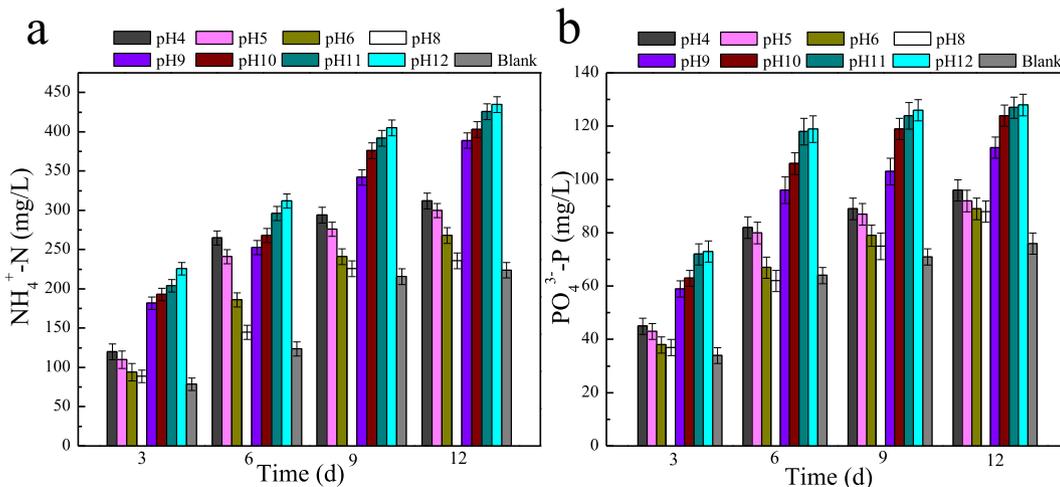
**Table 2**  
Comparison of specific activities of key enzymes involved in SCFA production among different fermentation reactors under their optimal conditions.<sup>a</sup>

	pH4	pH5	pH6	pH8	pH9	pH10	pH11	pH12	Blank
Protease	5.09 ± 0.12	4.85 ± 0.05	4.28 ± 0.03	4.12 ± 0.04	5.12 ± 0.05	5.86 ± 0.03	6.14 ± 0.02	6.03 ± 0.03	3.15 ± 0.05
α-glucosidase	0.196 ± 0.003	0.175 ± 0.003	0.142 ± 0.002	0.145 ± 0.004	0.206 ± 0.002	0.224 ± 0.004	0.248 ± 0.002	0.231 ± 0.002	0.115 ± 0.004
PTA	0.189 ± 0.002	0.168 ± 0.001	0.125 ± 0.002	0.134 ± 0.004	0.216 ± 0.002	0.241 ± 0.004	0.264 ± 0.002	0.172 ± 0.003	0.112 ± 0.003
AK	1.69 ± 0.03	1.62 ± 0.04	1.31 ± 0.02	1.38 ± 0.04	1.84 ± 0.03	1.98 ± 0.02	2.15 ± 0.04	1.76 ± 0.03	1.06 ± 0.02
OAAATC	0.429 ± 0.003	0.315 ± 0.004	0.211 ± 0.004	0.237 ± 0.002	0.642 ± 0.003	0.685 ± 0.003	0.728 ± 0.002	0.584 ± 0.003	0.125 ± 0.004
CoA transferase	0.567 ± 0.003	0.428 ± 0.002	0.215 ± 0.004	0.223 ± 0.006	0.648 ± 0.004	0.752 ± 0.003	0.845 ± 0.003	0.592 ± 0.004	0.084 ± 0.005

<sup>a</sup> The specific enzyme activity was defined as unit of enzyme activity per milligram of VSS. Results are the averages and their standard deviations of triplicate measurements.

**Table 3**  
The statistical analysis results of initial pH12 affecting soluble COD, soluble protein, soluble carbohydrate and VSS reduction (compared with initial pH11 on day 3).

