Alkaline Pretreatment of Banana Stems for Methane Generation: Effects of Temperature and Physicochemical Changes

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The effects of NaOH pretreatment temperature on the physicochemical characteristics and methane production of anaerobically digested banana stems were investigated in this paper. With the increase of pretreatment temperature from 0 °C to 100 °C, the chemical oxygen demand (COD) of the soak liquid in the treated biomass approximately linearly increased from 5.9 g/L for the untreated stems to 34.0 g/L. A weight loss of 5.1% was observed for the untreated material, while it was up to 31.2% for the sample treated at 100 °C. The removal of lignin and hemicellulose accounted for the majority of the weight loss. The removal rates of lignin and hemicellulose increased from 15.0% to 41.6% and 1.9% to 23.6% when the treatment temperature increased from 0 °C to 100 °C, respectively. Moreover, the crystalline index (CI) of the banana stems also increased with rising temperature, resulting from the dissolution of amorphous cellulose with increasingly harsher alkaline environments. The optimal pretreatment temperature for banana stems was confirmed at 50 °C. In these conditions, methane was produced *via* anaerobic digestion with 239.9 mL/g total solid (TS) yield, which represented an increase of 66.7% over untreated banana stems.

Keywords: Alkaline pretreatment; Temperature; Anaerobic digestion; Banana stems; Biogas

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INTRODUCTION

Bananas are one of the most important fruits grown in tropical and subtropical regions and are widely cultivated all over the world. They represent a unique perennial single-harvest crop and are one of the largest herbaceous plants in the world (Ploetz *et al*. 2007). Banana stems are the main by-product after banana harvesting, with about 100 metric tons per hectare being produced annually (Zhang *et al.* 2013); this waste is usually crushed and left to degrade on the farms to replenish some of the nutrients in the soil (Padam *et al*. 2014). However, this disposal method generates a large amount of greenhouse gases and unpleasant odors (Pei *et al.* 2014). Furthermore, these approaches can induce an outbreak of Panama disease, a type of banana Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum*. Infection poses serious problems because the pathogen is resistant to fungicide and cannot be controlled chemically (Getha and Vikineswary 2002; Xiao *et al.* 2013). It would therefore be highly useful if the biomass from banana stems could be converted into useful biogas, which would simultaneously solve any environmental or pathogen-related problems. Banana stems have been proposed as useful biomass in paper production, animal feed production, compost, renewable materials, waste water purification, and biofuel production (Deng *et al.* 2014; Khan *et al.* 2014; Madhu *et al.* 2014; Mullassery *et al.* 2014; Padam *et al.* 2014; Noremberg *et al*. 2017). Among these options, biogas production *via* anaerobic digestion (AD) is a viable way of converting banana stem biomass into a useful energy source (Padam *et al.* 2014). In addition, banana stems produced higher biogas yields than corn and straw stalks (Han *et al.* 2016). AD is the most efficient method of energy generation from biomass in terms of the energy output/input ratio (28:1) compared with other biological and thermo-chemical routes of energy production (Deublein and Steinhauser 2011). The AD process can be divided into four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Campanaro *et al.* 2016). In most cases, methanogenesis is the rate-limiting step of the whole process (Chen *et al.* 2008); however, for lignocellulose biomasses, hydrolysis is believed to be the rate-limiting step (Azman *et al*. 2015). Thus, pretreatment prior to AD is usually required to reduce structural and compositional impediments of lignocellulosic biomass degradation, which requires the full exposure of the cellulose and hemicellulose biopolymer chains to microbial and enzymatic attack in order to increase the biodegradation rate (Zheng *et al.* 2014). Common pretreatment options include physical, chemical, biological, and physicochemical methods (Zheng *et al.* 2014). Alkaline pretreatment can change the composition and structure of plant cells, alter cellulose crystallinity, and break down and dissolve lignin, thereby improving AD performance of the lignocellulosic biomass (Zheng *et al.* 2009). In addition, moderate operating conditions and the ability to perform the procedure without specialized equipment make alkaline pretreatment more suitable for industrial biogas production from lignocellulosic biomass.

The key factors in alkali pretreatment include the dosage and type of alkali used, the operating temperature, reaction time, and moisture content. For instance, with regards to the effect of the pretreatment temperature, Mittal *et al*. (2011) reported that treatment at higher temperatures (130 °C to 140 °C compared to 25 °C) resulted in a more crystalline hydrolyzed biomass. While the pretreatment temperature has been clearly established as a key factor during alkaline hydrolysis (Mirahmadi *et al.* 2010), the effect varies based on the feedstock characteristics. Taherdanak and Zilouei (2014) determined that the methane yield after NaOH pretreatment increased with temperature, but decreased beyond 75 °C. With antibiotic mycelial residues, the final cumulative methane yield after anaerobic digestion increased with rising pretreatment temperatures (60 °C to 100 °C) (Li *et al.* 2016). However, there is little information available about the impact of NaOH pretreatment temperature on changes in the morphology and methane production from pretreated banana stems.

This study investigated the effect of temperature on the NaOH pretreatment of banana stems, which then underwent anaerobic digestion, generating methane for possible use as a renewable energy source. Changes in the morphology and structure of the treated stems were investigated through scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The optimal pretreatment temperature was established by investigating various anaerobic digestion indicators, such as methane production performance and biodegradability. In addition, changes in weight loss, chemical oxygen demand (COD), and composition (cellulose, hemicellulose, and lignin) were also used to rationalize the trends in methane production with pretreatment temperature.

EXPERIMENTAL

Feedstock and Inoculum

Banana stems were obtained from a banana plantation (19°76′ N, 110°12′ E) in Chengmai county, Hainan Province, China. Stems were cut into 0.5 cm lengths and openly air dried for 5 days. Then they were used for pretreatment and AD. Before AD, stems were stored in an air-tight bag at -20 °C. The inoculum used in this study is anaerobic sludge obtained from the mesophilic anaerobic tank from a wastewater treatment plant in Beijing. The total solid (TS, based on wet solid) and volatile solid (VS, based on dry matter) of the banana stems and anaerobic sludge and the elemental analysis of the banana stems are presented in Table 1. It should be pointed that, in a certain condition, the C/N ratio of inoculum could affect the anaerobic digestion results. In order to obtain an optimal anaerobic digestion performance, the C/N ratio of inoculum should be considered.

Table 1. TS, VS, and Elemental Contents[a] of Feedstock and Inoculum

	TS (%)	VS (%)	C (%)	H (%)	O (%)	N (%)
Banana stems	95.5 ± 0.4	89.3 ± 0.5	43.7 ± 0.1	4.9 ± 0.11	42.3 ± 0.9	0.6 ± 0.1
Inoculum	4.1 ± 0.1	2.1 ± 0.2	-	-	-	-

[a] Values are based on TS.

