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Paper No. CSBE17043

Prerequisite – a hot air oven pretreatment for anaerobic digestion of lignocellulose waste material

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Written for presentation at the
CSBE/SCGAB 2017 Annual Conference
Canad Inns Polo Park, Winnipeg, MB
6-10 August 2017

ABSTRACT This study was focused on obtaining the best pretreatment conditions (temperature and time) and followed by an anaerobic digestion process for the biological bioconversion of pulp and paper mill sludge into biogas in batch assay. The effectiveness of pretreatment study was studied by chemical and compositional analyses. The pretreatment at 80°C for 90 min shows highest impact on sludge solubilization rate. The soluble chemical oxygen demand and volatile fatty acid were increased by 1.71 and 1.95 times respectively. In addition to that XRD and FT-IR spectroscopic characterization shows the development of aliphatic, unsaturated and carbonyl carbon functionalities in the pretreated samples. FESEM picture also confirms the change in structural alteration after thermal pretreatment. Thus hot air oven pretreatments serve to disrupt the lignocellulosic structure, making the cellulose easily accessible to acidogenic microorganisms. Biochemical methane potential test was carried out to determine the efficacy of pretreated and untreated pulp and paper mill sludge. The methane production potential was increased from 264 to 303 mL of CH_4 per g $VS_{removed}$ in pulp and paper mill sludge after pretreatment.

Keywords: Thermal pretreatment, anaerobic digestion, lignocellulose, bioprocessing, biorefinery.

INTRODUCTION An anaerobic digestion is a mature technology widely applied for bioenergy production from the sewage sludge, animal manure, agricultural residue, industrial sludge and energy crops in developing and developed countries (Li and Khanal, 2016). These substrate has high levels of biodegradable organics make it an ideal feedstock for anaerobic digestion. Due to the continuous production in the industrial level, there is a huge accumulation of waste especially in pulp and paper mill industry, the enormous amount of sludge was produced. Sludge handling and management of this produced sludge from effluent treatment plant of pulp and paper mill has account more than 60% of the total wastewater treatment plant operating cost (Monte et al., 2009; Xu and Lancaster, 2009).

The lignocellulose material consists mainly of cellulose, hemicellulose, and lignin. Due to the inherent recalcitrant structure of lignocellulose material towards enzymatic deconstruction pretreatment is necessary to make the holocelluloses amenable to enzymatic hydrolysis and fermentation (Yang and Wyman, 2008; Sharma et al., 2015). In recent years there has been renewed interest in pretreatment of lignocellulose biomass and waste for anaerobic digestion (Veluchamy et al., 2017). The several structural and compositional properties of lignocellulosic content render it resistant to biodegradation. Even though there has been an extensive research on the effect of pretreatment, the correlation between the degradability of lignocellulose material, the structural and compositional properties remain unclear and contradictory (Zheng et al., 2014). Different pretreatment technologies i.e., physical, chemical, and biological pretreatments have been studied and applied to increase the conversion of organic matter to valuable energy source as biogas (Elliott and Mahmood, 2007). The aim of these pretreatment was to improve the accessibility and/or solubilize the organic fraction of sludge. As hydrolysis is a rate-limiting step in anaerobic digestion of pulp and paper mill sludge (PPMS) (Elliott and Mahmood, 2012; Wood et al., 2009) and also in previous study by Veluchamy and Kalamdhad (2017a) reported that pretreatment is necessary to fasten the hydrolysis stage because of the longer digestion time. To accelerate the hydrolysis step, a hot air oven pretreatment was investigated.

Due to the compositional difference between the municipal sludge and PPMS, a systematic comparison of pretreatment on sludge solubilization and methane yield is essential in order to assess the options in treating PPMS. To delineate the effect of hot air oven pretreatment condition such as temperature and time study was performed to fasten the hydrolysis step in anaerobic digestion. Therefore in the light of literature, the objective of this study is to the investigate the effect of hot air oven pretreatment conditions (temperature and time), also to elucidate the changes produced in the structure and composition of the PPMS before and after pretreatment, followed by the batch anaerobic digestion process for the biological conversion of PPMS into biogas.

