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Two-stage mesophilic anaerobic digestion from waste activated sludge enhanced by low-temperature thermal hydrolysis

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ABSTRACT

Two-stage mesophilic anaerobic digestion of waste activated sludge was conducted to enhance methane production by low-temperature thermal hydrolysis. Two steady stages were evaluated: the first acidogenic stage was operated at sludge retention times of 2 d, and the second methanogenic stage was controlled at hydraulic retention times of 8 d. Thermal hydrolysis results showed that more chemical oxygen demand, proteins and carbohydrates were released from sludge as temperature increased from 50 to 120°C. Protein-like substances were major components from three-dimensional (3D) excitation–emission matrix analysis, and their fluorescence intensities were matched with temperatures. Sludge extracellular structure was disintegrated from scanning electron microscope analysis when the temperature was beyond 80°C. The volatile fatty acids (VFAs) and methane production had positive relation to the soluble organic matters after low-temperature hydrolysis. About 100°C was the suitable temperature for sludge digestion, and corresponding VFAs and biogas productions were 1,672 mg/L and 123 mL/gVSS, respectively.

Keywords: Waste activated sludge; Mesophilic anaerobic digestion; Thermal hydrolysis; Methane production

1. Introduction

Large amounts of waste activated sludge (WAS), generated from the activated sludge process, draw public attention as environmental regulations become more stringent [1]. Sludge treatment and disposal have become an essential part of wastewater treatment plants (WWTPs), accounting for 50% of operational costs [2]. Therefore, the emphasis of sludge treatment has been moved toward recycle as a useful energy source [3]. In this respect, anaerobic digestion (AD) is of particular interest in sludge treatment since it can reduce the overall amount of biosolid (40%), recover biogas, improve sludge stabilization and dewaterability, and kill pathogens [4]. However, WAS, primarily

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composed of micro-organisms, is difficult to be degraded through AD due to the refractory nature of extracellular polymeric substances (EPS) and glycan strands in cell walls [4]. Accordingly, the interest in finding more efficient ways to improve AD of WAS has been increased.

Numerous pretreatment methods, including mechanical, chemical, thermal, and biological aiding, have been employed for sludge hydrolysis under the assumption that these methods are capable of accelerating cell lysis and releasing the intracellular organic material into the liquid phase [5–7]. Actually, these methods played an important role in improving solubilization of complex particulate matters, digestion reaction rate, and biogas production in the subsequent AD process [8]. Among these methods, thermal hydrolysis was used to facilitate methane production from sludge, because it accelerates the breakdown of sludge gel structure and the release of intracellular organic matters [9]. As is reported, thermal hydrolysis, ranging from 60 to 270˚C, can disintegrate sludge cells more effectively [10,11]. In Europe, the sludge is usually pretreated at 170˚C and 7 bar pressure for 30 min before AD, and the reduction of sludge volatile solid is improved from 30–45% to 50–60% [12]. However, temperatures higher than 180˚C lead to the production of recalcitrant soluble organic matters or toxic intermediates, hence reduce the biodegradability of sludge [10]. Especially, the recovered energy from increased biogas production is largely used for compensating energy consumption of high temperature hydrolysis, which largely reduces the overall profitability of AD [11]. It has been reported that combined low-temperature pretreatment with AD overcame the drawbacks of high temperature and increased biogas production of primary and secondary sludge [13].

As we know, the sludge AD process usually includes three stages of hydrolysis, acidification, and methanogenesis [14]. The acidogenic micro-organisms were substantially different from the methanogenic ones in the following aspects: nutrient requirements, physiology, and environmental conditions (pH-value, etc.) [15]. So, it is reasonable to divide the AD process into two reactors for acquiring optimal environmental conditions for each group of micro-organisms. That is, volatile fatty acids (VFAs) were produced in the first reactor, and converted into methane and carbon dioxide by methanogenic micro-organisms in the second reactor. Some studies showed that splitting and separately optimizing hydrolysis/acidogenesis and methanogenesis could enhance the overall reaction rate, maximize biogas yields, and make the process easier to be controlled, both in meso- and thermophilic conditions [16,17]. According to some literatures, the biogas production and operational stability of sludge AD were enhanced by combining two-stage AD with high pressure homogenization hydrolysis or alkaline hydrolysis [18,19].