Alkaline Pretreatment

Air dried banana stems (83.47 g) were pretreated with 8% (w/w) NaOH based on their dry weight, and the moisture of the pretreatment mixture was adjusted to 90% (w/w) by adding deionized water to ensure sufficient mixing in 1000 mL serum bottles. Bottles were tightly sealed and incubated in a water bath at 0 °C, 25 °C, 50 °C, 75 °C, and 100 °C for 24 h and were manually shaken every 3 h. It should be pointed that, for alkaline pretreatment, the optimal pretreatment time was significantly affected by the raw material size, alkaline dosage, and pretreatment temperature (Pei *et al*. 2014; Taherdanak and Zilouei 2014). According to the cited work, 24 h was selected as the pretreatment time in this experiment. For a certain pretreatment temperature, the corresponding optimal pretreatment time could be determined according to the further experiments. After 24 h of pretreatment, the banana stems were manually squeezed to reduce the mean moisture content to 25%. Anaerobic digestion took place with unwashed pretreated banana stems; after digestion, they were stored at -20 °C for analysis. Banana stems were washed five times with deionized water to wash out the soluble materials generated during the alkaline pretreatment, and they were analyzed for compositional and morphological changes. The COD and pH of the soak solution were then analyzed. Banana stems soaked in deionized water without NaOH were used as the control group.

Anaerobic Digestion

Anaerobic digestion was conducted in 500 mL serum bottles with a working volume of 400 mL and a gas-tight rubber stopper. The headspace was flushed with nitrogen prior to AD to ensure an anaerobic environment. The biogas produced during AD was collected in 1-L sealed bottles filled with 2% NaOH, which absorbed carbon dioxide and other acid gases produced during fermentation. The volume of methane produced was measured by the water displacement method. The ratio of inoculum to solids (both expressed in g of VS) was 1:2, to avoid potential substrate inhibition and low microbial density, while the total solid (TS) of the fermentation system was 5%. NH_4Cl and $NaHCO_3$ were added to adjust the C/N ratio and alkalinity of the fermentation system to 25:1 and 3000 mg $CaCO_3$/L, respectively. All of the digesters were incubated in a water bath at 37 ± 1 °C for 25 days. Blank tests were carried out with the inoculum in order to ascertain the amount of endogenous methane produced from the sludge. All the AD experiments were carried out in triplicate.

Analytical Methods

Chemical analysis

The TS and VS of banana stems and the COD of the soak liquid were analyzed according to the procedure reported by Rice *et al.* (2012). Elemental analysis of banana stems was obtained according to the industry standard procedure (ISO17247-2005 2005). The structural carbohydrates and lignin contents were measured according to a standard procedure (Sluiter *et al.* 2008). Weight loss and component removal rate were calculated with Eqs. 1 and 2, respectively, as follows,

$$\text{Weight loss (\%)} = W_\text{O} - W_\text{AP} / W_\text{O} \times 100 \qquad (1)$$

$$\text{Component removal rate (\%)} = (W_\text{O} \times C_\text{O} - W_\text{AP} \times C_\text{AP})/ (W_\text{O} \times C_\text{O})\times 100 \qquad (2)$$

where W_O and W_AP are the total solid weight (g) before and after alkaline pretreatment, respectively; C_O and C_AP are the relative contents of cellulose, hemicellulose, or lignin in the banana stems (%) before and after alkaline pretreatment, respectively.

X-ray diffraction

X-ray powder diffraction was performed with a D8 Advance diffractometer (Bruker, AXS, Karlsruhe, Germany) using Cu Kα radiation (λ = 0.154 nm) generated at a voltage of 40 kV and a current of 40 mA. The scan angle (2θ) ranged from 10° to 40°. The crystalline index (CI) of cellulose was calculated according to Eq. 3 for native cellulose (Park *et al.* 2010).

$$\text{CI\%} = (I(002) - I18.0°) / I(002) \times 100 \qquad (3)$$

where CI is the crystalline index (%), $I(002)$ is the maximum intensity of the (002) lattice diffraction, and $I18.0°$ is the intensity diffraction at $2\theta = 18.0°$.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy (Spectrum GX; Perkin-Elmer, Waltham, MA, USA) was used to determine the changes in the structure of banana stems after pretreatments. Spectra were recorded with a resolution of 4 cm^{-1} from 4000 cm^{-1} to 400 cm^{-1} using air as the background reference and were analyzed with Origin software (OriginLab Co., Northampton, MA, USA).

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed with a high resolution microscope (Zeiss, Jena, Germany) at 15.0 kV.

RESULTS AND DISCUSSION

Characteristics of Feedstock and Inoculum

TS, VS, and elemental analysis of the banana stems are presented in Table 1. The C/N ratio of banana stems was 71.6, which is much higher than the optimal ratio required for AD to proceed efficiently. High C/N ratios usually lead to poor conversion of biomass to biogas, both in terms of daily methane production and methane yield (Ge *et al*. 2016); it was therefore deemed necessary to add an extra nitrogen source to the system prior to anaerobic digestion. According to a formula based on the elements content (Sobotka *et al.* 1983), banana stems are considered to be suitable for biogas production when the theoretical methane yield (TMY) reaches 429.2 mL/g.

Alkaline Hydrolysis of Banana Stems

Alkaline pretreatment chemically modifies the content of complex sugars (celluloses and starches) and increases the removal of lignin present in various biomasses (Bali *et al.* 2015). Furthermore, cellulose and hemicellulose are hydrolyzed under alkaline conditions, releasing sugars that might also decompose under harsher conditions (Taherdanak and Zilouei 2014). Therefore, the COD of the soak liquid and the weight loss of the banana stems were used to determine the level of alkaline hydrolysis during pretreatment (Fig. 1).

