

EFFECT OF LOW-TEMPERATURE THERMAL-ALKALINE PRETREATMENT WITH ALKYL POLYGLUCOSIDES (APGS) FOR LOW-ORGANIC-CONTENT SLUDGE ON ANAEROBIC DIGESTION

LIU, X.^{1,2} – ZHANG, C.^{1,2*} – YU, Z.^{1,2}

¹*College of Environmental Science and Engineering, Hunan University, Changsha 410082, China*

²*Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, China*

**Corresponding author*

e-mail: zhangchang@hnu.edu.cn; phone/fax: +86-731-8882-2312

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Abstract. Low-temperature thermo-alkaline pretreatment with alkyl polyglucosides (APGs) was employed for improving the utilization of low-organic-content sludge in anaerobic digestion (AD). An orthogonal experiment (L_9 (3^4)) was designed to estimate the influence of four factors on pretreatment, and the order determined by significance level was: pH > temperature > APG dosage > time. Under the conditions of pH 11, 60 °C, and 60 min, the content of organic matter dissolved from sludge increased as the APG dosage increased to 18 g/L. When fermenting at a dosage above its critical micelle concentration (CMC), however, APG inhibited aerogenesis due to the adverse impact on methanogen activity. Thus, the optimal APG dosage was fixed at 3.0 g/L. After 35 days of mesophilic AD, the biogas volume, the methane volume fraction, and the organics utilization rate reached their respective peaks. The methane volume fraction (45.8%) was lower than normal biogas (at least 53%), so further evaluation is needed.

Keywords: *surfactant, CMC, mesophilic, methanogenesis, orthogonal experiment*

Introduction

The large amount of sludge generated by wastewater treatment plants (WWTPs) has been a great challenge and has raised significant concerns in China (Yang et al., 2015). Disposal methods, including sanitary landfill, land application, and incineration, are the main final destinations of sludge after treatment (Murakami et al., 2009; Song and Lee, 2010; Hale et al., 2012). Processes such as thickening, conditioning, dewatering, stabilization, and drying are commonly employed as sludge treatment methods (Yang et al., 2015). Stabilization, a well-developed approach, is an important way to reduce the environmental risks of sewage sludge. In particular, anaerobic and aerobic digestion are the most common stabilization methods, and they are usually applied in cases of small WWTPs, although the majority of produced sludge is treated anaerobically (Kelessidis and Stasinakis, 2012).

Anaerobic digestion (AD) is a complex process which is generally considered to have a rate limited by the hydrolysis of sludge (Appels et al., 2008). Therefore, pretreatments facilitating sludge hydrolysis enhance the efficiency of AD. Generally speaking, pretreatment methods include biological, thermal, mechanical (ultrasonic treatment, lysis-centrifugation, liquid shear, and grinding), and chemical (oxidation and alkalization) (Carrere et al., 2010) treatments, among which thermal treatment has been paid much attention. Thermal pretreatment can be mainly classified into two categories

based on the temperature applied, i.e., pretreatment higher than 100 °C (need a high-pressure installation) (Bougrier et al., 2008; Ennouri et al., 2016; Jae et al., 2018) and pretreatment lower than 100 °C (under atmospheric pressure) (Appels et al., 2010; Fernández-Marchante, 2018). However, treatment in a mild temperature is often combined with a chemical method (Mendez et al., 2013), most commonly with an alkali. Some researchers (Kim et al., 2015; Zhang et al., 2015; Li et al., 2016; Du et al., 2019) have concluded that a low-temperature thermo-alkali pretreatment significantly enhances the efficiency of AD.

From studies on sludge dewatering, it was found that surfactants were highly effective in releasing extracellular polymeric substances (EPSs) from sludge (Wang et al., 2014; Liu et al., 2019). EPSs consist of high-molecular-weight secretions from microorganisms, and products of cellular lysis and macromolecule hydrolysis. A higher EPS content in sludge results in greater sludge stability (Sheng et al., 2010), so the release of EPSs from cells can facilitate the dissolution of organics, thus making a positive contribution to the hydrolysis of sludge. In short, surfactants can be chosen for sludge pretreatment (Guan et al., 2017), but their environmental friendliness must be taken into consideration.

Alkyl polyglucoside (APG) is a nonionic surfactant synthesized from renewable raw materials, and it has excellent ecotoxicological profiles, so it is readily biodegradable (Geetha and Tyagi, 2012). Luo et al. (2015) reported that adding APG to an anaerobic treatment system of waste activated sludge (WAS) improved the production of short-chain fatty acids remarkably. However, so far, no one has tried to pretreat sludge with APG before AD.

WAS with comparatively low organic content has not been qualified for AD. This study, therefore, explored the effect of low-temperature thermo-alkaline pretreatment, optimized by APG dosing of low-organic-content sludge, on AD. An orthogonal experiment was firstly designed to estimate the effects of pH, temperature, APG dosage, and time on the pretreatment, and single factor experiments were also conducted to meet the shortcomings of the orthogonal experiment. More importantly, the role of APG dosage in AD was investigated by a series of fermentation experiments.