MATERILS AND METHODS

Materials

PPMS was obtained from the Nagaon paper mill located at Jagiroad, Assam, India. PPMS collected from the filter house of effluent treatment plant was used as a substrate. Collected PPMS was stored in refrigerator at 4^{o}C before further use. Cow dung collected from farm present nearer to the Indian Institute of Technology Guwahati, North Guwahati, India, was used as the inoculum for the batch study. Table 1 shows the initial chemical characterization of substrate and inoculum.

Table 1. The initial chemical characterization of pulp and paper mill sludge and cow dung

Parameter	Pulp and paper mill sludge	Cow dung
Moisture content (%)	73.75±1.36	79.73±1.46
Total solid (TS) (%)	26.25±0.40	20.27±0.76
Volatile solid (VS) (%)	18.88±0.75	16.62±1.36
VS/TS	71.92	81.98
pH	6.53±0.5	7.30±0.36
Total Nitrogen (%, b.d.w[a])	1.03±0.4	3.08±0.04
Organic carbon (%,b.d.w[a])	35.16±1.14	46.34±1.36
C/N ratio	34	15

[a]b.d.w. based on dry weight

Numbers are mean ± standard deviation from three replicates.

Pretreatment of PPMS

For hot air oven pretreatment study, 50 g of PPMS is added to 150 mL of distilled water and mix properly to get slurry form. The pretreatment study is split into two approach. The first approach based on temperature as variable, the prepared samples were kept at different temperatures for a particular time. After obtaining the suitable temperature, the second approach based on time as variable, the prepared samples were kept at varied time interval with the selected best temperature inside the hot air oven. The sample kept outside without any pretreatment is served as control.

Hot air oven (SONNU-220/230 volt AC; temperature 0 to 200^{o}C) was used for the pretreatment of PPMS. Based on preliminary and literature (Wood et al., 2009) pretreatment temperature and time were selected. To study the influence of hot air on sealed conical flasks containing PPMS at varied temperature 70, 80, 90, 100 and 110^{o}C for 30 min were used. To study the effect of exposure time, different time duration such as 30, 60, 90, 120, and 150 min were used with the best temperature evaluated in temperature study.

Anaerobic biodegrability setup

To test the rate and extent of anaerobic biodegradability of untreated (control) and thermally pretreated PPMS batch anaerobic reactor was performed. Anaerobic reactors (1,000 mL) were loaded with the mixture of pretreated PPMS and cow dung (set 1), and the mixture of untreated and cow dung (set 2) at a fixed food to microorganisms ratio equals to 2 based on the previous study (Veluchamy and Kalamdhad, 2017a). After adding essential macro and micro nutrients, all the reactor were purged with nitrogen in order to make anaerobic condition. Then it was coupled to another aspirator bottle having 1.5 N sodium hydroxide (NaOH) solution that absorbs the CO_2 and H_2S and displaces NaOH solution that represent methane (CH_4) production (Veluchamy and Kalamdhad, 2017b). All the reactor were maintained at room temperature varies from 30 to 38^{o}C. Manual mixing was done three to four time in a day. The experiment was conducted in triplicate for 35 d.

Analytical methods

The different parameter i.e, pH, soluble chemical oxygen demand (sCOD), total solids, moisture content, and volatile solids, were analysed using standard protocols according to APHA (2005). Volatile fatty acid was analysed by pH titration method adopted by the DiLallo and Albertson (1961). Lignin was analysed using National renewable energy laboratory (NREL) procedure (Ehrman, 1996; Templeton and Ehrman, 1995). Cellulose and hemicellulose were analysed according to the protocol adopted by Updegraff (1969) and Goering and Van (1975) respectively. To determine the significant in dissimilarities between unpretreated and pretreated, analysis of variance (ANOVA) was used in the Origin Pro 9.0. Pretreatment experiments were conducted thrice and their samples were analysed in triplicate.