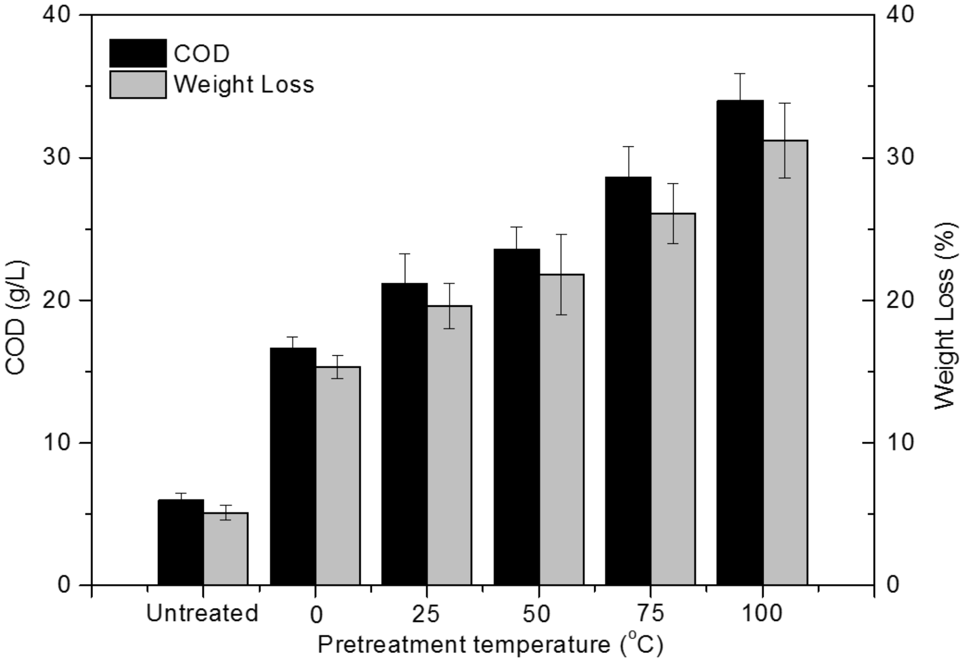

Fig. 1. COD of soak liquid and weight loss of banana stems with different pretreatment temperature

After pretreatment, the pH values of all alkali-pretreated samples were still higher than 10, indicating that a sufficient amount of NaOH was used, and therefore, the only parameter that remained to be optimized was the pretreatment temperature. The COD of the soak liquid and the weight loss observed in the untreated banana stems were 5.9 g/L and 5.1%, respectively, indicating only a small amount of biomass had undergone decomposition, with little organic matter diffusing into the surrounding liquid. As expected, the COD and weight loss in the alkali-pretreated samples were higher than in the untreated biomass, with both increasing in an almost linear fashion with pretreatment temperature (Fig. 1). As the temperature increased from 0 °C to 100 °C, the weight loss of the banana stems increased from 15.3% to 31.2%, which was 3.0 and 6.1 times higher, respectively, than in the untreated biomass. Upon treatment, the COD underwent an increase from 16.6 g/L at 0 °C to 34.0 g/L at 100 °C. Treatment with NaOH resulted in the hydrolysis of large polymeric substances, forming smaller, more soluble molecular matter, and thereby led to the observed increase in the COD of the treated soak liquid with temperature. Based on an extrapolation of the data, it is possible that still higher COD and weight loss values might be obtained under harsher hydrolysis conditions at more elevated temperatures.

Change in Biopolymer Content in Treated Banana Stems

The cellulose in plant cells is coated with hemicellulose and lignin, which protect the cells against microbial and enzymatic attack; these three biopolymers constitute the main constituents in lignocellulosic biomass (Yang *et al*. 2015). Untreated banana stems contain more cellulose (48.7%) and less lignin (17.4%, TS%; Table 2) than other common types of agricultural waste (Han *et al.* 2016). These qualities render banana stems suitable waste for anaerobic digestion by avoiding the poor biodegradability and spatial obstacles between microorganisms and carbohydrates commonly found in biomass with low cellulose and high lignin contents (He *et al.* 2008; Frigon and Guiot 2010; Han *et al.* 2016). The cellulose content increased with pretreatment temperature, reaching 57.7% at 100 °C. However, the hemicellulose content decreased from 14.1% to 11.3% as the temperature increased but it was still higher than in the untreated sample (10.90%) (Table 2). The lignin content of pre-treated samples decreased significantly as the temperature increased, decreasing by 5.7% to 21.3% at the highest treatment temperature compared to the untreated stems; interestingly, alkaline-pretreated wheat plants exhibited a similar decrease in lignin content with increasing treatment temperatures (Taherdanak and Zilouei 2014). Under higher temperatures, the decomposition of lignin and hemicellulose increase relative to that of cellulose, increasing the TS% of cellulose (Girio *et al.* 2010). In addition, alkaline pretreatment at higher temperatures promotes anaerobic digestion of the banana stems.

Table 2. Relative Content of Three Main Components of Banana Stems (TS%)

Sample	Cellulose	Hemicellulose	Lignin
Untreated	48.7 ± 0.8	10.9 ± 0.3	17.4 ± 0.1
0 °C pretreated	49.7 ± 0.2	14.1 ± 0.8	16.4 ± 0.3
25 °C pretreated	50.4 ± 0.3	12.6 ± 0.3	15.7 ± 0.4
50 °C pretreated	54.0 ± 1.5	11.5 ± 1.0	14.5 ± 0.2
75 °C pretreated	54.2 ± 1.7	11.4 ± 0.1	13.8 ± 0.6
100 °C pretreated	57.7 ± 1.7	11.3 ± 0.4	13.7 ± 0.7

According to Eq. 2, the removal rates of the three main components (cellulose, hemicellulose, and lignin) observed at different alkali-pretreated temperatures are shown in Fig. 2. There was little variation in the hydrolysis of cellulose under all pretreatment temperatures (8.1% to 12.3%), with negligible change from 25 °C to 100 °C. The results indicated that increasing the temperature had a much greater effect on the hydrolysis of hemicellulose and lignin (with decomposition rates varying from 1.9% to 23.6% and 15.0% to 41.6%, respectively) compared with that of cellulose, which showed the lowest decomposition rates at the higher temperature range, in particular at 100 °C. This result suggested that the increasing weight losses observed in treated banana stems and the increase in the COD of soak liquid with temperature (Fig. 1) can be mainly attributed to the hydrolysis of lignin and hemicellulose during pretreatment, in particular at the higher temperature range (50 °C to 100 °C). As observed in Fig. 2, at the highest temperatures, the rate of cellulose decomposition was lower than that of the other components, resulting in a higher relative content of cellulose that allowed microorganisms to act more efficiently on the treated biomass, and therefore, which led to higher amounts of biogas production (He *et al.* 2008).