Materials and methods

Test materials

Waste activated sludge

The sludge used in this study was collected from Kaifu Wastewater Treatment Plant (Changsha, China) through a Modified Sequencing Batch Reactor (MSBR). It was dewatered by belt filter press in the plant, and it was then stored in a plastic hermetic bag at 4 °C in the laboratory. The moisture content of the sludge was $81.3 \pm 0.05\%$.

Before each experiment, the dewatered sludge was diluted with deionized water into approximate 15 g/L (total solids), and the ratio of volatile solids (VS) to total solids (TS) was $51.32 \pm 0.10\%$, which is comparatively low (VS/TS of WAS for AD in the literature was generally above 70%). The main characteristics of the sludge solution were total chemical oxygen demand (TCOD) = 6976.8 ± 77.5 mg/L, soluble chemical oxygen demand (SCOD) = 85.3 ± 7.7 mg/L, soluble total organic carbon (STOC) = 60.45 ± 5.38 mg/L, soluble total phosphorus (soluble TP) = 10.18 ± 0.20 mg/L, pH = 6.96 ± 0.01 .

Inocula

For the anaerobic digestion tests, the sludge solution was anaerobically cultivated at 37 °C for 3 days in a constant-temperature incubator in order to activate the anaerobe, and to prepare for the inocula (it is feasible to use raw WAS as inocula, as shown in the literature; Nguyen et al., 2014). Its main characteristics were TCOD = 6821.8 ± 155.0 mg/L, SCOD = 100.8 ± 23.3 mg/L, STOC = 66.45 ± 3.62 mg/L, soluble TP = 14.30 ± 0.11 mg/L, pH = 6.91 ± 0.01 .

Alkyl polyglucoside

The surfactant alkyl polyglucoside (APG0810), which was a faint yellow thick fluid (solid content $\geq 50\%$), was purchased from Shanghai Fine Chemical CO., LTD and was used with no further purification. Thus, APG concentration in this paper refers to the concentration of the fluid. Its carbon content was $34.9 \pm 0.3\%$.

Critical micelle concentration (CMC) is an important characteristic of a surfactant, and it is defined as the concentration of surfactants above which micelles form, and all additional surfactants added to the system form micelles (IUPAC, 1997). Before reaching the CMC, the surface tension changes strongly with the concentration of the surfactant. After reaching the CMC, the surface tension remains relatively constant, or it changes at a slower rate.

The surface tensions of the surfactant aqueous solution and sludge solution were measured and are shown in *Figure 1*. The CMC of the APG aqueous solution was about 1.5 g/L, which corresponds with the data provided by the manufacturer, while the CMC of the APG sludge solution was approximately 3.0 g/L. *Figure 1* also indicates that NaOH treatment nearly had no effect on the surface tension of the sludge solution.

Methods

Low-temperature thermo-alkaline pretreatment with APG

The pretreatment tests were conducted in 150 mL conical flasks with covers (to avoid water loss by evaporation) fed with 100 mL of sludge solution. Different volumes of 1 mol/L NaOH and 100 g/L APG solution were added to adjust the pH and APG concentration, respectively. The volume of NaOH added for reaching a set pH was determined by a preliminary experiment, whose results are shown in *Table 1*.

Table 1. The relationship between pH and volumes of NaOH

Volume of NaOH (mL/100 mL)	0.0	0.6	1.4	2.0
pH	7	10	11	12

All flasks were maintained in a digital thermostat water bath oscillator, and the solution temperatures were detected by a thermometer. The timer was started as soon as the temperature reached the desired value. Each test was repeated twice.

After completion, samples were centrifuged at 8000 rpm ($3785 \times g$) for 15 min, the supernatant was filtered by a 0.45 μm microporous membrane, and the soluble total organic carbon and soluble total phosphorus were analyzed. In the present study, mixed-STOC = the data from the experimental analysis which included organic carbon both from sludge and APG, and STOC = mixed-STOC – APG $\times 349$ (1 g APG = 349 mg TOC, which can be deduced from section 2.1.3), which included the TOC dissolved just from sludge.