Instrumental characterization

To study the structural changes happen on the PPMS material surface before and after pretreatment, the instrumental characterization was carried out. The surface alteration was identified by field emission scanning electron microscopy (FESEM (Zeiss, Sigma)). The changes in lignocellulosic fraction was studied by using Fourier transform infrared (FT-IR). The FT-IR spectrum (PerkinElmer Spectrum 2) was recorded from 4000 to 400 cm^{-1} at 16 scan with a resolution of 4 cm^{-1}. Moreover, the cellulose crystallinity has been characterized by X-ray diffractograms (XRD) with the Bruker, D-8 advance from 5^{o}C to 35^{o}C of diffracting angle (2θ) at the scanning speed of 5^{o}/min. The crystallinity index was measured from the XRD pattern by an empirical method (Veluchamy et al., 2017) using the following equation.

$$I_C = \frac{I_{cr} - I_{am}}{I_{cr}} \quad (1)$$

Where I_C is the crystallinity index, I_{cr} is the maximum diffraction intensity at peak position at 2θ = 31^{o} and I_{am} is the intensity at 2θ = 18^{o}.

RESULTS AND DISCUSSION

Pretreatment effect on chemical characteristics

- The effect of temperature and time in hot air oven pretreatment

The effect of temperature and time on PPMS heated by the hot air in hot air oven was shown in Figure 1(a) and (b). It was observed from the Figure 1(a), increase in temperature has shown increase in solubilization rate which was measured in the form of sCOD and VFA. The sCOD and VFA increased up to 80°C and then it got reduced. The reduction was attributed by the higher vaporization of volatile compound produced from the pretreatment effect than the solubilization rate (Appels et al., 2010; Ariunbaatar et al., 2014). The pretreated sample at 80°C has high sCOD and VFA. For the time study, the samples were kept at the temperature of 80°C for the different time duration. From the time study Figure 1(b), the sample kept at the 90 min showed the highest sCOD with the increment of 1.71 times and VFA of about 1.95 times increment in hot air oven pretreatment. Ariunbaatar et al. (2014) also reported that the pretreatment at lower temperature need high pretreatment exposure time (minimum of 1 h) for the better solubilization rate. Wood et al. (2009) depicted that the hydrothermal pretreatment of sulfite and Kraft pulp mill waste activated sludge at 170°C for 60 min had the better solubilization.

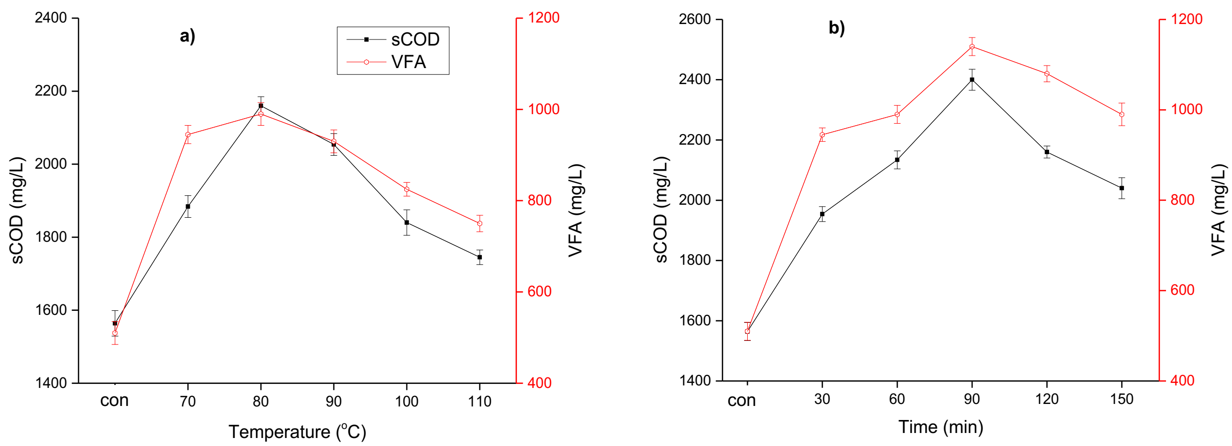


Figure 1. The effect of temperature and time on sCOD and VFA of pulp and paper mill sludge sample kept inside hot air oven.

a) The effect of temperature on the sCOD and VFA in hot air oven for 30 min.
b) The effect of time on the sCOD and VFA in hot air oven for 80°C.

