Effects of Thermal-Alkaline Pretreatment on Solubilisation and High-Solid Anaerobic Digestion of Dewatered Activated Sludge

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The effects of thermal-alkaline pretreatment on dewatered activated sludge (DAS) solubilisation and subsequent high-solid anaerobic digestion were studied by response surface methodology (RSM) from 105 to 135 °C and between 5 and 35 mg alkaline/g total solid (TS) DAS. Soluble chemical oxygen demand (SCOD), soluble carbohydrates, and protein concentrations were significantly enhanced in thermal-alkaline pretreated DAS samples. Daily methane yield increased at the middle of digestion, and cumulative methane yield (CMY) significantly increased after thermal-alkaline pretreatment. A first-order linear model of temperature and alkaline was significant for SCOD by RSM, and the determination coefficient (R^2) was 94.62%. The quadratic model of temperature and alkaline was also significant for methane yield. R^2 of 99.80% confirmed that the model used in this study fit the experimental variables very well. Using the model, the optimum pretreatment condition of methane yield was obtained at 134.95 °C and 23.77 mg alkali. Therefore, RSM was an effective tool in predicting the DAS pretreatment condition for optimum methane yield.

Keywords: High-solid anaerobic digestion; Thermal-alkaline pretreatment; Dewatered activated sludge; Methane yield

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INTRODUCTION

In China, approximately 30 million tons of sewage sludge (80% moisture content) are generated every year, and nearly 80% of this sludge is not appropriately stabilised (Duan *et al.* 2012), resulting in large societal and environmental burdens. Anaerobic digestion converts some organic matter in the sludge into biogas, which is an effective biological stabilisation technology (Francioso *et al.* 2010; Meester *et al.* 2012; Dai *et al.* 2014; Fahad *et al.* 2014; Zhao *et al.* 2014). Traditional anaerobic digestion is carried out at a low-solid state (TS < 15%). However, more than 80% of the sludge is dewatered before further disposal or treatment (Dai *et al.* 2014), and the total solid (TS) in sludge is usually higher than 15%. Hence, high-solid anaerobic digestion (TS ≥ 15%) (Guendouz *et al.* 2008) is a potential solution to the sludge disposal problem, as it avoids secondary sludge dehydration in low-solid anaerobic digestion. In addition, high-solid anaerobic digestion is advantageous because of its smaller reactor volume, lower energy input for heating, and less material handling (Domenec 2009; Duan *et al.* 2012; Fernández Rodríguez *et al.* 2012; Zhang *et al.* 2015).

Recently, high-solid anaerobic digestion of sludge has been investigated and found to be feasible (Duan *et al.* 2012; Zhang *et al.* 2015). However, high-solid anaerobic digestion decreases methane yield per g volatile solid (VS). Hydrolysis is a rate-limiting step in the anaerobic digestion of sludge (Batstone *et al.* 2009). Some combinations of pretreatment methods, including thermal and alkaline, alkaline and ultrasonic, and alkaline and microwave, enhance sludge solubilisation. Alkaline pretreatment is often combined with thermal pretreatment for WAS solubilisation, as the combined pretreatment reduces the required quantity of alkaline and energy (Shehu *et al.* 2012). The synergistic effects of thermal-alkaline pretreatment have been reported on WAS solubilisation (Kim *et al.* 2003; Valo *et al.* 2004; Shehu *et al.* 2012; Kim and Lee 2013; Xu *et al.* 2014). Shehu *et al.* (2012) discovered that the thermal-alkaline pretreatment (88.50 °C; 2.29 M NaOH) enhances sludge solubilisation and increases biogas yield by about 36% v/v. Thermal-alkaline pretreatment (90 °C; 0.2 M NaOH) improves sludge solubilisation to 75.6%, which is 48.3% greater than the 90 °C pretreatment alone (Kim *et al.* 2013). However, these studies have focused on low-solids pretreatment.

Response surface methodology (RSM) employs statistical methods to study the combined effects of thermal and alkaline treatments (Kim and Lee 2013). The synergistic effects of combined pretreatments on sludge solubilisation and methane yield have been investigated by RSM, using variables including TS, pH, and reaction time and the thermal, alkaline, and microwave methods (Kim and Lee 2013; Yang *et al.* 2013; Cho *et al.* 2014). However, the effectiveness of combined thermal-alkaline pretreatment on sludge solubilisation and subsequent anaerobic digestion at high-solid state has not been evaluated. This study examined the synergetic effects of thermal and alkaline on WAS solubilisation and subsequent anaerobic digestion at high-solid state using RSM. The concentrations of COD, carbohydrates, and proteins were monitored before and after pretreatment to determine its effects on DAS solubilisation. To better understand the effect of pretreatment on biogas production, daily methane yield was monitored during anaerobic digestion. COD, carbohydrates, and proteins were also measured in pretreated DAS samples after anaerobic digestion.

EXPERIMENTAL

Raw Materials

Dewatered activated sludge was obtained from the Xianyanglu Wastewater Treatment Plant (Tianjin, China) and stored at 4 °C before use. The inoculum (mesophilic seed sludge) came from an anaerobic reactor and was centrifuged before inoculation. Characteristics of the DAS and inoculum are listed in Table 1.