Therefore, the objective of the current work is to study the solubilization of organic matters after low-temperature thermal hydrolysis and the enhancement of biogas production in two-stage AD process. The mechanism of sludge disintegration at low temperature was analyzed by three-dimensional (3D) excitation–emission matrix (EEM) fluorescence spectroscopy and scanning electron microscope (SEM). The changes of VFAs and biogas of thermal hydrolysis sludge are also monitored in two-stage AD reactors.

2. Materials and methods

2.1. Experimental materials

The sludge used in this study was obtained from the secondary sedimentation tank of Wenchang municipal WWTP in Harbin, China. Prior to thermal hydrolysis, the sludge was concentrated and stored at 4˚C. The detailed characteristics of the concentrated sludge are shown in Table 1. The anaerobic sludge was collected from an upflow anaerobic sludge blanket reactor in our laboratory.

2.2. Thermal hydrolysis

Thermal hydrolysis was conducted for 2 h at 50, 80, 100, and 120˚C, respectively, in a thermal reactor (controlled in temperature) with a sample volume of 1.0 L. The sludge was introduced in the thermal reactor at room temperature and it took less than 5 min to heat it to the set temperature. The sludge was continuously gently stirred during the thermal hydrolysis to avoid temperature gradients. After hydrolysis, sludge samples were stored at 4˚C for the following tests.

Table 1
Characteristics of concentrated WAS used in experiment

Item	Value
Total suspended solids (TSS) (mg/L)	30,000 ± 200
Volatile suspended solids (VSS) (mg/L)	15,700 ± 100
Total chemical oxygen demand (TCOD) (mg/L)	21,000 ± 200
Soluble chemical oxygen demand (SCOD) (mg/L)	217.8
Soluble proteins (mg COD/L)	78.4
Soluble carbohydrates (mg COD/L)	24.7
pH	7.0 ± 0.3

2.3. Acidogenic and methanogenic batch test

In the acidogenic test, five identical continuous stirred tank reactors with working volume of 10 L were established. About 8 L thermal hydrolysis sludge and 1 L anaerobic sludge were added into reactors, and the sludge without thermal hydrolysis was set as the control. The sludge retention time was 2 d and the temperature was 35 ± 1℃. The pH was regulated at 9 for VFAs accumulation in the acidogenic process [20]. The acidification liquid was collected by centrifugation at 4,000 g for 10 min, and pHs of acidification liquid were regulated to 7 by 2 M sodium hydroxide (NaOH) or 2 M hydrochloric acid (HCl) before methanogenic test. Methanogenic stage was operated in five reactors with working volume of 20 L (internal diameter of 250 mm and height of 400 mm). The hydraulic retention time was 8 d and the temperature was 35 ± 1℃. The headspace was purged with nitrogen gas for 10 min to maintain anaerobic condition. The gas produced in each reactor was collected by gasbag, and the total volume was measured using a glass syringe.

All the experiments were conducted in triplicate with average and standard deviation reported. The cumulative biogas yield was evaluated according to equation:

$$\text{Cumulative biogas yield} = \frac{\text{cumulative biogas volume (mL)}}{\text{VSS}_{\text{WAS add}}\,(\text{gVSS})} \quad (1)$$

2.4. Analytical methods

The TCOD, SCOD, TSS, and VSS were measured according to Standard Methods [21]. The carbohydrates were determined using the anthrone sulfuric method with glucose as the standard [22], and the proteins were quantified using a modified Lowry method with bovine serum albumin as the standard [23]. The composition of VFAs was analyzed by means of gas chromatography (Agilent 6890 N) using a flame ionization detector and DB-WAXETR column (30 m × 0.53 mm × 1.0 μm) [24], and the total VFAs was recorded as the sum of acetic, propionic, n-butyric, iso-butyric, n-valeric, and iso-valeric acids. The biogas composition was measured by a gas chromatograph (Agilent 7890 N) equipped with a thermal conductivity detector and a column carbon molecular sieve. Nitrogen was used as carrier gas at a rate of 25 mL/min. The temperatures in column temperature, injection temperature, and detector temperature were 40, 50, and 50℃, respectively. The sludge EPS was extracted using ultrasonic extraction method [25].