Pretreatment effect on composition and characterization of PPMS

- Compositional analysis

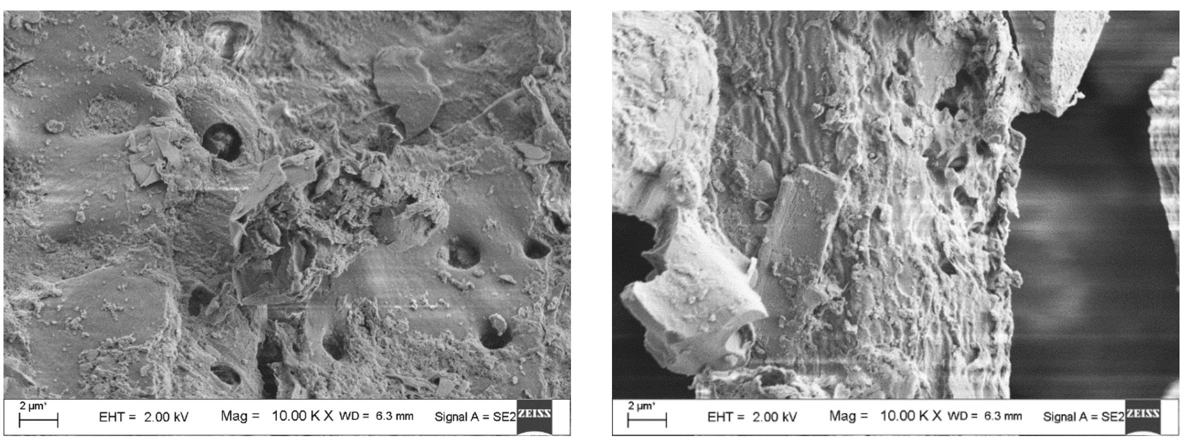
The compositional analysis was performed to identify the changes in composition of cellulose, hemicellulose and lignin, at the before and after the pretreatment of PPMS. The pretreatment conditions and lignocellulose contents of untreated and pretreated PPMS (Table 2). From Table 2, it was perceived that the increase in acid soluble lignin, insoluble lignin, and cellulose and reduction in the hemicellulose content. The increase in acid soluble and cellulose represents the hot air oven pretreatment has the higher liquefaction and delignification characteristic. Similar results also obtained in the previous literature in thermal pretreatment (Li et al., 2007). Suggesting that the presence of higher acid soluble lignin could be easily fermentable by the fermentative microorganisms (Patel et al., 2015) and cellulose present in the pretreated PPMS has been easily accessed by the microbes when compared to the untreated PPMS. The reduction in the hemicellulose content may leads to the formation fermentation inhibitor such as furfural from xylose, hydroxymethylfurfural (HMF) from glucose, and acetic acid from acetate groups attached to the hemicellulose (Gaur et al., 2016; Sharma et al., 2015). During hot air oven pretreatment, hemicellulose is breakdown mainly into xylose and small amount of glucose. Thus, higher liquefaction and delignification of lignocellulose matter by the hot air oven pretreatment could speed-up the hydrolysis step, with the enhanced biogas production in anaerobic digestion.

Table 2. Pretreatment conditions and lignocellulose contents of untreated and thermally pretreated PPMS

Pretreatment	Temperature (^oC)	Time (min)	Acid inslouble lignin (%)	Acid soluble lignin (%)	Hemicellulose (%)	Cellulose (%)
Control	-	-	2.30±0.12	3.53±0.12	6.27±0.75	32.75±1.26
Hot air oven	80	90	3.01±0.11	6.27±0.08	5.89±0.59	38.75±1.37

- FESEM

The morphological structural changes over the untreated (control) and hot air oven pretreated PPMS was studied by FESEM. Figure 2 shows the FESEM images of control and hot air oven pretreated PPMS. There was a greater change in morphological structure. The control sample has firm trampled plane surface with some batches, whereas in hot air oven pretreated sample show a morphological change with fissures and large hollows on their surface layer. Due to the morphological alteration, there was a release of easily biodegradable substance that enhance the bioaccesibility of cellulose in the pretreated sample. Similar finding has been identified for the lignocellulose material using different heating devise (Gabhane et al., 2011; Zhen et al., 2014).