Methods

Pretreatment conditions designed by RSM

Response surface methodology is a statistical approach used to simultaneously optimise independent variables that are correlated with each other. In this study, the central composite design with RSM investigated the interaction of independent variables, such as temperature and alkaline dose, on DAS solubilisation and methane yield. The detailed experimental runs are shown in Table 2. As previously determined (Zhang *et al.* 2015), the optimal alkaline (NaOH) dose is 20 mg•g^{-1} TS with alkaline treatment alone, and the best temperature is 120 °C in thermal pretreatment alone. In the present study, the alkaline dose was set to 5, 20, and 35 mg•g^{-1} TS and the temperature was set to 105, 120, and 135 °C.

Table 1. Characteristics of the Dewatered Activated Sludge and Inoculum in the Thermal-Alkaline Pretreatment

Parameters	DAS	Inoculum
pH	7.45 ± 0.05	8.15 ± 0.03
TS (%)	18.94 ± 0.21	15.23 ± 0.19
VS/TS (%)	56.94 ± 0.18	65.34 ± 0.20
COD (g/kg)	148.95 ± 6.38	154.59 ± 5.19
SCOD (g/kg)	3.01 ± 0.32	24.57 ± 0.49
Total carbohydrates (g/kg)	13.14 ± 0.48	20.42 ± 0.52
Total proteins (g/kg)	51.67 ± 1.25	57.51 ± 1.09

Note: g/kg, g/kg wet base

Table 2. Response Surface Analysis Experimental Design Matrix and Data

Run no.	Coded variables		Experimental Variables		Observations	
	X_1	X_2	X_1 (°C)	X_2 (mg/g TS)	SCOD (g/kg)	CMY (mL/g VS)
1	1	-1	135	5	24.57 ± 0.46b	104.16 ± 5.50de
2	-1	1	105	35	19.21 ± 0.31ef	89.31 ± 5.38g
3	-1	0	105	20	18.59 ± 0.16e	95.45 ± 4.69f
4	1	1	135	35	25.32 ± 0.48a	113.58 ± 2.90b
5	0	0	120	20	20.92 ± 0.23cd	106.63 ± 1.28cd
6	0	-1	120	5	20.41 ± 0.14d	95.36 ± 3.86f
7	0	0	120	20	21.23 ± 0.44c	108.38 ± 1.45c
8	1	0	135	20	24.94 ± 0.21b	118.32 ± 4.36a
9	-1	-1	105	5	17.70 ± 0.20f	85.08 ± 4.05h
10	0	1	120	35	20.55 ± 0.27d	102.40 ± 2.21e
Control	-	-	-	-	3.01 ± 0.32g	73.52 ± 1.20i

Note: The letters (a, b, c……) behind the data represent the significant difference ($p<0.05$) among different groups.

The experiment was first implemented in a beaker with a working volume of 1.0 L, and the DAS was mixed evenly with alkaline substance. The mixed samples were placed into thermal reactors with a working volume of 0.5 L and incubated for 1 h in an autoclave. Finally, the treated samples were chilled (4 °C) for 23 h prior to being neutralised to an initial pH of 7.82 with 6 M HCl. An untreated sample was used as a reference.

Batch experiments of anaerobic digestion

A 300-mL pressure bottle was used as the digestion reactor. There were three parallel reactors for each treatment, and each reactor was fed with 75 g of DAS and 25 g of inoculum. After removing oxygen in the reactors with nitrogen gas for 5 min, the reactors were sealed with butyl rubber stoppers. The time of mesophilic anaerobic digestion was 30 days at 37 ± 1 °C. Methane production was measured every day before day 14 and every two days after day 14, and the volume of biogas produced was based on a pressure measurement. The TS of the digestion substrate was 17.10 ± 0.32%.

Analytical method

DAS was heated at 105 °C for 24 h to obtain TS data and then to 550 °C for 4 h to procure VS data. The soluble fractions of DAS were obtained by centrifuging the DAS at 10000 rpm for 10 min at 4 °C. The supernatant was filtered through a microfiber membrane with a pore size of 0.45 µm. SCOD, soluble carbohydrates, and proteins were analysed by the dichromate reflux method (CODcr), Anthrone method, and Coomassie brilliant blue method, respectively (APHA 1998). The biogas composition was examined using a Thermal Trace-1300 gas chromatograph (Thermo, USA) equipped with a molecular sieve column (length 2 m, diameter 2 mm) and a thermal conductivity detector. The pressure in reactors was measured by pressure gauges (GMH 3111, Greisinger, Germany).