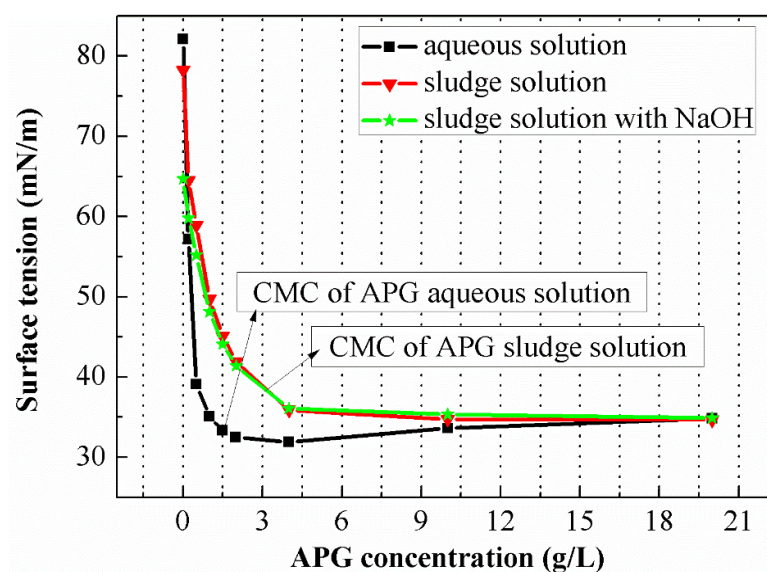


Figure 1. Surface tension of different APG concentrations

STOC represents the effect of the pretreatment. Soluble TP indicates the disintegration of the microbial cells due to those in the WWTPs, polyphosphate accumulation organisms (PAOs) are able to store phosphate as intracellular polyphosphate, leading to P removal via PAO cells in WAS (Oehmen et al., 2007). As a result, soluble TP comes almost entirely from the cellular interior, having dissolved when cells were destroyed.

Orthogonal experiment design

For the pretreatment tests, sludge was mainly affected by temperature, time, pH, and APG concentration. It was assumed that the four factors had significant influences on pretreatment. The results described herein are from an orthogonal experiment, which was designed for evaluating such experimental factors. Each factor includes three levels, as follows. Temperature: 40, 60, and 80 °C; time: 30, 60, and 90 min; pH: 7, 10, and 12 (neutral, alkaline, and strongly alkaline); and APG: 1.5, 3, and 4.5 g/L (below CMC, CMC, and above CMC). Since there were four experimental factors and three levels for each factor, the orthogonal matrix denoted as $L_9(3^4)$ was chosen as the experimental scheme to arrange the tests. Each test was repeated two times, and SPSS software was used for the experiment design and statistical analysis.

Anaerobic digestion tests

The experiments were conducted using conical flasks in duplicate with a working volume of 500 mL. The bottles contained a magnet rotor (for stirring automatically once a day) and were filled with 200 mL inocula and 300 mL pretreated sludge. All pretreatments were under the conditions of pH = 11, temperature = 60 °C, time = 60 min, APG = 0, 1.5, 3.0, and 5.0 g/L (preliminary experiments had proved that when pretreating at APG > 5 g/L, there is no gas produced in the AD system). The bottles of APG were named T0, T1.5, T3.0, and T5.0, respectively. For comparison, a blank test was run in a flask filled with 200 mL inocula and 300 mL raw sludge solution.

All flasks were incubated at 35 °C in an electrically heated thermostatic water bath for 35 days until gas was no longer produced. The gas was collected by aluminum foil gas-collecting bags, and gas volume was measured using a syringe. Moreover, the biogas composition was analyzed using gas chromatography (GC). Each test was repeated twice.

Analysis methods

The analyses of sludge moisture content, TS, VS, COD, TP, and pH were conducted according to standard methods (APHA, 2005). The surface tension was determined by pulling escape using a JZ-200A automatic interface tensiometer. By using a Shimadzu TOC-Vcph/cpn instrument, TOC was detected by catalytic oxidation nondispersive infrared analysis. The methane was analyzed by a gas chromatograph (Agilent, 6890N) equipped with a flame ionization detector (FID) and a 30 m × 0.32 mm capillary column (HP-5). The temperature of the injector, detector, and column were kept at 100 °C, 180 °C, and 80 °C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL/min. Three parallel samples were used for each analysis.

Data calculation formula

The energy efficiency from the single factor experiments was calculated as in Equation 1.

$$\text{energy efficiency} = \frac{c \times v}{m \times (T - 20) \times C} \quad (\text{Eq.1})$$

where *c* is the average concentration value when time ≥ 30 min, *v* is the volume, *m* is the mass, *T* is the desired temperature, 20 is the room temperature, and *C* is the specific heat capacity of water (4.2 kJ/°C·kg). The unit for energy efficiency is mg/kJ, which refers to the dissolved quantity of sludge per kJ energy consumed. The heat loss that occurs during the constant temperature process was ignored because of its small value and the difficulties in its calculation.

Results and discussion

Orthogonal experiment

The results of the orthogonal experiment for estimating the effects of temperature, time, pH, and APG concentration on the pretreatment of sludge are summarized in Table 2. The F-value shown in Table 2 indicates that the order of the effects of factors on STOC is pH > temperature > APG concentration > time. For soluble TP, the order of pH and temperature was the same, but the order of the other two factors (APG concentration and time) was opposite. This distinction indicates that the dissolution of organics was not caused solely by cell disintegration, but was also related to the dissolution of EPSs.