(a) Untreated (control) (b) Hot air oven pretreated

Figure 2. FESEM images of untreated and thermally pretreated pulp and paper mill sludge.

- FTIR

With the help of characteristic absorbance band at FTIR spectrum, the structural and components change in cellulose, hemicellulose, and lignin can be extensively studied. Morán et al. (2008) had been well documented the characteristic FTIR bands of cellulose, hemicellulose, and lignin. The characteristic band changes in relative absorbance were applied to determine the structural and components change after pretreatment. FTIR spectra of pretreated and untreated PPMS was shown in Figure 3. The variation in the characteristic band in relative absorbance of lignocellulose content was addressed on Table 3. The efficiency of hot air oven pretreatment effect on structural changes has been studied by comparing with Morán et al. (2008) (as denoted by wavenumber reported) and the present pretreatment study (as denoted by wavenumber picked) with the corresponding assignment are enlisted (Table 3). After pretreatment the relative absorbance band of the lignin characteristic bands are slightly decreased, whereas the relative absorbance band of the cellulose are slightly increased, indicating a changes in the relative structural and components of lignocellulose content. There was no study used the FTIR analysis for the structural changes at pretreatment stage, whereas the study related to reduction in organic content and aliphatic chain during anaerobic digestion (Yang et al., 2009) has been studied.

Table 3. Characteristics FTIR bands of pretreated pulp and paper mill sludge

Fiber component	Wavenumber reported (cm^{-1})	Wavenumber picked (cm^{-1})	Functional group	Assignment
Cellulose	1048	1014	C-OH	O-H bond bending
	1270-1280	1252	C-O-C	Aryl-alkyl ether vibration
	1640	1631	Fiber-OH	Absorbed water bending
	2890	2875	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3346	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching
Hemicellulose	1048	1014	C-OH	O-H bond bending
	2890	2860	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3337	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching
Lignin	700-900	684	C-H	Aromatic hydrogen deformation
	700-900	800	C-H	Aromatic hydrogen deformation
	1048	1014	C-OH	O-H bond bending
	1158	1160	C-O	Phenyl bending
	1430	1433	$O-CH_3$	Methoxyl-$O-CH_3$ deformation
	2890	2860	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3333	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching

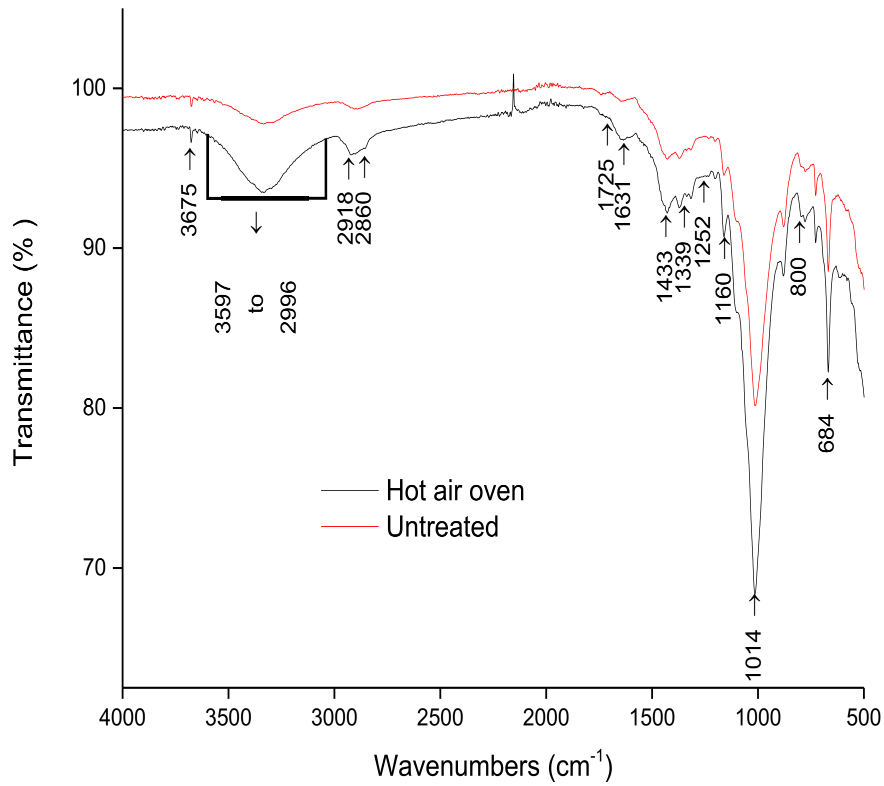


Figure 3. FTIR spectra of untreated and thermally pretreated pulp and paper mill sludge.

- XRD

By studying the changes in cellulose crystallinity, the effectiveness of pretreatment on PPMS was evaluated. The XRD spectra of control and pretreated PPMS (Figure 4) has shown great variation in their peaks reduction. The sharp peaks in control sample indicates the higher crystalline in nature whereas in pretreated PPMS retain less peaks that indicates the amorphous nature. Reduced peaks (Figure 4) after pretreatment depicts the reduction in their cellulose crystallinity. There was a severe reduction in cellulose crystallinity after pretreatment.

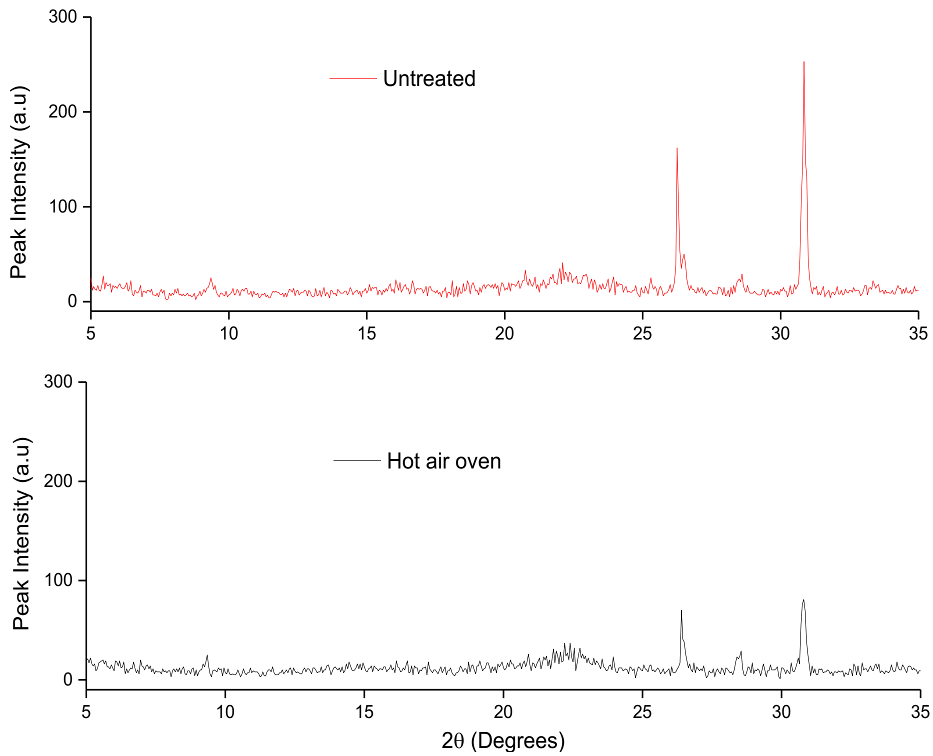


Figure 4. XRD of the untreated and thermally pretreated pulp and paper mill sludge.

It was observed mathematically that the cellulose crystallinity reduced from 0.9137 in control to 0.7941 in hot air oven pretreated PPMS. Yang et al. (2009) depicted that the reduction in cellulose crystallinity would contribute to an increased fragility and susceptibility of cell wall for the cellulolytic microorganisms. These results are in accordance with previous pretreatment studies for ethanol production (Gabhane et al., 2011; Yang and Wyman, 2008).