Statistical significance was determined by analysis of variance (ANOVA, $p < 0.05$) using SPASS 22.0. Multiple comparisons were performed with Tukey’s test ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of Thermal-Alkaline Pretreatment on DAS Solubilisation

COD solubilisation in DAS

Thermal-alkaline pretreatment resulted in the disruption of chemical bonds in macromolecular matter. Subsequently, Extracellular Polymeric Substances (EPS) and intracellular organic matter in the sludge were degraded and released, resulting in COD solubilisation (Appels *et al.* 2010; Yan *et al.* 2013). The effect of thermal-alkaline pretreatment on DAS solubilisation is summarised in Table 3. With the same alkaline dose, the SCOD concentration was significantly increased with increasing thermal pretreatment temperature. Under the same temperature conditions there was a little variation in the SCOD concentration with increasing alkaline dose. When the temperature was 105 or 120 °C, the SCOD concentration first increased, then decreased, with increasing alkaline dose, resulting in the highest SCOD concentration with 20 mg alkaline. With the 135 °C pretreatment, the SCOD concentration increased with more severe alkaline pretreatments. The highest SCOD concentration of 25.32 mg/kg was obtained with the 135 °C/35 mg alkaline pretreatment. These results suggest that thermal pretreatment combined with alkaline pretreatment improved the hydrolysis rate and increased the SCOD concentration. While the greater amount of SCOD may not be completely disintegrated during anaerobic digestion, the increased amount of available substrates results in greater sludge disintegration (Wang *et al.* 2005). Thus, the thermal-alkaline pretreatment enhanced DAS disintegration. However, thermal pretreatment was more effective than alkaline pretreatment in this effect. The correlation coefficient of temperature and SCOD concentration was 0.9687, while that of alkaline dose and SCOD concentration was only 0.0892 (data not shown), probably because the alkaline pretreatment was non linear response. When 35 mg alkaline/g TS DAS was added, the pH of DAS was lower than 9.80. When the pH is 11.0, hydrolysis is more efficient (Vlyssides and Karlis 2004; Uma Rani *et al.* 2012; Cho *et al.* 2014). A combined thermal and alkaline pretreatment (90 °C; 0.2 M NaOH) significantly enhances the disintegration of sludge solids (up to 75.6%) compared with thermal pretreatment alone (Kim *et al.* (2013). Increasing the alkaline dose is necessary for DAS solubilisation, but a higher alkaline dose inhibits methanogenic activity in the subsequent anaerobic digestion (Rinzema *et al.* 1988; Chen *et al.* 2008; Fang *et al.* 2014; Zhang *et al.* 2015).

Table 3. Concentration and Solubilisation of COD in DAS after Thermal-Alkaline Pretreatment

Treatments	COD (g/kg)	SCOD (g/kg)	Solubilisation (%)
$T_{(5\ mg, 105\ °C)}$	158.27 ± 6.32	17.70 ± 0.20 f	9.28 ± 0.49 d
$T_{(5\ mg, 120\ °C)}$	154.91 ± 3.46	20.41 ± 0.14 d	11.23 ± 0.19 bc
$T_{(5\ mg, 135\ °C)}$	148.43 ± 2.96	24.57 ± 0.46 b	14.52 ± 0.18 a
$T_{(20\ mg,\ 105\ °C)}$	151.82 ± 3.15	18.59 ± 0.16 e	10.26 ± 0.17 cd
$T_{(20\ mg,\ 120\ °C)}$	153.57 ± 2.76	20.92 ± 0.23 cd	11.66 ± 0.37 b
$T_{(20\ mg,\ 120\ °C)}$	151.82 ± 3.28	21.23 ± 0.44 c	12.00 ± 0.16 b
$T_{(20\ mg,\ 135\ °C)}$	151.10 ± 0.84	24.94 ± 0.21 b	14.51 ± 0.12 a
$T_{(35\ mg, 105\ °C)}$	147.31 ± 6.33	19.21 ± 0.31 ef	11.00 ± 0.74 bc
$T_{(35\ mg,\ 120\ °C)}$	150.10 ± 0.36	20.55 ± 0.27 d	11.69 ± 0.24 b
$T_{(35\ mg, 135\ °C)}$	153.18 ± 5.19	25.32 ± 0.48 a	14.56 ± 0.19 a
Control	156.98 ± 2.79	3.01 ± 0.32 g	0 e

Note: The letters (a, b, c……) behind the data represent the significant difference ($p<0.05$) among different groups.

Soluble proteins and carbohydrates

Proteins and carbohydrates are the main constituents of sludge, and they are converted to soluble forms during sludge pre-treatment (Appels *et al.* 2010; Yan *et al.* 2013). Because thermal-alkaline pretreatment was an effective method for sludge solubilisation, the effects of thermal-alkaline pretreatment on the soluble carbohydrates and proteins were monitored as well (Fig. 1).

The soluble carbohydrates were significantly increased after thermal-alkaline pretreatment (Fig. 1a). With the same alkaline dose, the concentration of soluble carbohydrates gradually increased with increasing pretreatment temperature. With the 105 °C condition, the soluble carbohydrates did not significantly increase with increasing alkaline dose; the 120 °C condition produced similar results.

For treatment at 135 °C, the soluble carbohydrate concentration significantly increased with increasing alkaline dose. The lowest soluble carbohydrates concentration (864.88 mg/kg) occurred after the 105 °C/5 mg alkaline pretreatment. The soluble carbohydrates concentration was the highest (2137.07 mg/kg) after the 135 °C/35 mg alkaline pretreatment.

These two conditions produced soluble carbohydrate concentrations that were 7.21- and 18.19-fold higher, respectively, than the control. Thus, higher temperature and higher alkaline dose conditions were favorable for the solubilisation of DAS carbohydrates.