2.5. 3D-EEM analysis of soluble organic matters

3D-EEM spectra of soluble organic matters after different thermal hydrolysis were measured using a molecule fluorescence spectrometer (FP-6500, Jasco, Japan). Scanning emission spectra from 220 to 550 nm at 1 nm increments was obtained by varying the excitation wavelength from 220 to 450 nm at 5 nm increments. The excitation and emission slits were, respectively, maintained at 5 and 3 nm, and the scanning speed was set at 2,000 nm/min for all samples. The influence of the Raman and Rayleigh scattering was decreased by subtracting the EEM spectra of a control (Milli-Q water) from the EEMs of all samples [26]. Origin 8.0 software was employed to process EEM data, with the resulting EEM spectra visualized as contour plots, where the *X*-axis and *Y*-axis represent the emission and excitation spectra, respectively.

2.6. SEM analysis

The surface morphology of thermal hydrolysis sludge was observed by a electron microscope (QANTA200, FEI, USA). The samples were washed three times with PBS (pH 6.8) and fixed with 2.5% glutaraldehyde (pH 7.2–7.4) overnight at 4℃, then washed with PBS (pH 6.8) for three times and 15 min per session before being dehydrated by successively passaging through 50, 70, 80, 90, and 100% ethanol. Then the sludge samples were further washed by ethanol–isoamyl acetate (1:1) and isoamyl acetate, respectively, followed by drying with a freeze drier (BT2KXL, VIRTIS, USA) and metalizing with a thin gold layer with a Sputter Coater (SCD005, BAL-TEC, Switzerland).

3. Results and discussion

3.1. Effect of thermal hydrolysis on organic matters solubilization

Sludge pretreatments accelerate the transfer from particles organic matters to supernatant [9]. The changes of SCOD, soluble proteins and carbohydrates after thermal hydrolysis were shown in Fig. 1. The SCOD concentrations increased with increase in temperatures. For example, the SCOD concentration increased to 5,874 mg/L as thermal temperature increased to 120℃, which was almost three times higher than that at 50℃. Especially, the solubilization of chemical oxygen demand (COD) was more pronounced as temperature increased from 50 to 80℃, although it was lower than that of 100 and 120℃. For further analysis of sludge disintegration degree, soluble organic matters released from thermal hydrolysis

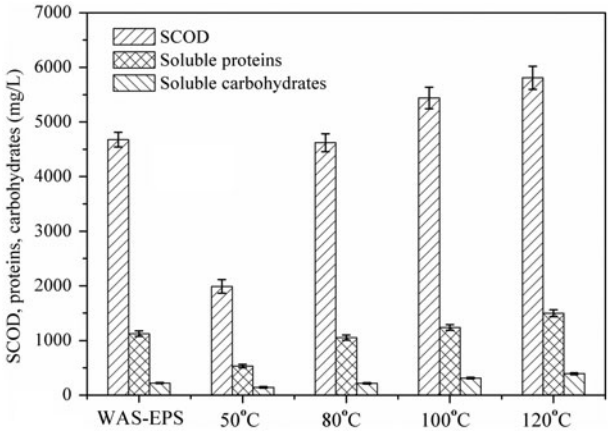

Fig. 1. Solubilization of sludge after different thermal hydrolysis.

sludge were compared with that extracted from sludge EPS. It was clear to see that the SCOD concentration with temperature over 80˚C was higher than that in EPS (4,679 mg/L), indicating that sludge EPS was destroyed effectively [9]. At this time, the release of organic matters was not only from EPS, but also from intracellular organic matters. It was also obvious that the concentrations of soluble proteins and carbohydrates after thermal hydrolysis had a trend similar to the changes of SCOD. That was because proteins and carbohydrates were main components of sludge, which were released accompanying with the disintegration of EPS and cell walls [7].

3.2. 3D-EEM analysis of thermal hydrolysis sludge

Biochemical transformation of organic compounds takes place in the water-soluble phase [13]. Therefore, it is crucial to investigate the characteristics of soluble organic matters in thermal hydrolysis sludge for understanding the AD. The 3D-EEM fluorescence spectroscopy is a useful technique to rapidly determine the fluorescence compounds such as humic-like and protein-like compounds during alkaline anaerobic fermentation of WAS [27]. Typical EEM fluorescence spectra of thermal hydrolysis sludge are depicted in Fig. 2. Three main peaks, marked as peaks A, B, and C, were identified from EEM fluorescence spectra of thermal hydrolysis sludge. Based on the regions determined in previous literature [14], the Peaks A and B occurred at excitation/emission wavelengths (Ex/Em) of 220–240/280–360 nm and 250–290/280–360 nm, respectively, were reported as tyrosine