Pretreatment effect on biogas production

The hot air oven pretreatment of PPMS at 80°C for 90 min showed the higher rate of solubilisation. Hence, to study the rate and efficiency of hot air oven pretreatment for enhanced methane production, batch anaerobic study was conducted for pretreated and unpretreated PPMS. The steep curve of cumulative methane production of hot air oven pretreated PPMS provides the evident for superior degradation than the untreated PPMS (Figure 5). The cumulative methane production on the 35th day of hot air oven pretreated PPMS was 2478±10 mL of methane whereas for untreated PPMS was 2045±12 mL of methane. The pretreatment at 80°C for 90 min alter the lignocellulose structure in PPMS leading to production of fermentable sugars that can be quickly and efficiently biodegraded with the enhanced methane production. This rapid degradation was due to the presence of easily accessible soluble organic matter for the acidogenic and methanogenic microorganisms for the enhanced methane production (Hu et al., 2016). The biodegradation of untreated PPMS took more time with less methane production. This is because of the inherent recalcitrant structure of lignocellulose content (Zheng et al., 2014) in PPMS. Thus, it depicting the necessity of pretreatment to cut down (or) speedup the hydrolysis step in anaerobic digestion of PPMS for the enhanced quantity of methane production. It was calculated mathematically that the specific methane production potential rate was increased from 264±5 to 303±4 mL methane /g VS in PPMS.

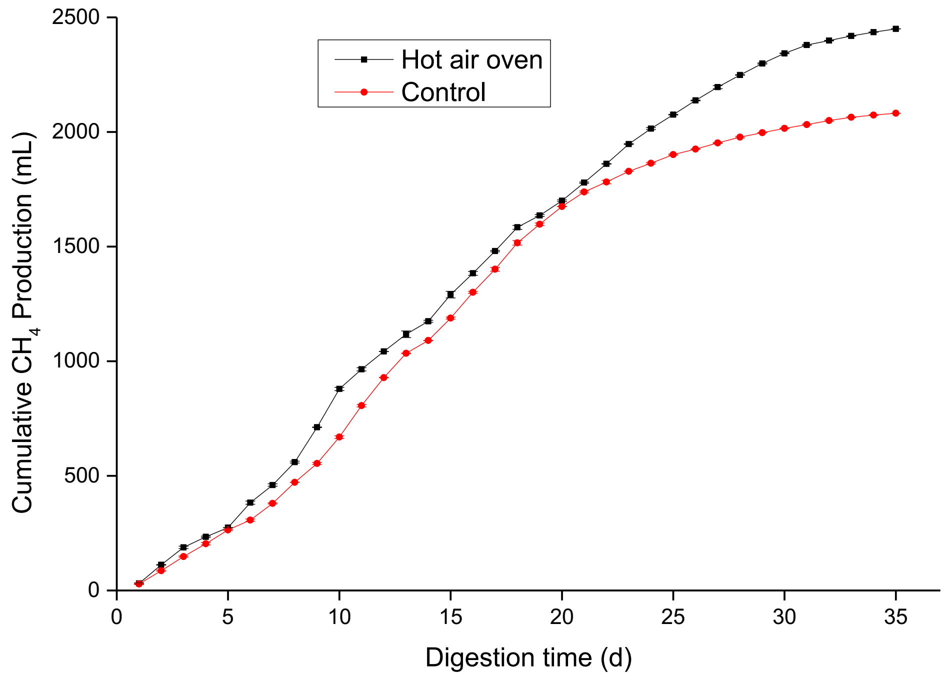


Figure 5. Cumulative methane production of hot air oven pretreated and untreated (control) pulp and paper mill sludge.

CONCLUSION This study exposed that the hot air oven pretreatment has significant effect on lignocellulose content in speedup the hydrolysis step in anaerobic process. It was experienced that the organic and inorganic compounds were efficiently solubilized at 80^{o}C for 90 min in hydrothermal pretreatment. Compositional and instrumental analysis such as FESEM, XRD, and FT-IR spectra showed hot air oven pretreatment has strongly affect the lignocellulose structures. Batch study naked the methane production potential was increased from 264±5 to 303±4 mL methane /g VS in pulp and paper mill sludge after pretreatment.

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