However, dissolution of EPSs contributes to the destruction of cells, and, as a result, the effects of the factors on STOC and soluble TP have a lot in common. As shown in Figure 2, pH (the most important factor) and temperature (the second most important factor) have similar effects, agreeing with the previous literature (Kim et al., 2015) that an increase of pH and temperature both enhance the dissolution of organics and

phosphorus from sludge. Taking into consideration the effects of pH and temperature on anaerobic digestion (Zhao et al., 2015a) and the energy consumption of the pretreatment, the final pH of the pretreatment was adjusted to 11 for both pretreatment efficiency and the reducing effect of alkalinity on AD, but the temperature needed some additional experiments.

Table 2. Tests of between-subject effects

Source	Dependent variable: STOC R Squared = .996 (Adjusted R Squared = .992)		Dependent variable: soluble TP R Squared = .999 (Adjusted R Squared = .997)	
	F	Sig.	F	Sig.
Corrected Model	260.748	.000	785.335	.000
Intercept	11235.690	.000	17368.888	.000
Temp	186.670	.000	199.503	.000
Time	3.603	.071	25.473	.000
APG	25.808	.000	7.907	.010
pH	826.909	.000	2908.456	.000

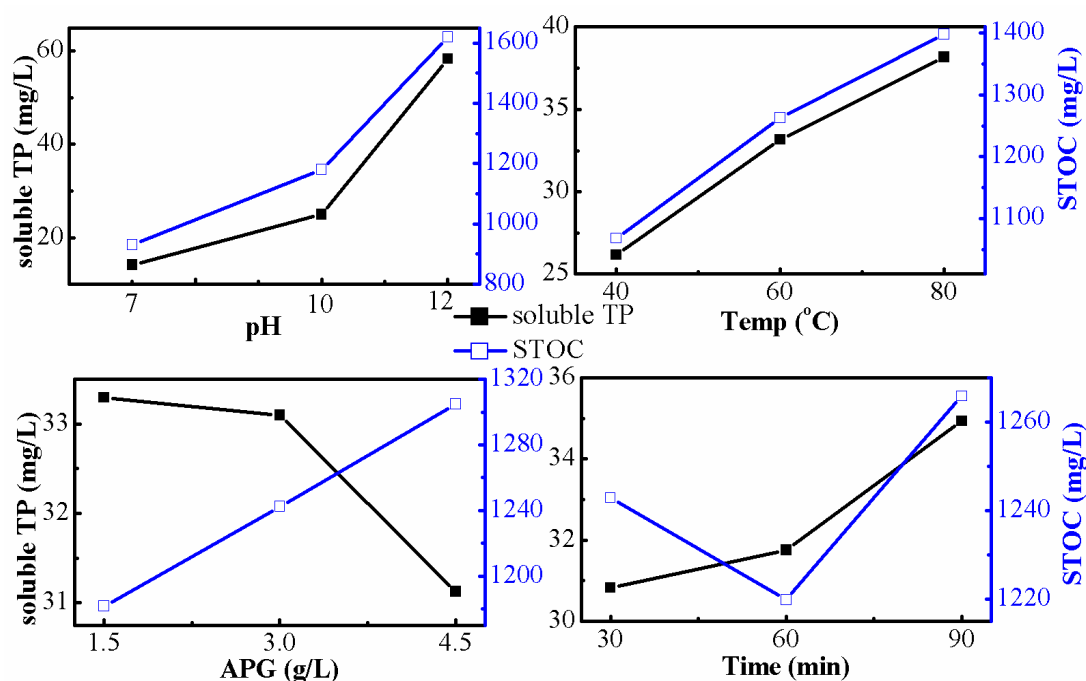


Figure 2. Level effects of each factor on STOC and soluble TP

Figure 2 also shows that for STOC and soluble TP, the least significant factors (time of STOC, APG concentration of soluble TP) have abnormal data, which could be explained by that time and APG concentration were easily affected by the other factors. The orthogonal design has two fundamental limitations: one is that it considers only first-order effects, and the other is that it does not account for the interaction among factors (Zeng et al., 2004). As a result, single factor experiments were required for demonstrating the effects of those two factors.

Single factor experiments

Effect of time and determination of appropriate temperature

The effect of time at various temperatures is shown in *Figure 3a* for the conditions: pH = 11; APG = 5 g/L; and temperature = 40 °C, 60 °C, and 80 °C. Once alkali and APG were added to the sludge solution, organic matter and phosphorus dissolved in the water rapidly until 30 min had elapsed, then the dissolving speed slowed down considerably. Referring to the literature (Kim et al., 2013; Ruffino et al., 2016), 60 min was chosen as a pretreatment time to ensure that the pretreatment was comparatively complete.

It is shown in *Figure 3a* that temperature enhanced the dissolution of organics and phosphorus in the sludge, which is consistent with the result of the orthogonal experiment. However, in *Figure 3b*, the energy efficiency decreased as the temperature increased, which negates the notion that the hotter the better. Although the dissolved quantity of sludge at 80 °C was higher than that at 60 °C, the amount of energy consumed at 80 °C was higher, or the energy efficiency was lower at 80 °C. Besides, it shows that the energy efficiency at 40 °C was the highest, but the dissolved quantity was compromised (the lowest). Thus, to obtain both the optimal dissolved quantity and energy efficiency, 60 °C was selected as the optimal temperature.