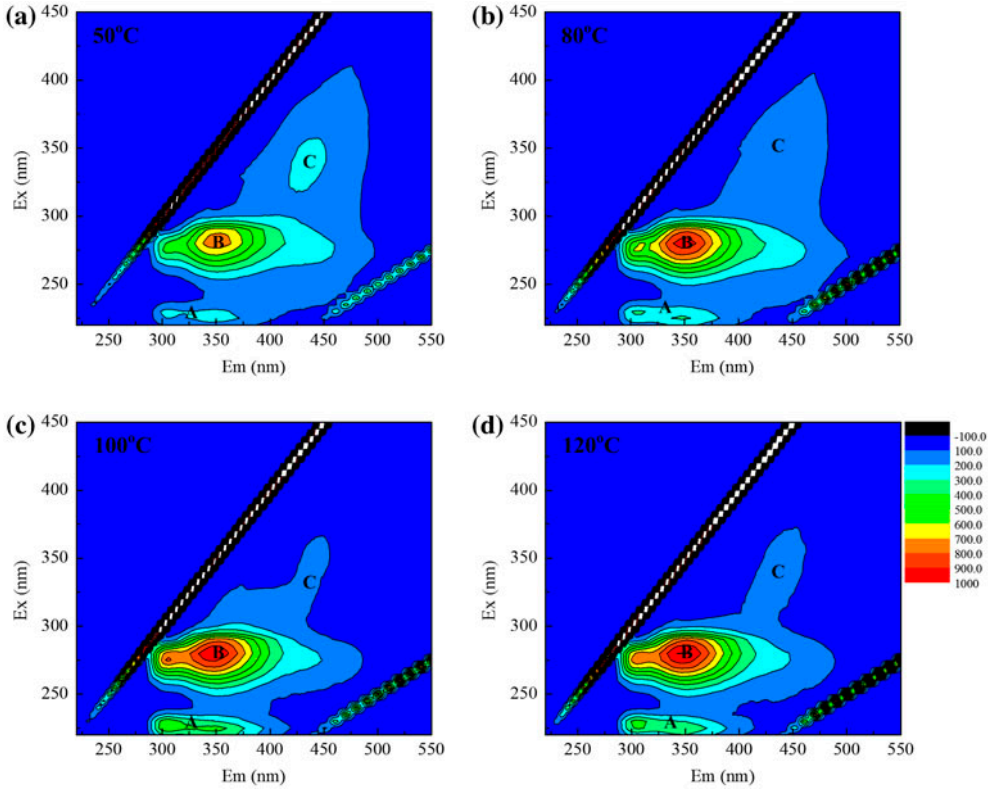

Fig. 2. EEM fluorescence spectra of soluble organic matters after thermal hydrolysis of (a) 50˚C, (b) 80˚C, (c) 100˚C, and (d) 120˚C.

protein-like and tryptophan protein-like substances. The Peak C located at Ex/Em of 330–370/420–460 nm represented humic-like substances.

As shown in Fig. 2, the peak with the maximum fluorescence intensity was Peak B, indicating that the tryptophan protein-like substance was the main soluble component after thermal hydrolysis. As a result of thermal hydrolysis, the sludge flocs were disrupted and the cell was dissolved, which resulted in the release of extracellular and intracellular protein-like biopolymers. This result was consistent with previous report [13], where they found that more soluble organic matters were released into the liquid phase after thermal hydrolysis, and these released organic matters were beneficial for methane production. It was also found that the fluorescence intensities of Peaks A, B, and C were quite different with each other in four trials. For example, the intensities of Peaks B sharply increased as the temperature increased from 50 to 120˚C, which suggested that more protein-like substances were released to the supernatant accompanying with the sludge destruction. The fluorescence intensity of Peak C decreased with increase in temperature, and the intensities of Peak A first increased and then decreased at the temperature of 100˚C. The distinct differences in fluorescence intensity of these Peaks presumably due to sludge disintegration caused by thermal hydrolysis [13].