The soluble protein concentration significantly increased after thermal-alkaline pretreatment (Fig. 1b). With unvarying alkaline dose, soluble proteins gradually increased with increasing pretreatment temperature. In the 105 °C condition, soluble protein concentration significantly increased when the alkaline dose increased from 5 to 20 mg alkaline/g TS DAS, but there was little increase when the alkaline dose rose from 20 to 35 mg alkaline/g TS DAS. With the 120 °C pretreatment, the results were similar. In the 135 °C condition, soluble proteins significantly increased with increasing alkaline dose.

The soluble protein concentration was the lowest (188.29 mg/kg) after the 105 °C/5 mg alkaline pretreatment and highest (452.65 mg/kg) after the 135 °C/35 mg alkaline pretreatment. These two conditions produced soluble proteins concentrations that were 6.65- and 15.98-fold higher, respectively, than the control.

These results demonstrate that trends in soluble protein concentration were similar to those of soluble carbohydrates. Thus, the pretreatment conditions employed in this study improved the solubilisation of both carbohydrates and proteins in DAS, which was similar to a previous study (Xu *et al.* 2014). A possible explanation for these results is EPS solubilisation and cell lysis at high temperature (Appels *et al.* 2010).

High-solid Anaerobic Digestion after Thermal-Alkaline Pretreatment

The batch high-solid anaerobic digestion was conducted for 30 days, and daily methane yield curves and CMY curves were calculated. There were significant differences ($p < 0.05$) in daily methane yield and CMY between treatments.

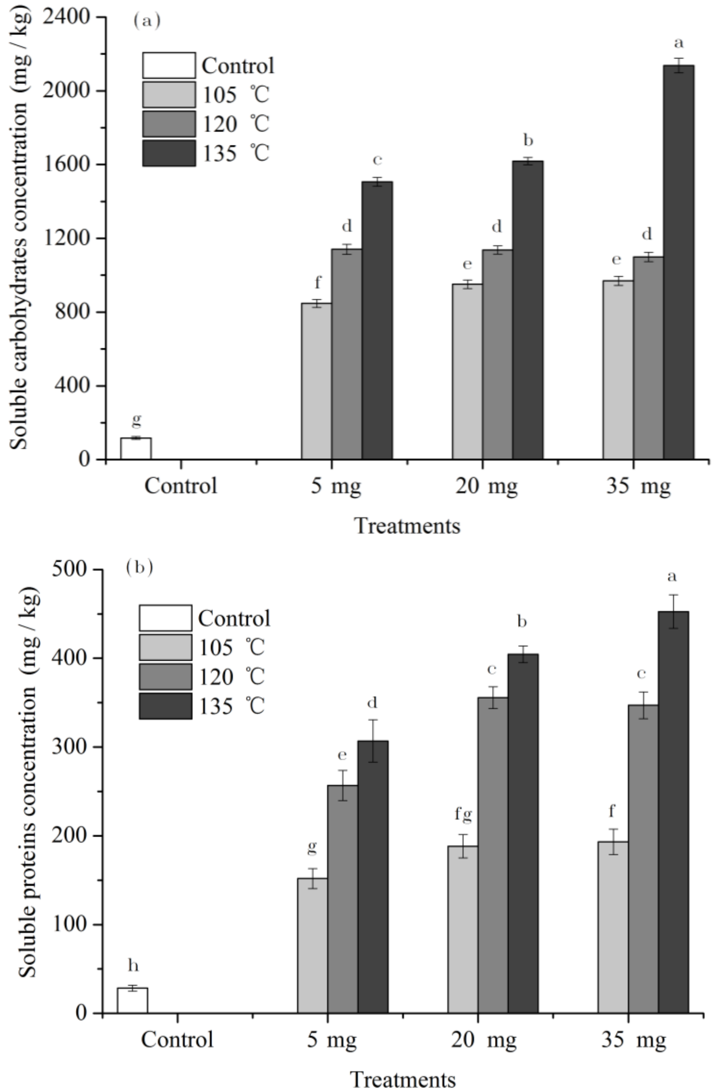

Fig. 1. Concentrations of the soluble carbohydrates (a) and proteins (b) in the DAS after thermal-alkaline pretreatment. Bars indicate the standard deviation (SD). Note: The letters (a, b, c……) behind the data represent the significant difference (p<0.05) among different groups.

Daily methane yield

Thermal-alkaline pretreatment resulted in an improved daily methane yield (Fig. 2), as there were more soluble carbohydrates and proteins readily available as substrates for methanogenic bacteria (Fig. 1). There was only one remarkable peak in daily methane yield during the high-solid anaerobic digestion of the control sample. The peak value appeared at day 4 (6.86 mL/(g VS•d)), after which the daily methane yield decreased. For the thermal-alkaline pretreatment, there were several remarkable peaks, and the first peak appeared at days 4 through 6. Afterwards, daily methane yield fluctuated greatly for many days. This result differed from thermal pretreatment alone (Zhang *et al.* 2015), which may be related to the addition of alkaline. Under the 105 °C condition (Fig. 2a), there were two peaks for the 5 mg alkaline pretreatment and three peaks for the 20 and 35 mg alkaline pretreatments. For the 35 mg/105 °C pretreatment, the first peak was at 8.01 mL/(g VS•d), which was the highest of the four treatments.