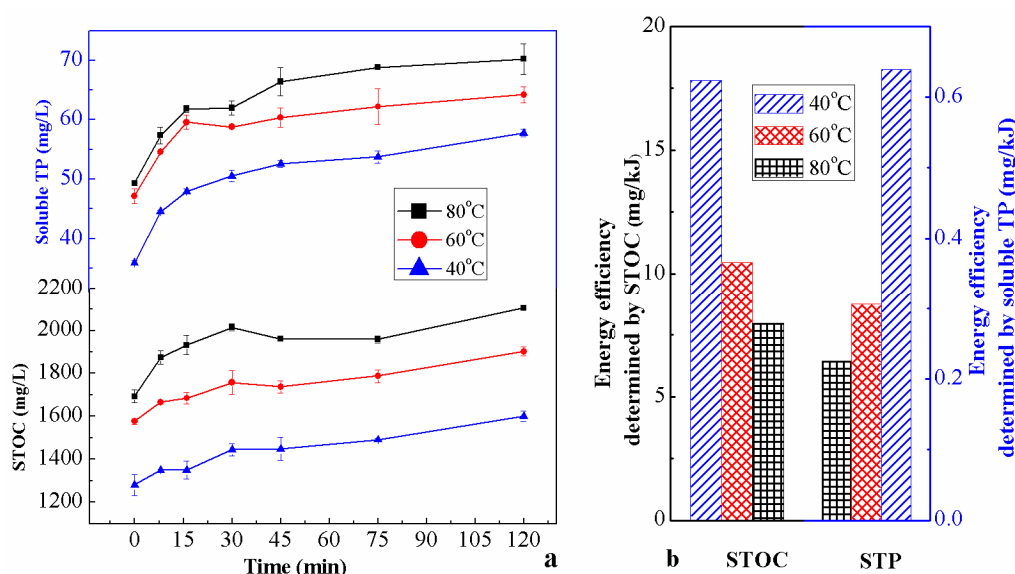


Figure 3. Effects of time on pretreatment

Effect of APG concentration

The effect of APG is shown in *Figure 4* for the conditions of pH = 11, temperature = 60 °C, and time = 60 min. The mixed-TOC (TOC from both sludge and APG) had a good linear relationship with APG (*Table 3*), but there is a discontinuity when APG = 18 g/L (*Fig. 4a*). The trend of STOC is from rising to declining, which could be explained as follows: Setting APG as x and mixed-STOC as y , then $y = kx + b$, and setting STOC as y' , then $y' = (k - 349)x + b$. It is easy to conclude from the equation that the slope coefficient of y' ($k - 349$) changed due to the change of the coefficient of y (k , from 446.1 to 275.4) at the point where APG = 18 g/L. Also, it is

clear from the figure that at the point where APG = 18 g/L, the efficiency of APG decreased slowly and then rapidly.

Table 3. Linear fitting equation of APG (x) and mixed-STOC (y)

APG concentration (mg/L)	Linear equation	R ²
[0,18]	$y = 446.1x + 970.7$	0.999
[18,30]	$y = 275.4x + 4017$	0.998