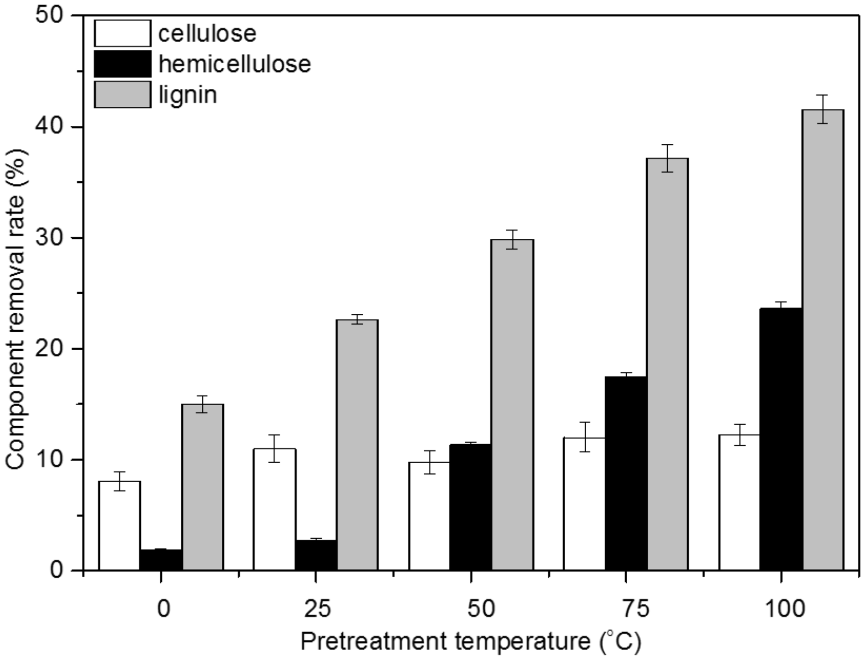

Fig. 2. Component removal rates of the main components of pretreated banana stems

Physicochemical Changes in Pretreated Banana Stems

The physicochemical characteristics of banana stems pretreated at three different temperatures (0 °C, 50 °C, and 100 °C) were characterized by SEM, XRD, and FTIR. In the untreated banana stems, individual plant cells were stacked in an orderly arrangement, with clearly visible lignin between individual cells (Fig. 3a). After alkaline pretreatment, a deterioration in the surface structure and formation of lamella was observed, even at a low temperature (0 °C; Fig. 3b). As the temperature increased to 50 °C and 100 °C (Fig. 3c and Fig. 3d, respectively), some independent fibers were exposed, and there was a clear further deterioration of the cell walls, resulting in an increased surface area.

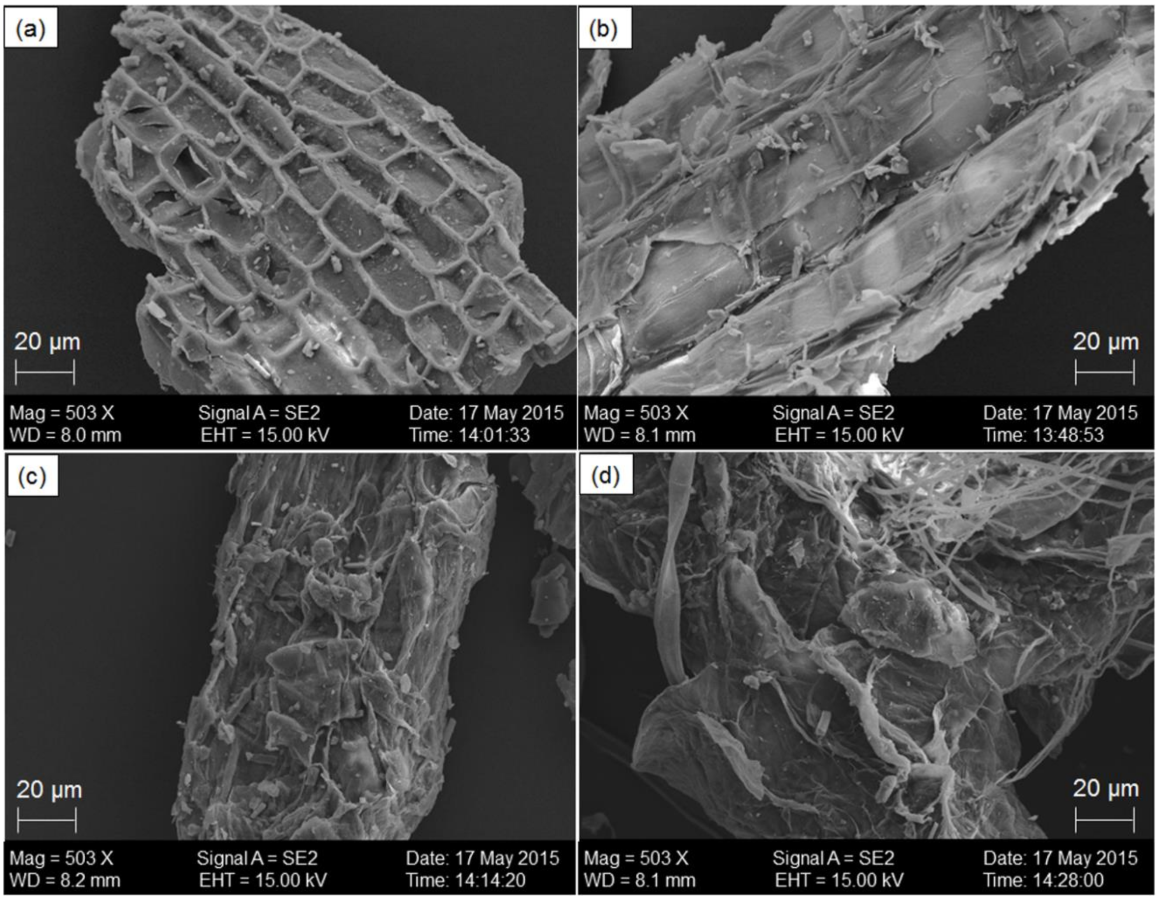

Fig. 3. SEM images of banana stems (a, untreated; b, 0 °C; c, 50 °C; and d, 100 °C)

Table 3. Assignment of the Main Bands In the FTIR Spectra

Wavenumber (cm^{-1})	Functional Group	Assignment
2919	C-H stretching	Cellulose
1610	C=C stretching of aromatic ring	Lignin
1510	C=C stretching of aromatic ring	Lignin
1430	CH_2 bending	Cellulose
1375	C-H bending	Cellulose
1158	C-O-C glycoside asymmetric stretching	Cellulose and hemicellulose
898	Asym., out of phase ring stretching (glucose)	Cellulose

FTIR spectroscopy was used to qualitatively investigate the structural changes in pretreated banana stems in comparison to the untreated biomass. Seven bands were assigned to cellulose, hemicellulose, and lignin (Fig. 4, Table 3). The intensity of the bands at 1430 and 1375 cm^{-1} assigned to cellulose increased with pretreatment temperature, while the bands at 1610 and 1510 cm^{-1} assigned to lignin gradually faded, which indicated that alkaline pretreatment results in lignin decomposition, leading to larger amounts of cellulose being exposed on the surface of the stems.