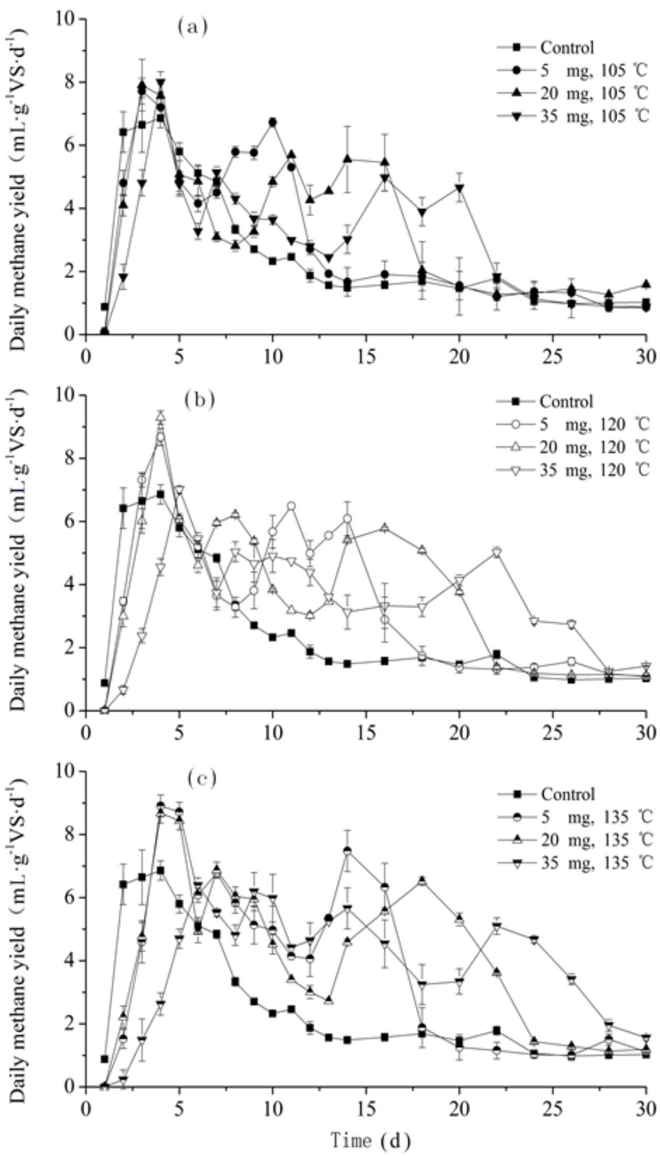

Fig. 2. Daily methane yield during high-solid anaerobic digestion after thermal-alkaline pretreatment

Daily methane yield was lower for the 35 mg/105 °C pretreatment than the other treatments during the first two days of digestion. This result may reflect the alkaline addition, which caused a longer lag period for digestion. The lag period depends on the acclimation of microorganisms to their proper substrates and environmental conditions (Lay *et al.* 1997), and therefore it is possible that microorganisms were affected by highly alkaline conditions. For the 5 mg/105 °C, 20 mg/105 °C, and 35 mg/105 °C pretreatments, daily methane yield dropped to 2 mL/(gVS•d) at days 14, 20, and 24, respectively. However, daily methane yield from the 35 mg/105 °C pretreatment was lower than the 20 mg/105 °C pretreatment for most of the time before day 16. Thus, the addition of alkali delayed methane production, and 35 mg alkaline was too much to improve daily methane yield. With the 120 °C and 135 °C conditions (Fig. 2b, Fig. 2c), similar results were observed. For the 5 mg/120 °C, 20 mg/120 °C, and 35 mg/120 °C pretreatments, daily methane yield dropped to 2 mL/(g VS•d) at days 16, 22 and 28, respectively. In the 5 mg/135 °C, 20 mg/135 °C, and 35 mg/135 °C pretreatments, daily methane yield dropped to 2 mL/(g VS•d) at days 18, 24, and 30, respectively. These results demonstrated that the time of maintaining high daily methane yield gradually increased with increasing temperature and alkaline dose. Thus, thermal-alkaline pretreatment was effective in improving daily methane yield. Under the 35 mg alkaline condition, the daily methane yield was lower during digestion than the other treatments because the high alkaline dose potentially inhibited methanogenic activity (Rinzema *et al.* 1988). Daily methane yield improved for the 35 alkaline pretreatment during later timepoints, which may reflect enhanced Na^+ resistance in methanogenic bacteria during an extended digestion time.