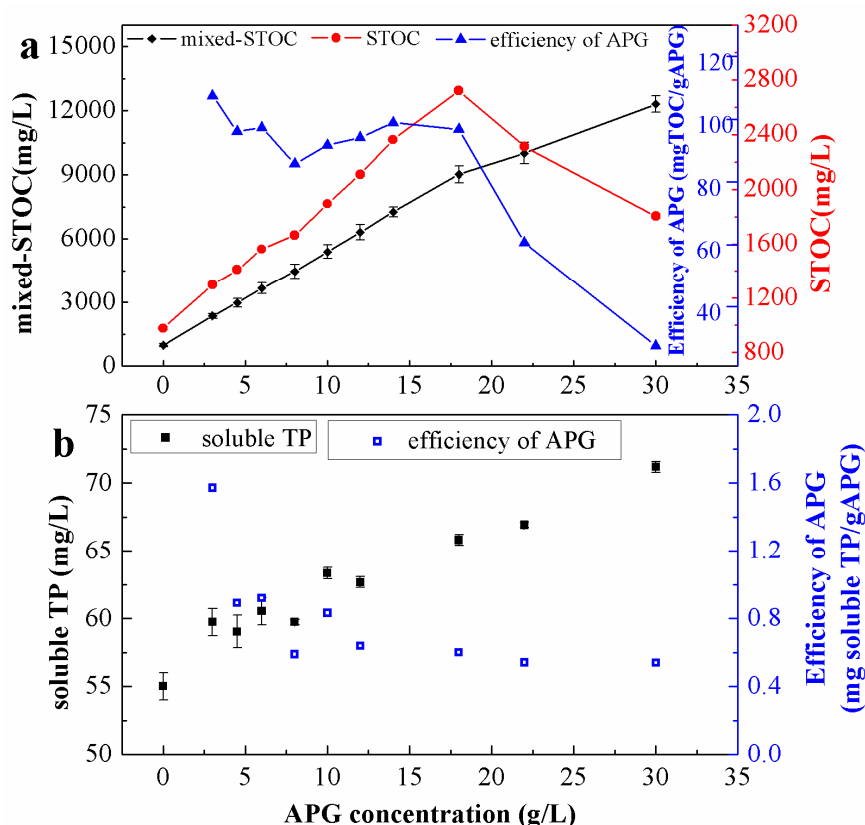


Figure 4. Effects of APG concentration on STOC (a) and soluble TP (b). In (a), the efficiency of APG = $(STOC - STOC_0)/APG$, where the $STOC_0$ is STOC with APG = 0 mg/L. In (b), the efficiency of APG = $(soluble\ TP - soluble\ TP_0)/APG$, where the $soluble\ TP_0$ is soluble TP with APG = 0 g/L

In Figure 4a, when APG < 18 g/L, the higher the APG concentration, the more organics dissolved from the sludge. However, when APG concentration is over 18 g/L, STOC inversely decreases as APG increases. This could be explained as follows: When the concentration of a surfactant is above its CMC, micelles transform from spherical to rod-like. The rod-like micelles would even become associated if continuously adding the surfactant (Rakshit, 2008). In other words, a concentration above the CMC induces the formation of bigger micelles. When the micelles are large enough to be blocked by a microporous membrane, the organics in these micelles may not appear in the dissolved form. In this solution system, 18 g/L was the critical concentration, at which there were micelles blocked by a 0.45 μ m microporous membrane.

In *Figure 4b*, soluble TP increases with increasing APG dosage, which indicates that more APG led to more cell disintegration. Phosphorus was not contained in micelles, so big micelles blocked by the microporous membrane did not affect it; therefore, there was no discontinuity insoluble TP.

Similar to other surfactants, some APG molecules formed micelles when the concentration was above its CMC. Thus, the quantity of effective APG molecules did not have a linear increase with its concentration, resulting in the decreasing efficiency of APG in both *Figure 4a* and *b*.

Anaerobic digestion

Gas production

The biogas production from AD is shown in *Figure 5* as a function of fermentation time (on the x-axis) and cumulative gas volume (on they-axis). Comparing the blank with T0 shows that after the low-temperature thermo-alkaline pretreatment without APG, there was higher biogas production efficiency of T0, which is consistent with the literature (Li et al., 2016). However, pretreatment with different dosages of APG had a significant effect on the gas production.

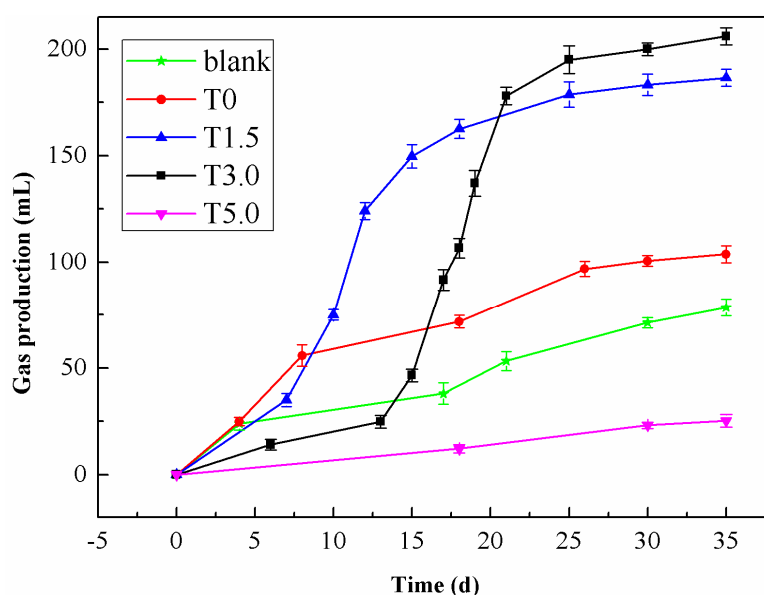


Figure 5. Total volume of biogas in AD

Comparing T0 with T1.5, it was found that adding APG to the pretreatment had a greater impact on AD than adding solely alkali. Moreover, the gas production of T1.5 increased rapidly about 7 days later, which means that the microorganisms in T1.5 had an adaptive phase of 7 days.

In comparison with T1.5, it was observed that the gas production rate of T3.0 started rising rapidly on the 13th day. The longer adaptive phase defines the inhibiting effect of APG on the microorganisms in the anaerobic system. When the APG concentration is less than the CMC, the adaptive phase increases with increasing APG dosage.