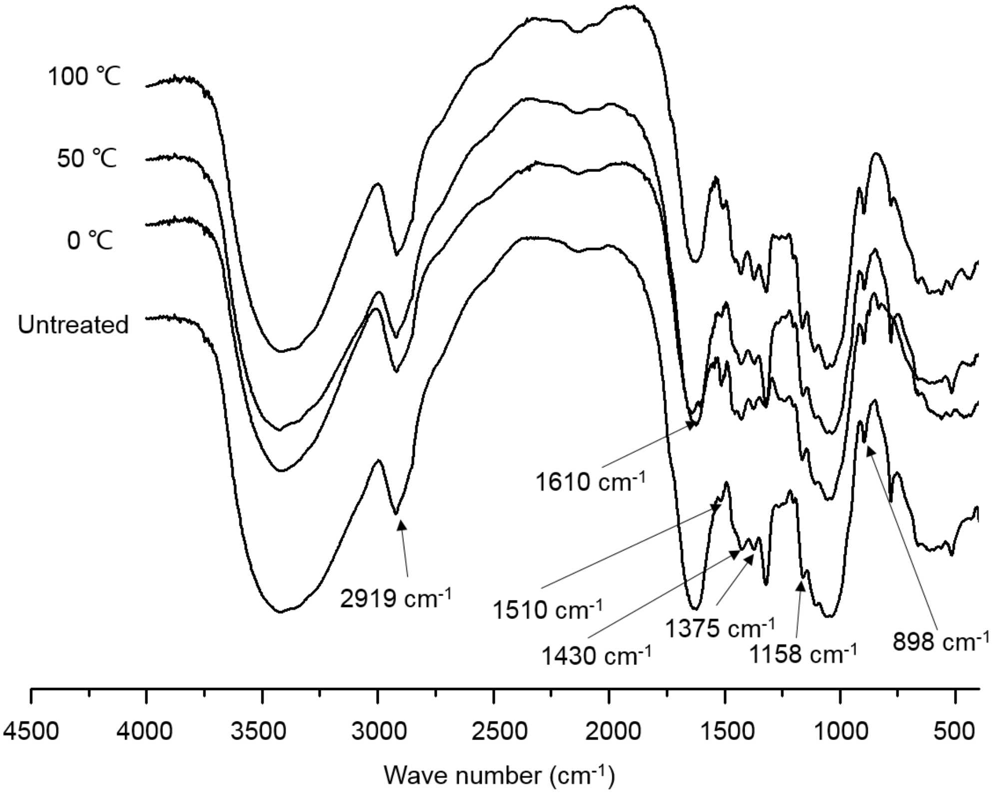

Fig. 4. FTIR spectra of pretreated and untreated banana stems

Banana stems with or without pretreatment had similar X-ray diffraction patterns, but the intensity of the two main peaks at $2\theta = 18.0°$ and 22.4° varied as the temperature rose, indicating that the temperature of alkaline pre-treatment influenced the crystallinity index of the banana stems (Fig. 5). The crystallinity as determined from the CI, which was 44.7% for the untreated sample, and it increased gradually to 49.9%, 50.7% and 57.1% for the samples pretreated at 0 °C, 50 °C, and 100 °C, respectively. These values represented an increase of 11.7%, 13.5%, and 27.7%, respectively, over the untreated sample. Increasing amounts of hydrolysis of the glycosidic linkages under harsher NaOH catalyzed conditions in the accessible regions of the cellulose fibers resulted in an increase of CI, and it was also presumably related to the removal of amorphous substances, hence increasing the fraction of crystalline polymer. The same trend in CI with temperature has been observed previously, for example, with pretreatment of mustard with NaOH (Sindhu *et al.* 2012; Yang *et al.* 2014; Kapoor *et al.* 2015). However, the opposite trend with temperature was observed with wheat plant (grain and straw) pretreated with NaOH (He *et al.* 2008; Taherdanak and Zilouei 2014). These results indicated that the trends in CI with temperature cannot be extrapolated to different sources of biomass, but were unique to each type of plant source.