3.3. SEM analysis of thermal hydrolysis sludge

SEM is a physical method, as opposed to chemical methods such as COD and proteins, to ascertain sludge disintegrated by intuitively investigating the surface morphology of micro-organisms. As shown in Fig. 3, there were significant differences in the appearance of the sludge flocs and cells of thermal hydrolysis sludge. It is well-known that the photo of untreated sludge was relatively smooth and integrated, with no structural damage. After thermal hydrolysis, the morphology of the microbes were not broken obviously, but it was clear that as the temperature increased, the flocs structures were disintegrated, and the cells were swelled and softened. As reported in previous literature [9], the sludge disintegration degree was significantly correlated with soluble proteins and carbohydrates. In this study, the change of sludge surface morphology was consistent with the result of SCOD, proteins, and carbohydrates, suggesting that the thermal hydrolysis had the capability to denature sludge EPS and break cells, and thus some intracellular substances covered by cell wall were released into the solution.

3.4. Acidification performance of thermal hydrolysis sludge

The soluble proteins and carbohydrates are hydrolyzed into low molecular-weight intermediates such as amino acid and glucose which are in turn degraded to VFAs during acidification process [14]. In the acidification process, the composition of acidification liquid was presented in Fig. 4(a). The soluble proteins and carbohydrates concentrations increased with increase in temperature (50–120˚C), indicating that the hydrolysis rate of proteins and carbohydrates was higher than the acidification rate in the acidification stage. Simultaneously, a similar trend was observed in VFAs concentrations. An explanation of VFAs increase in temperature of 50–120˚C could be that some soluble proteins and carbohydrates generated from thermal hydrolysis were converted to VFAs by acid-forming bacteria [10].

It is well-known that acetic acid can be degraded into methane and carbon dioxide directly by methanogens [18], therefore, it is necessary to study the composition of VFAs in acidification liquid. The change of individual VFAs in sludge fermentation liquid was summarized in Fig. 4(b). It was obvious that acetic and propionic acids were the main products in all reactors, accounting for more than 90%, and they were obviously changed by thermal temperatures.

3.5. Methane production

The optimal conditions for methane production may be high COD solubilization and low organic matter mineralization, but increased solubilization does not always lead to an enhanced methane potential [28]. Therefore, the effects of thermal hydrolysis and acidification on methanogenic stage are needed to be assessed. As shown in Fig. 5, the cumulative biogas production increased with the increase in temperature. For example, in methanogenic stage, the cumulative biogas production of untreated sludge was only 35 mL/gVSS on the 5th day, however, the cumulative biogas production were 54, 91, 123, and 125 mL/gVSS, respectively, at thermal temperature of 50, 80, 100, and 120˚C. The results suggested that thermal hydrolysis effectively enhanced biogas production, and the biogas production had positive relation to soluble COD, proteins, and carbohydrates. Clearly, no obvious growth of biogas production was observed between 100 and 120˚C. In the view of the energy consumption, 100˚C was the suitable temperature for biogas production. Also, in Fig. 5, the methane percentage was changed by thermal hydrolysis, and slight decrease in methane percentage was observed with temperature increasing from 50 to 120˚C. The average value of methane percentage in biogas production was 65.3%.