Assuming that there was no wastage of APG during the process of pretreatment, APG concentration in the fermentation broth was 0.9 g/L for T1.5, 1.8 g/L for T3.0, and

3.0 g/L for T5.0 (APG was diluted 5/3 times by inocula). It is shown in *Figure 5* that T5.0 produced a small amount of biogas, which suggests that the aerogen can hardly adapt to the APG concentration at CMC (the CMC of the APG sludge solution was approximately 3.0 g/L, section 2.1.3). Considering the result of preliminary experiments indicating that there were no bubbles in the fermentation liquor when pretreating at $\text{APG} > 5.0$ g/L, it is the APG micelles that have a negative impact of activity on the aerogen. However, in the study by Xia and Onyukse (2000), they found that it was the surfactant monomer, not the micelle, that went through the cell membrane to damage the cell. Therefore, as they stated, “micelles may act as a depot to continuously replace aqueous surfactant monomers taken up by the membrane.”

Property analysis of fermentation liquor and biogas

The compositions of the liquor and biogas during fermentation are shown in *Figure 6*. The sludge solutions of T0, T1.5, T3.0, and T5.0 pretreated by the low-temperature thermo-alkaline method (initial pH = 11) were mixed with inocula. All pH was between 8.8 and 8.9, as shown in *Figure 6a*, and all pH values were significantly reduced after 35 days. The AD of the organic material basically follows hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Appels et al., 2008). It could be deduced that the main cause of the pH reduction was acidogenesis and acetogenesis.

It is known that methanogens are extremely sensitive to pH, with an optimum between 6.5 and 7.2 (Appels et al., 2008), and the optimal pH of hydrolysis and acidogenesis is between 5.5 and 6.5 (Ward et al., 2008). As shown in *Figure 6a*, the pH values of the blank, T0, T1.5, and T3.0 after AD were between 6.5 and 7.2, which may indicate that the maximal activity strain in the fermentation reactor was a methanogen. However, *Figure 6b* also shows that it is only in T1.5 and T3.0 that there was a relatively large CH_4 volume fraction. Only 1.66 vol% methane in the blank was produced, which is because the poor efficiency of hydrolysis made acidogenesis difficult (the pH changed just from 6.97 to 6.80), so of course acetogenesis and methanogenesis were limited. In T0, the pretreatment facilitated hydrolysis, so acidogenesis and acetogenesis made the pH drop sharply from 8.80 to 7.13. The low methane yield may be caused by the limitation of low organic substrate concentration, since the ratio of VS to TS of sludge used in this study was just $51.32 \pm 0.10\%$ (shown in section 2.1.1). Adding APG to the pretreatments more effectively promoted the dissolution of organics in the sludge, and APG could even act as an organic substrate, thus leading to the relatively large amount of CH_4 in T1.5 and T3.0, compared to that in T0.

Meanwhile, the pH of T5.0 was at the optimum for hydrolysis and acidogenesis (5.5–6.5, *Fig. 6a*), and there was no methane detected (*Fig. 6b*), both of which suggest that the activity of the methanogens in the T5.0 anaerobic system was extremely low. This conclusion could be also deduced by its STOC reduction rate (*Fig. 6c*).

The STOC reduction rate is unequal to the carbon utilization rate of methanogens, because hydrolysis, acidogenesis, acetogenesis, and methanogenesis simultaneously occur in a mixed anaerobic system. That is to say, when soluble carbon was being converted into CH_4 , and CO_2 volatilizing from the liquor, the carbon in the suspended solids was being dissolved into the water by hydrolysis and acidogenesis. However, it is only soluble carbon that methanogens can utilize, so the STOC reduction rate is, to some extent, equal to the activity of methanogens. *Figure 6c* shows that T5.0 had a STOC reduction rate far below that of the other tests, showing its low methanogen

activity. Taking the conclusion of section 3.3.1 into consideration, APGs have more of a negative impact of activity on methanogens than on other strains in the AD system. The literature (Luo et al., 2015) reports that there was far more Firmicutes in WAS anaerobic reactors with APG than without APG (42.6% vs. 9.8%), from which it could be deduced that a firm cell wall can help to protect the bacteria from the toxic effect of APG. Methanogens belong to Archaea, whose cell wall is comparatively weak, so APG monomers easily go through cell membranes and cause them damage.