Cumulative methane yield (CMY)

Cumulative methane yield was calculated by summing daily methane yields. The thermal-alkaline pretreatment resulted in an improved CMY (Fig. 3). In the 105 °C condition (Fig. 3a) with 5 mg, 20 mg, and 35 mg alkaline pretreatment, CMY increased by 15.72%, 29.83%, and 21.47%, respectively, compared with the control. On the other hand, under the 120 °C condition (Fig. 3b), for 5 mg, 20 mg, and 35 mg alkaline pretreatments, CMY increased by 29.70%, 46.23%, and 39.27%, respectively. With the 135 °C/5 mg, 135 °C/20 mg, and 135 °C/35 mg pretreatments (Fig. 3c), CMY increased by 41.67%, 60.93%, and 54.50%, respectively. The maximum methane yield was 118.32 ml/g VS DAS. It has been reported that at 0.05 mol/L alkalinity with high pressure homogenization (HPH) pretreatment, the methane yield reached into 247 ml/g VS and improved 107% relative to HPH pretreatment alone (Fang *et al.* 2014). Thermo-alkaline pretreatment of sewage sludge at optimized condition could increase the biogas yield by about 36% (Shehu *et al.* 2012). At the same temperature, CMY in 20 mg alkaline pretreated samples was the highest; methanogenic activity was promoted by Na^+ 20 mg alkaline/g TS DAS but inhibited by 35 mg alkaline/g TS DAS (Zhang *et al*. 2015). At 5 g/L, Na^+ inhibits 10% of the maximum specific aceticlastic methanogenic activity in granular sludge (Rinzema *et al.* 1988). For 35 mg alkaline pretreatment in this study, the Na^+ concentration was 4.60 g/L in the high-solid anaerobic digestion system, which caused a slight inhibition. At the same alkaline dose, CMY gradually increased with the more severe thermal pretreatments, as the increased amount of soluble carbohydrates and proteins were readily available to bacteria for methane production (Fig. 1; Yan *et al.* 2013). The CMY of thermal-alkaline pretreated samples exceeded that of the control after day 8, which was consistent with the subsequent appearance of peaks in daily methane yield. These results indicated that the thermal-alkaline pretreatment dramatically enhanced methane production at the middle of digestion, probably because the pretreatment improved DAS biodegradability. In sum, the thermal-alkaline pretreatment increased CMY during high-solid digestion. However, alkaline addition must be limited to a certain range so that Na^+ does not inhibit methanogenic activity (Zhang *et al.* 2015).

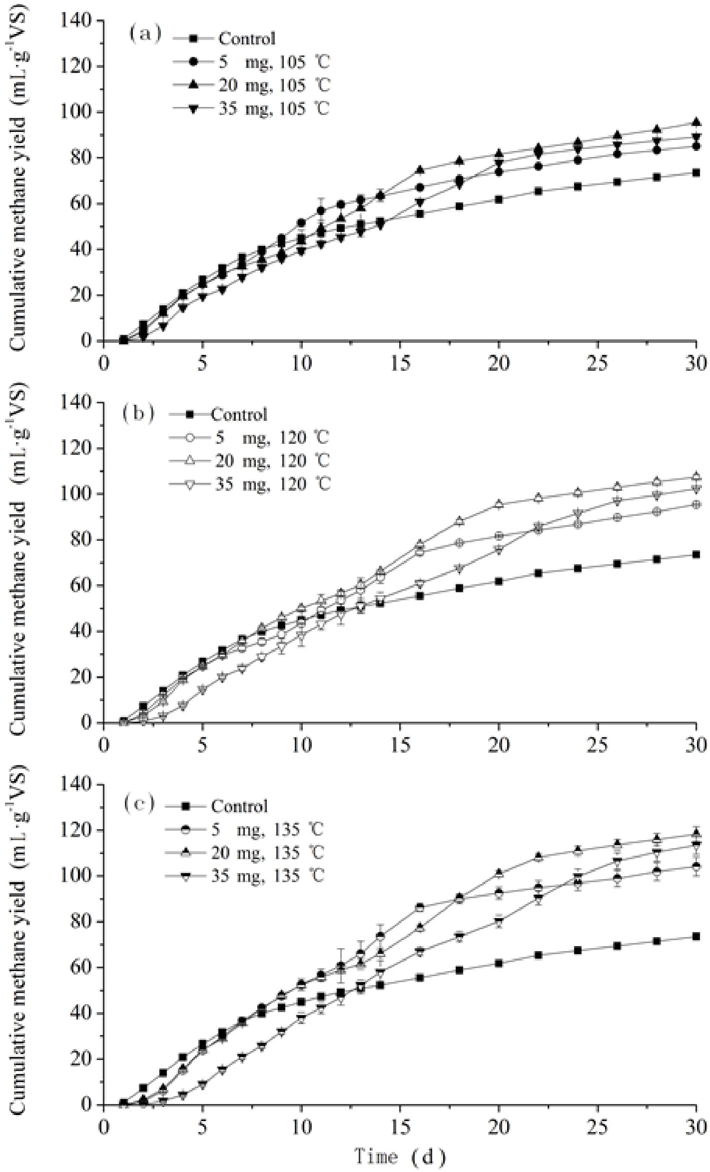

Fig. 3. Cumulative methane yield during high-solid anaerobic digestion after thermal-alkaline pretreatment

RSM Analysis of SCOD and Methane Yield

Analysis of variance for SCOD

A first-order linear equation was used to fit the experimental data (Table 2) suggesting the best-fit model describing the SCOD response surface, as shown below,

$$Y_{SCOD} = -4.77 + 0.21X_1 + 0.02X_2 \quad (1)$$

where Y_{SCOD}, X_1, and X_2 were SCOD concentration (g/kg), temperature (°C), and alkaline dose (mg/g TS DAS), respectively.