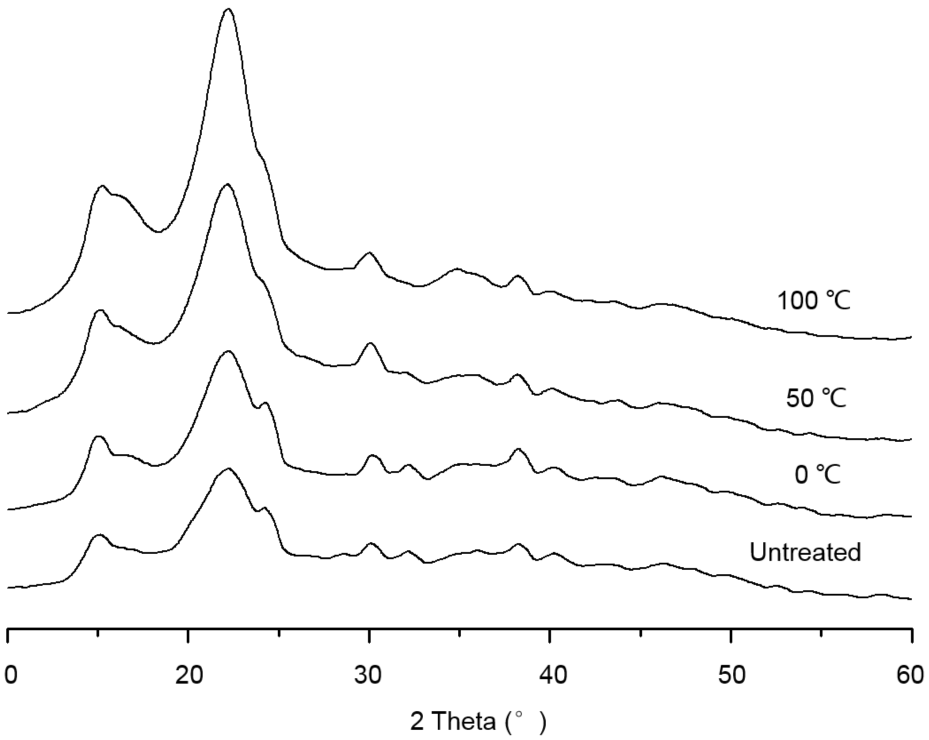

Fig. 5. X-ray diffraction of pretreated and untreated banana stem

Methane Production from Banana Stems

Daily methane production (DMP) and cumulative methane production (CMP) were recorded and calculated to investigate the effect of pretreatment temperature on methane production from banana stems. DMP from treated and untreated samples both exhibited peak methane production during the first ten days (with maximum production on the fourth day). Gas production sharply tapered off after the ninth day and stayed nearly constant after this time period. Generally, these observations were similar to those reported elsewhere (Zheng *et al.* 2009; Chen *et al.* 2014; Zhang *et al*. 2016). Except for the first day of anaerobic digestion and after peak DMP production (first ten days), NaOH pretreatment increased significantly the DMP and shortened the AD period. For example, the DMP from the treated stems on the 4th day was 108% to 242% higher than the control, and the highest DMP (304.7 mL) was obtained at 50 °C (Fig. 6A). Pretreatment also increased the CMP from banana stems, which increased up to the maximum temperature, although the CMP levels at 50 °C and 75 °C were nearly identical (Fig. 6B). As the temperature increased from 0 °C to 50 °C, the CMP increased from 1361.5 mL to 1756.4 mL, representing an increase of 26.3% to 62.9%, respectively, compared with the control (1078.2 mL). Based on the CMP, and taking into consideration the preference for using milder hydrolysis conditions which reduced energy requirements and the overall cost of the pretreatment process, the optimal pretreatment temperature was 50 °C. Even though the highest CMP was observed at 100 °C, increasing the temperature from 50 °C to 100 °C only afforded an improvement in the CMP of 4.6%. According to the data of DMP and CMP, 12 day could be regarded as a reasonable retention time in the experiment conditions.

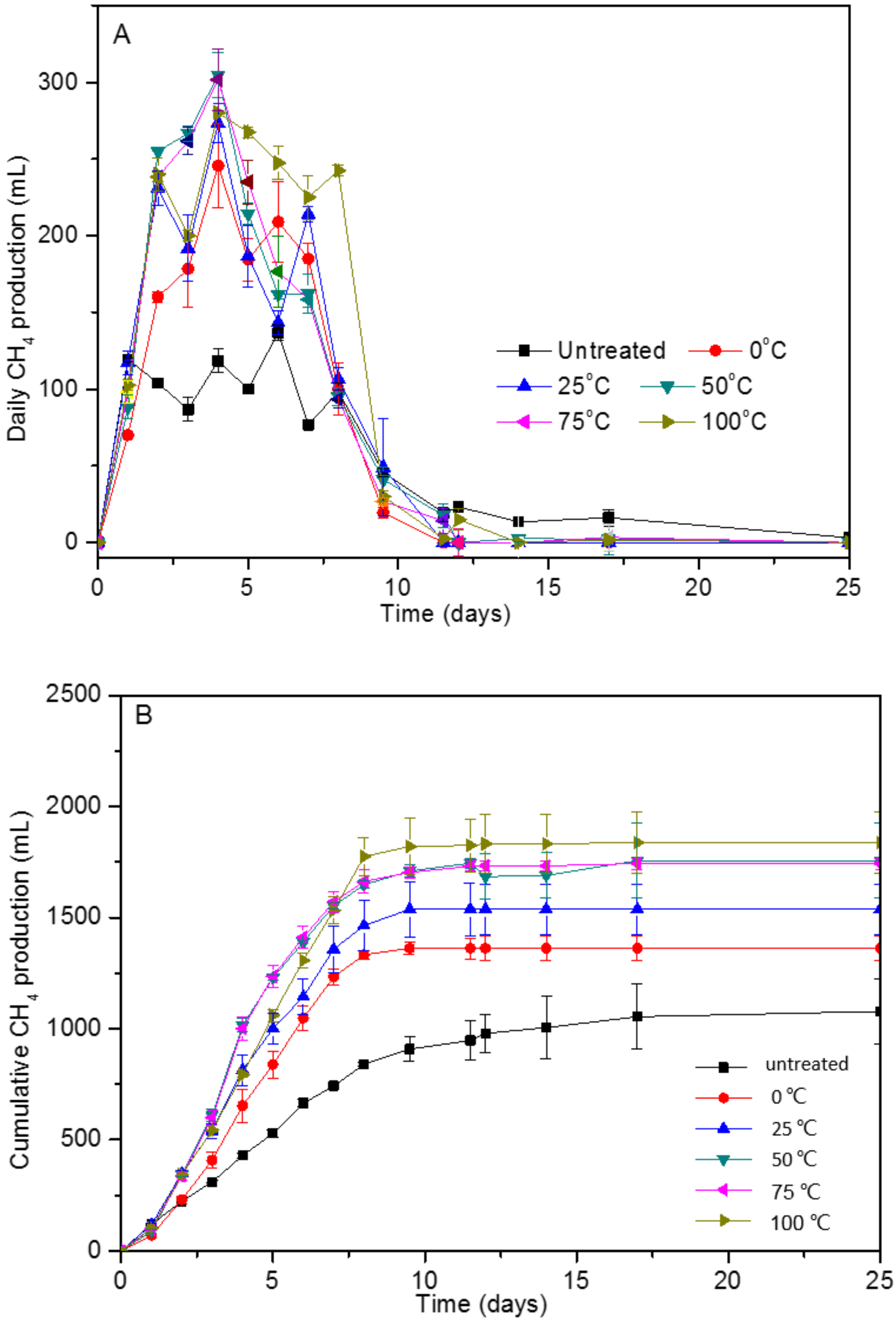

Fig. 6. Effects of NaOH pretreatment at different temperatures on daily methane production (A) and cumulative methane yield from banana stems (B)

Biodegradability Improvement

The reduction in TS, VS, methane yield (mL/g TS), methane production based on the amount of VS digested (M/VSd), and digestion time (DT_{90}) in pretreated (over a temperature range of 0 °C to 100 °C) over control stems was investigated to evaluate the biodegradability of the banana stems pretreated under different conditions (Table 4). In the treated stems, TS and VS reduction significantly increased from 67.9% to 74.2% and from 68.5% to 75.0%, indicating that more solid components in the banana stems were degraded, dissolving into the surrounding liquid or were converted to gas products such as methane and carbon dioxide.

As expected, the untreated banana stems had the lowest TS and VS reductions, at 64.1% and 64.8%, respectively. These results were similar to those obtained with some other biomass, *i.e.*, corn stover, asparagus stem, and banana stem co-digested with swine manure (Zheng *et al.* 2009; Zhang *et al.* 2013; Chen *et al.* 2014). Similar to the small increase in CMP from 50 °C to 100 °C, the increase in methane yield was also lower in this temperature range than that observed from 0 °C to 50 °C.