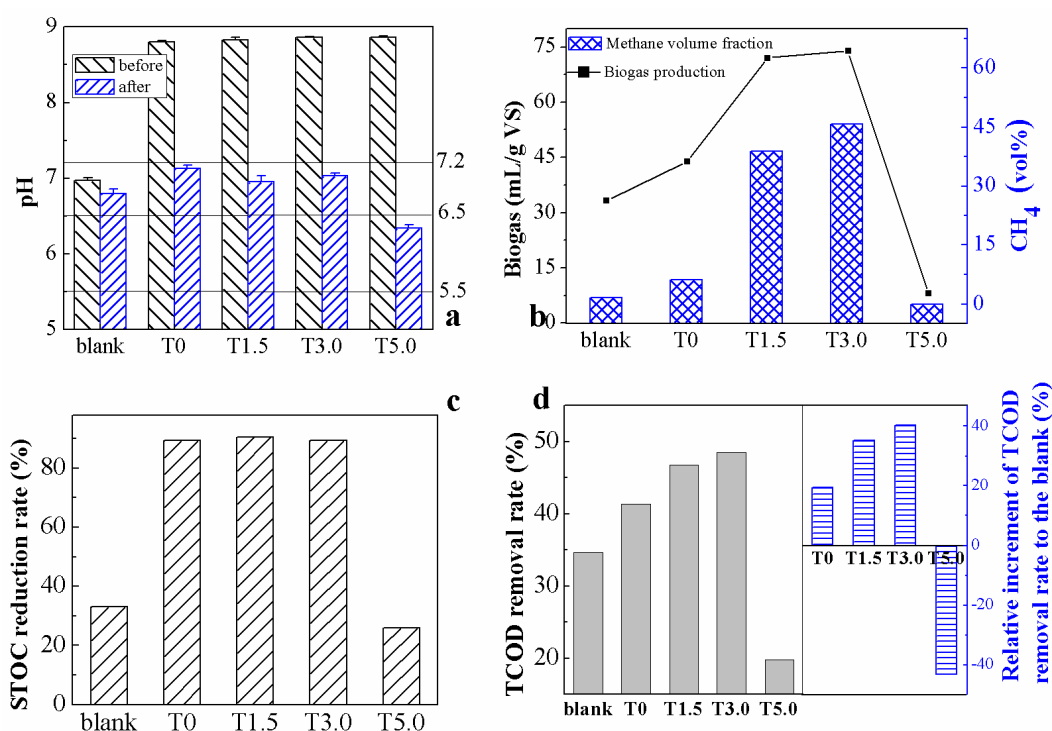


Figure 6. Property analysis of fermentation liquor and biogas. (a) The change of pH before and after AD. (b) Biogas yield and volume fraction of CH₄. In (c), STOC reduction rate = $(STOC_b - STOC_a)/STOC_b$, where $STOC_b$ is soluble total organic carbon in the fermentation broth before fermenting, and $STOC_a$ is after fermenting. In (d), TCOD removal rate = $(TCOD_b - TCOD_a)/TCOD_b$, where $TCOD_b$ is total chemical oxygen demand in the fermentation broth before fermenting, and $TCOD_a$ is after fermenting. The relative increase of the TCOD removal rate, compared to the blank = $TCOD$ removal rate of $(T\alpha - \text{blank})/\text{blank} \times 100\%$, which quantifies the comparison between TCOD removed by AD with pretreatment (T0, T1.5, T3.0, T5.0) and without pretreatment (blank)

The rate of TCOD removal can be considered to represent the organics utilization rate in the AD system. It is observed from Figure 6d that the APG dosage increased the organics utilization rate when the APG concentration was under the CMC. However, when the APG was plentiful enough to generate abundant micelles (APG concentration $\geq$ CMC), the micelles sharply lowered the activity of the methanogens to inhibit methanogenesis, so the organics utilization rate of T5.0 decreased obviously. This is why Zhao et al. (2015b) and Luo et al. (2015) added APG to anaerobic fermentation systems to inhibit methanogenesis in order to accumulate short-chain fatty acids (SCFAs).

The relative increase of the TCOD removal rate compared to the blank (*Fig. 6d*) suggests that pretreatment does improve the efficiency of AD. The relative increase of T0 was 19.37%, and that of T3.0 was 40.30%. It was easy to calculate that 40.30 is 108.07% greater than 19.37, significantly demonstrating that 3.0 g/L is a reasonable selection for the optimal APG dosage. This is due to the fact that, during pretreatment, APG at the CMC promotes dissolution of EPSs, which accelerates hydrolysis. Further, when fermenting, APG is diluted to below CMC, so its toxicity to methanogens is reduced.

In addition, the methane volume fraction of T3.0 was 45.84% (*Fig. 6b*), which is lower than normal biogas (CH₄ volume fraction of digestion biogas was 53–70%; Appels et al., 2008). In comparison with the blank (1.66%) and T0 (6.17%), T3.0 nearly realized sludge recycling.

Although there was an adaption phase in the AD system because of APG, this disadvantage could be overcome by additional high-efficiency gas production studies in a continuous fermentation system (not in a sequencing batch reactor, as in the present experiment). Further, although the methane content was low because of the lack of organic substrate, other degradable organic waste, such as food waste, could be used to increase the organic substrate concentration. However, these suppositions need to be confirmed by more experiments.

Conclusion

A low-temperature thermo-alkaline pretreatment with APG for WAS with low organic content could greatly improve the sludge utilization in AD systems. More APG led to more dissolution of organic matter during the pretreatment, but in fermentation, APG above the CMC severely inhibited methanogenesis. As a result, 3.0 g/L was chosen as the optimal APG dosage. Under optimum conditions, pretreated sludge was fermented anaerobically at mesophilic temperatures to obtain the best biogas production, CH₄ volume fraction, and organics utilization rate. Although there was an adaptive phase of 13 days and the methane content was low, additional experiments could be conducted to find solutions that overcome these disadvantages.

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