An analysis of variance (ANOVA) was used to determine the adequacy and significance of the SCOD predictive model (Table 4). The model *F*-value of 61.61 implied that the model was significant. There was only a less 0.01% chance that a "Model *F*-value" this large could occur due to noise. Value of "P" less than 0.05 indicated model terms were significant. This implied a very significant effect on the process of thermal-alkaline degradation. "P-value" of the linear term of temperature (X_1) was lower than 0.0001, indicating it had the most significant influence on the model. The first-order alkaline term (X_2) was less significant at the 5% level ("P" = 0.3426). These results mean effects of temperature on SCOD was more significant than that of alkaline concentration. These were different with the previous study (Kim *et al.* 2013; Uma Rani *et al.* 2012), because of high temperature and low alkaline dose. The "Lack of Fit", having an *F*-value of 1.65 and P-value of 0.5347, showed that the Lack of Fit was not significant relative to the pure error, and this implied that the model had a good fit for prediction. The determination coefficient (R^2) of the model was 0.9462, demonstrating that the model was appropriate. Adequate precision (AP) is a measure of the range of estimated responses relative to the average estimation error, *i.e.*, a signal-to-noise ratio (Kim *et al.* 2013). A high AP value demonstrates the adequacy of the model, and the recommended AP value is greater than 4. In this study, the AP was 17.995.

Table 4. ANOVA Results of SCOD for a Linear Response Surface Model

Source	Coefficient	F -value	P-value
Model		61.61	< 0.0001
Intercept	-4.77		
X_1 (Temperature)	0.21	122.18	< 0.0001
X_2 (Alkaline)	0.02	1.04	0.3426
Lack of fit		1.65	0.5347
R^2=0.9462， AP=17.995			

Analysis of variance for methane yield

By applying regression analysis, methane yield results were fitted to a second-order polynomial equation, as shown below:

$$Y_{MY} = -35.05 + 1.42X_1 + 1.09X_2 + 5.77 \times 10^{-3}X_1X_2 - 3.33 \times 10^{-3}X_1^2 - 3.89 \times 10^{-2}X_2^2 \quad (2)$$

where Y_{MY}, X_1, and X_2 were methane yield (mL/g VS), temperature (°C), and alkaline dose (mg/g TS DAS), respectively.

The methane yield predictive model was examined by ANOVA (Table 5). The model *F*-value of 389.62 with a very low probability (p) value of < 0.0001 implied that the model was significant. This implied that thermal-alkaline pretreatment had a very significant effect on the methane yield. The linear term of temperature (X_1) and the quadratic term of alkaline concentration (X_2^2) showed low P-values of < 0.0001, indicating that the two terms had the most significant influence on the model. The first-order alkaline term (X_2) was significant at the 1% level. The interaction term (X_1X_2) of temperature-alkaline was significant at the 5% level. However, the quadratic term of temperature (X_1^2) in the model was the least significant at the 5% level. These results implied that thermal pretreatment mainly had a linear relation and alkaline pretreatment mainly had a quadratic relation with methane yield. Although X_1^2 was insignificant (P<0.05), it cannot be eliminated to support the hierarchy of the model, because the determination coefficient (R^2 = 0.9980) indicated that this model can explain up to 99.80% variability in the response. The "Lack of Fit" with *F*-value of 0.11 and P-value of 0.9409 indicated the Lack of Fit was not significant relative to the pure error, and the model had a good fit for prediction. The AP of 59.440 indicated that this model was adequate for navigating the design space.

Table 5. ANOVA of Methane Yield for a Quadratic Response Surface Model

Source	Coefficient	F -value	P-value
Model		389.62	< 0.0001
Intercept	-35.05		
X_1 (Temperature)	1.42	1424.49	< 0.0001
X_2 (Alkaline)	1.09	139.06	0.0003
X_1X_2	5.77×10^{-3}	13.13	0.0223
X_1^2	$- 3.33 \times 10^{-3}$	2.56	0.1850
X_2^2	$- 3.89 \times 10^{-2}$	348.59	< 0.0001
Lack of fit		0.11	0.9409
R^2=0.9980， AP=59.44			

Response surface plots for SCOD and methane yield

3D response surfaces and 2D contour plots described the trends of SCOD and methane yield with respect to temperature and alkaline (Fig. 4). Temperature and alkaline dose had significant effects on the SCOD and methane yield. The response surface of SCOD gradually increased with increasing temperature and alkaline dose, as previously reported (Vlyssides and Karlis 2004). A possible explanation is that increases in temperature and alkaline dose result in more solubilisation of DAS for thermal-alkaline pretreatment. The response surface of methane yield gradually increased with increasing temperature. However, methane yield exhibited an initial rise, a mid peak (23.77 mg/g TS DAS), and a final fall as alkaline dose increased from 5 to 35 mg/g TS DAS. The results indicated that there was a maximum value of methane yield for the quadratic model within the range of alkaline and temperature conditions in the study. The same trend regarding solubilisation had been reported (Kim *et al.* 2013; Yang *et al.* 2013). Higher alkaline doses result in higher SCOD (Uma Rani *et al.* 2012, 2014; Fang *et al.* 2014), indicating that alkaline pretreatment is a useful tool for enhancing sludge disintegration. However, higher alkaline did not mean higher methane yield (Zhang *et al.* 2013, 2015). As previously shown, 20 mg/g TS DAS results in the highest methane yield, whereas further increases in alkaline dose decreases methane yield (Zhang *et al.* 2015). Presumably, Na^+ inhibits methanogenic activity at higher alkaline doses (Rinzema *et al.* 1988).