	Soluble COD	Soluble protein	Soluble carbohydrate	VSS reduction
F <sub>observed</sub>	0.01	5.41	6.03	1.60
F <sub>significant</sub>	7.71	7.71	7.71	7.71
P <sub>(0.05)</sub>	0.92	0.08	0.07	0.27



**Fig. 4.** Release of NH<sub>4</sub><sup>+</sup>-N (a) and PO<sub>4</sub><sup>3-</sup>-P (b) during MBR sludge fermentation in the presence of APG at different initial pH values. Error bars represent standard deviations of triplicate determinations.

### 3.5. Effect of initial pH on $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ release

The effect of initial pH on the  $\text{PO}_4^{3-}\text{-P}$  and  $\text{NH}_4^+\text{-N}$  release is presented in Fig. 4. During the MBR sludge anaerobic fermentation process, the concentration of  $\text{NH}_4^+\text{-N}$  kept increasing with fermentation time in all investigated reactors. Furthermore,  $\text{NH}_4^+\text{-N}$  concentrations at alkaline pH were much higher than those at neutral or acidic pH, which was consistent with the soluble COD profile. As protein was the major component of MBR sludge (Wu et al., 2010), thus, the release of  $\text{NH}_4^+\text{-N}$  was mainly from the hydrolysis of sludge protein. It was reported that biosurfactant can change microorganism cell structure through making the cell materials leave the attached surface and then dissolving them in the aqueous solution (Sotirova et al., 2009). The MBR sludge hydrolysis could be improved at alkaline pH, thus more  $\text{NH}_4^+\text{-N}$  concentration in the system was detected.

Fig. 4b shows the profile of  $\text{PO}_4^{3-}\text{-P}$  concentration with time at different initial pH. It was found that  $\text{PO}_4^{3-}\text{-P}$  concentration increased gradually with time. The initial  $\text{PO}_4^{3-}\text{-P}$  concentration in all reactors was 5.5 mg/L, after 6 days fermentation time, the average  $\text{PO}_4^{3-}\text{-P}$  concentration in each reactor was 82 (pH4), 80 (pH5), 67 (pH6), 62 (pH8), 96 (pH9), 106 (pH10), 118 (pH11), 119 (pH12) and 64 (blank) mg/L, respectively. It should be highlighted that the average  $\text{PO}_4^{3-}\text{-P}$  concentration at alkaline pH was higher than that at neutral or acidic pH, which was in agreement with the  $\text{NH}_4^+\text{-N}$ .

The  $\text{NH}_4^+\text{-N}$  release was greater than that of  $\text{PO}_4^{3-}\text{-P}$  (291.8 (296–4.2) versus 112.5 mg/L (118–5.5)) at the initial pH11 on day 6. Similar results were also obtained at other initial pHs. Compared with the blank, more  $\text{NH}_4^+\text{-N}$  was released than  $\text{PO}_4^{3-}\text{-P}$  (172 (296–124) versus 54 (118–64) mg/L on 6th day) at initial pH11, which indicated that some of released  $\text{PO}_4^{3-}\text{-P}$  might precipitate at alkaline pH. It was reported that  $\text{PO}_4^{3-}\text{-P}$  could precipitate at alkaline pH (such as pH10) in the literature (Yan et al., 2010; Chen et al., 2007). In addition, the different composition between N and P in microbial structure proteins is also the reason for lower P concentration in fermentation liquid, as compared with N concentration.

## 4. Conclusion

Biosurfactant APG can significantly increase SCFA production during MBR sludge anaerobic fermentation, and the optimum APG dosage was 0.2 g/g DS with the corresponding SCFA production of 282.9 mg COD/g VSS, which was 4.8-fold of the blank. Furthermore, initial alkaline pH enhanced hydrolysis of proteins and carbohydrates and the production of SCFA from MBR sludge in the presence of APG. The optimum conditions for SCFA production were initial pH 11 and fermentation time of day 6. In addition, the activities of key enzymes for SCFA forming at alkaline pH were higher than those at neutral or acidic pH. On the one hand, APG can enhance SCFA generation from MBR sludge and shorten the fermentation time. On the other hand, it can be biodegraded during fermentation process and not cause secondary pollution in practice implication. Thus, the application of APG in MBR sludge treatment is promising.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ibiod.2015.06.012>.

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