The variable M/VSd combines methane yield and substrate digestion to assess the biodegradability of banana stems. The M/VSd of the alkaline-pretreated banana stems exhibited a significant increase (from 271.8 mL/g to 334.7 mL/g; Table 4) compared with the untreated banana stems (227.3 mL/g) due to the higher methane yield after alkali pretreatment. The highest M/VSd value was obtained at 50 °C, which represented an increase of 48.2% over the control conditions. Further elevating the temperature caused a slight drop in M/VSd since the increase in the percent reduction of VS was not matched by a corresponding increase in the methane yield.

Table 4. TS and VS Reduction, Methane Yield, M/VSd, and DT_{90} of the Digestion of Banana Stems with or Without Alkaline Pretreatment

	TS reduction (%)	VS reduction (%)	Methane yield (mL/g TS)	M/VSd (mL/g)	DT_{90} (days)
Untreated	64.1 ± 2.4	64.8 ± 2.6	147.3 ± 20.0	227.3 ± 13.6	11.5 ± 1.3
0 °C	67.9 ± 3.1	68.5 ± 3.3	186.2 ± 7.3	271.8 ± 3.9	7.0 ± 1.0
25 °C	68.8 ± 0.6	69.5 ± 0.8	210.2 ± 15.5	302.4 ± 7.4	7.0 ± 0.6
50 °C	70.5 ± 1.8	71.2 ± 1.7	239.9 ± 23.0	336.9 ± 9.6	7.0 ± 1.0
75 °C	70.6 ± 0.6	71.4 ± 0.5	238.2 ± 3.4	333.6 ± 1.4	6.5 ± 1.2
100 °C	74.2 ± 0.6	75.0 ± 0.2	251.0 ± 18.8	334.7 ± 7.5	7.0 ± 1.2

Digestion time (DT_{90}), which is defined as the fermentation time required to reach 90% of the maximum methane yield, is a common index to evaluate the biodegradability during AD (Zheng *et al.* 2009). The fermentation experiments in this study were allowed to run for 25 days, and the final volume of methane reached at the end of this time period was considered as the maximum methane yield. DT_{90} of the untreated banana stems was 11.5 days, which a was shorter time than previously reported (Zhang *et al.* 2013). A higher inoculum to feedstock ratio and lower organic loading rates were possible factors leading to the shorter digestion time observed in the pretreated samples in this study compared to the previous report. When banana stems were pretreated with NaOH at 0 °C, the DT_{90} decreased to nearly 7 days; however, increasing the temperature further did not result in further reductions in DT_{90}. In conclusion, in terms of methane yield, biodegradability and digestion time, the optimal pretreatment temperature was 50 °C. In addition, the cumulative methane yield only increased slightly at 100 °C. Thus, 50 °C can be established as the optimal temperature, which maximizes methane production while lowering the energy requirements of the experimental process.

CONCLUSIONS

1. Increasing the pretreatment temperature led to an almost linear increase in the weight loss of the biomass and COD of the soak liquid.
2. Lignin and hemicellulose were the most unstable components during alkaline thermal pretreatment, as determined by the component removal rate (%); this exposed greater amounts of cellulose to microbial attack and therefore aided the anaerobic digestion process.
3. TS and VS reduction increased with pretreatment temperature, with a concomitant improvement in the methane yield, while the optimal biodegradability and digestion did increase further after 50 °C.
4. The optimal results were observed with 8% (w/w) NaOH and a pretreatment temperature of 50 °C, leading to a methane yield of 239.9 mL/g, which was 66.7% higher than that of the untreated banana stems.

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REFERENCES CITED

Azman, S., Khadem, A. F., Lier, J. B. V., Zeeman, G., and Plugge, C. M. (2015). "Presence and role of anaerobic hydrolytic microbes in conversion of lignocellulosic biomass for biogas production," *Critical Reviews in Environmental Science and Technology* 45(23), 2523-2564. DOI: 10.1080/10643389.2015.1053727

Bali, G., Meng, X., Deneff, J. I., Sun, Q., and Ragauskas, A. J. (2015). "The effect of alkaline pretreatment methods on cellulose structure and accessibility," *Chemsuschem* 8(2), 275-279. DOI: 10.1002/cssc.201402752

Campanaro, S., Treu, L., Kougias, P. G., Francisci, D. D., Valle, G., and Angelidaki, I. (2016). "Metagenomic analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy," *Biotechnology for Biofuels* 9(1), 26. DOI: 10.1186/s13068-016-0441-1

Chen, X., Gu, Y., Zhou, X., and Zhang, Y. (2014). "Asparagus stem as a new lignocellulosic biomass feedstock for anaerobic digestion: Increasing hydrolysis rate, methane production and biodegradability by alkaline pretreatment," *Bioresource Technology* 164, 78-85. DOI: 10.1016/j.biortech.2014.04.070

Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). "Inhibition of anaerobic digestion process: a review," *Bioresource Technology* 99(10), 4044-4064. DOI: 10.1016/j.biortech.2007.01.057

Deng, X. K., Dong, C. M., Xin, K., Zhao, N., Yang, J. M., and Ruan, Y. Z. (2014). "Comparative study on effects of composting of banana stem, eucalyptus bark and swine manure relative to ratio of ingredients," *Journal of Ecology and Rural Environment* 30(5), 658-663.

Deublein, D., and Steinhauser, A. (2011). *Biogas from Waste and Renewable Resources: An Introduction*, John Wiley & Sons, Weinheim, Germany.

Frigon, J. C., and Guiot, S. R. (2010). "Biomethane production from starch and lignocellulosic crops: A comparative review," *Biofuels Bioproducts and Biorefining* 4(4), 447-458. DOI: 10.1002/bbb/229

Getha, K., and Vikineswary, S. (2002). "Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process," *Journal of Industrial Microbiology and Biotechnology* 28(6), 303-310. DOI: 10.1038/sj/jim/7000247

Girio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S., and Bogel-Lukasik, R. (2010). "Hemicelluloses for fuel ethanol: A review," *Bioresource Technology* 101(13), 4775-4800. DOI: 10.1016/j.biortech.2010.01.088

Han, Y., Zhang, C., Chen, X., Li, Y., Yue, R., Jiang, L., and Li, S. (2016). "Methane production performance comparison of different agricultural residues," *Chinese Society of Agricultural Engineering* 32(01), 258-264. DOI: 10.11975/j.issn.1002-6819.2016.01.036

He, Y., Pang, Y., Liu, Y., Li, X., and Wang, K. (2008). "Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production," *Energy and Fuels* 22(4), 2775-2781. DOI: 10.1021/ef8000967

ISO17247-2005 (2005). "Coal ultimate analysis," International Organization for Standardization, Geneva, Switzerland.