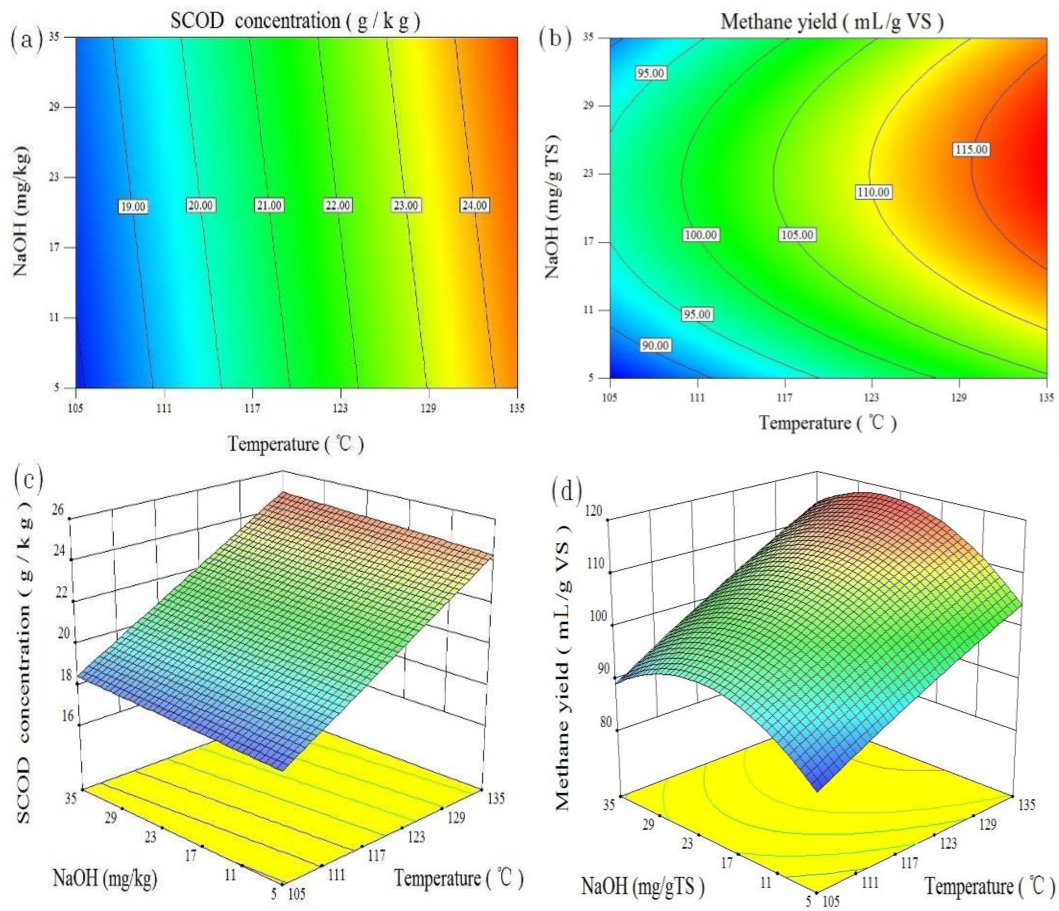

Fig. 4. Contour and 3D response surface plots: interactive effects of temperature and alkaline dose on SCOD concentration and methane yield

Optimum pretreatment condition

The conditions in the study were from 105 to 135 °C and between 5 and 35 mg alkaline/g TS DAS. SCOD and methane yield showed totally different responses to variations in alkaline dose and temperature, and thus their models suggested different optimum pretreatments. The maximum SCOD of 24.91 g/kg was estimated with a pretreatment temperature of 135.00 °C and an alkaline dose of 35.00 mg/g TS DAS; while the maximum methane yield of 118.47 mL/g VS was estimated with a pretreatment of 134.95 °C and 23.77 mg alkaline/g TS DAS within the range of conditions in the study. The objective of thermal-alkaline pretreatment was to improve the solubilisation of DAS, resulting in enhanced methane yield. These results suggested that pretreatments resulting in maximum methane yield were the optimum conditions for DAS digestion. After conducting verification experiments at the optimum pretreatment conditions (134.95 °C and 23.77 mg alkaline/g TS DAS), the actual methane yield was 126.56 mL/g VS, which was 6.83% higher than the predicted value. The difference in values was likely due to the differences in DAS in the high-solid anaerobic digestion.

CONCLUSIONS

1. Thermal-alkaline pretreatment increased the soluble organic matter in DAS, including SCOD, soluble proteins, and carbohydrates.

2. Thermal-alkaline pretreatment also enhanced methane yield from anaerobic digestion. Thus, it effectively improved high-solid anaerobic digestion of DAS.
3. RSM models were significant in verification experiments using the optimum pretreatment; the difference between actual methane yield and predicted methane yield was only 6.83%. These results indicated that RSM is an effective analytical method to investigate relationships between variables.

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