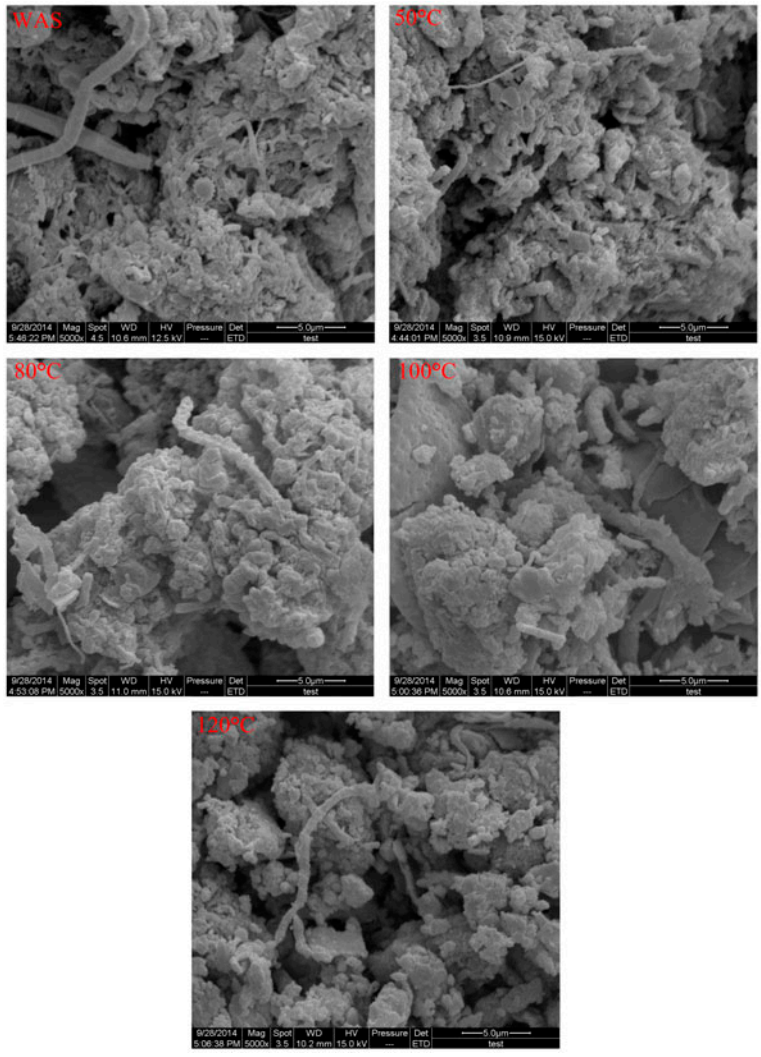

Fig. 3. The SEM images of sludge after different thermal hydrolysis.

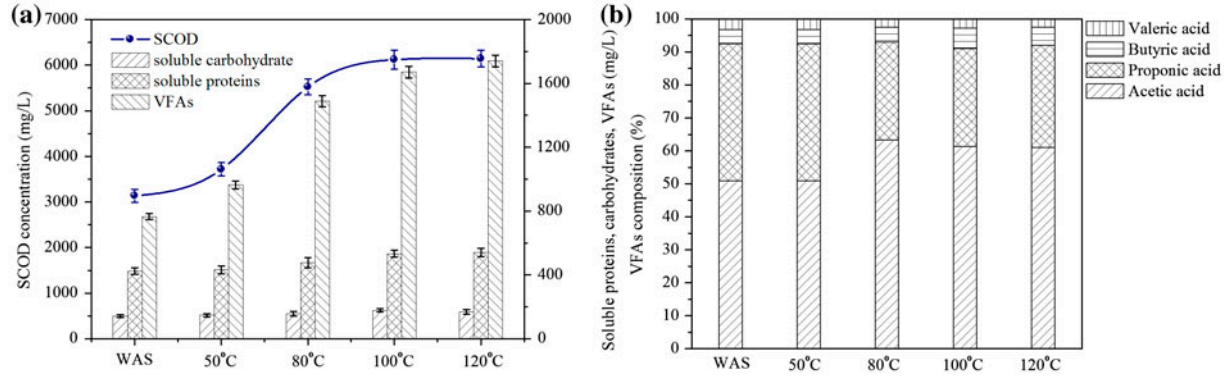

Fig. 4. The compositions of (a) acidification liquid and (b) VFAs in acidogenic stage of thermal hydrolysis sludge.

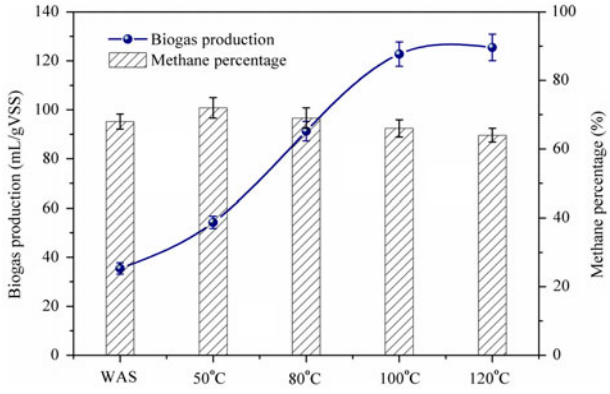

Fig. 5. Cumulative biogas production and methane percentage in methanogenic stage of thermal hydrolysis sludge.

4. Conclusions

In this study, sludge was pretreated through low-temperature thermal hydrolysis to provide an overall view of feasible scenarios for sludge treatment. Extracellular structure of sludge was destroyed by low-temperature thermal hydrolysis, and the more serious damages were observed at higher temperatures. The SCOD, proteins, and carbohydrates increased with the increase in temperature, and intracellular protein-like substances were released when the temperature was over 80˚C. The VFAs and methane production also increased with substrates solubilization. In terms of anaerobic biodegradation, 100˚C was the optimal temperature for methane production in range of 50–120˚C.

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