Kapoor, M., Raj, T., Vijayaraj, M., Chopra, A., Gupta, R. P., Tuli, D. K., and Kumar, R. (2015). "Structural features of dilute acid, steam exploded, and alkali pretreated mustard stalk and their impact on enzymatic hydrolysis," *Carbohydrate Polymers* 124, 265-273. DOI: 10.1016/j.carbpol.2015.02.044

Khan, M. Z. H., Sarkar, M. A. R., Al Imam, F. I., Khan, M. Z. H., and Malinen, R. O. (2014). "Paper making from banana pseudo-stem: Characterization and comparison," *Journal of Natural Fibers* 11(3), 199-211. DOI: 10.1080/15440478.2013.874962

Li, C. X., Zhang, G. Y., Zhang, Z. K., Ma, D. C., and Xu, G. W. (2016). "Alkaline thermal pretreatment at mild temperatures for biogas production from anaerobic digestion of antibiotic mycelial residue," *Bioresource Technology* 208, 49-57. DOI: 10.1016/j.biortech.2016.02.064

Madhu, R., Veeramani, V., and Chen, S. M. (2014). "Heteroatom-enriched and renewable banana-stem-derived porous carbon for the electrochemical determination of nitrite in various water samples," *Scientific Reports* 4. DOI: 10.1038/srep04679

Mirahmadi, K., Kabir, M. M., Jeihanipour, A., Karimi, K., and Taherzadeh, M. (2010). "Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production," *BioResources* 5(2), 928-938. DOI: 10.15376/biores.5.2.928-938

Mittal, A., Rui, K., Himmel, M. E., and Johnson, D. K. (2011). "Effects of alkaline or liquid-ammonia treatment on crystalline cellulose: Changes in crystalline structure and effects on enzymatic digestibility," *Biotechnology for Biofuels* 4(64), 1-16. DOI: 10.1186/1754-6834-4-41

Mullassery, M. D., Fernandez, N. B., and Anirudhan, T. S. (2014). "Removal of mercury(II) ions from aqueous solutions using chemically modified banana stem: kinetic and equilibrium modeling," *Separation Science and Technology* 49(8), 1259-1269. DOI: 10.1080/01496395.2013.876046

Noremberg, B. S., Silva, R. M., Paniz, O. G., Alano, J. H., Gonçalves, M. R., Wolke, S. I., Labidic, J., Valentinid, A., and Carreño, N. L. (2017). "From banana stem to conductive paper: A capacitive electrode and gas sensor," *Sensors and Actuators B: Chemical* 240, 459-467. DOI: 10.1016/j.snb.2016.09.014

Padam, B. S., Tin, H. S., Chye, F. Y., and Abdullah, M. I. (2014). "Banana by-products: an under-utilized renewable food biomass with great potential," *Journal of Food Science and Technology*, 1-19. DOI: 10.1007/s13197-012-0861-2

Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., and Johnson, D. K. (2010). "Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulase performance," *Biotechnology for Biofuels* 3(2), 103-111. DOI:10.1186/1754-6834-3-10

Pei, P., Zhang, C., Li, J., Chang, S., Li, S., Wang, J., Zhao, M., Jiang, L., Yu, M., and Chen, X. (2014). "Optimization of NaOH pretreatment for enhancement of biogas production of banana pseudo-stem fiber using response surface methodology," *BioResources* 9(3), 5073-5087. DOI: 10.15376/biores.9.3.5073-5087

Ploetz, R. C., Kepler, A. K., Daniells, J., and Nelson, S. C. (2007). "Banana and plantain—An overview with emphasis on Pacific island cultivars," *Permanent Agriculture Resources, Hawaii, USA*, 27, 1-27.

Rice, E. W. (2012). *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC.

Sindhu, R., Binod, P., Janu, K. U., Sukumaran, R. K., and Pandey, A. (2012). "Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol," *World Journal of Microbiology and Biotechnology* 28(2), 473-483. DOI: 10.1007/s11274-011-0838-8

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO.

Sobotka, M., Votruba, J., Havlik, I., and Minkevich, I. G. (1983). "The mass-energy balance of anaerobic methane production," *Folia Microbiologica* 28(3), 195-204. DOI: 10.1007/BF02884083

Taherdanak, M., and Zilouei, H. (2014). "Improving biogas production from wheat plant using alkaline pretreatment," *Fuel* 115, 714-719. DOI: 10.1016/j.fuel.2013.07.094

Xiao, R. F., Zhu, Y. J., Li, Y. D., and Liu, B. (2013). "Studies on vascular infection of *Fusarium oxysporum* F. Sp. cubense race 4 in banana by field survey and green fluorescent protein reporter," *ESci Journal of Plant Pathology* 2(1), 44-51.]

Yang, L. C., Xu, F. Q., Ge, X. M., & Li, Y. B. (2015). "Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass," *Renewable & Sustainable Energy Reviews* 44, 824-834. DOI: 10.1016/j.rser.2015.01.002

Yang, T., Yun, L., Hou, H., Xi, B., He, L., Wang, X., Huang, C., Wu, K., Zhao, Y., and Chen, B. (2014). "Spatial structure characteristic analysis of corn stover during alkali and biological co-pretreatment using XRD," *Bioresource Technology* 163, 356-359. DOI: 10.1016/j.biortech.2014.03.099

Zhang, C., Li, J., Liu, C., Liu, X., Wang, J., Li, S., Fan, G., and Zhang, L. (2013). "Alkaline pretreatment for enhancement of biogas production from banana stem and swine manure by anaerobic codigestion," *Bioresource Technology* 149, 353-358. DOI: 10.1016/j.biortech.2013.09.070

Zhang, C., Bi, S., Zhao, M., Chang, S., Li, Y., Pei, P., Gao, X., Zhang, L., Li, J., and Li, S. (2016). "Biogas production performance of different components from banana stems," 30(8), 6425-6429. DOI: 10.1021/acs.energyfuels.6b00657

Zheng, M., Li, X., Li, L., Yang, X., and He, Y. (2009). "Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment," *Bioresource Technology* 100(21), 5140-5145. DOI: 10.1016/j.biortech.2009.05.045

Zheng, Y., Zhao, J., Xu, F., and Li, Y. (2014). "Pretreatment of lignocellulosic biomass for enhanced biogas production," *Progress in Energy and Combustion Science* 42, 35-53. DOI: 10.1016/j.pecs.2014.01.001

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