Effects of Thermo-Chemical Pre-Treatment of Maize Leaves on Methane Production.

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ABSTRACT

Thermo-chemical pretreatment of dried Maize Leaves (ML) was examined at different NaOH loading rates and temperatures to determine effects of pretreatment on its bio-degradability in terms of the hydrolysis yield and degradation of ligno-cellulosic materials for biogas production. Temperature of 100^{o}C and NaOH loading rate of 6% is recommended as a proper ML pre-treatment conditions, under which biological methane production potentials of the pre-treated ML were improved by 38% in comparison with untreated ML.

Six identical column-type stainless steel leaching bed reactors (LBRs) with a working volume of 10-litre were set up to examine the hydrolysis and acidification of ML under three organic loading rates (OLRs). The highest ML hydrolysis yields of 51.5-58.2%, acidification yields of 57.2-60.3% and volatile solids (VS) removals of 62.1-66.3% were obtained in the conditions of addition of inoculum, leachate dilution with tap water (1:1 – v/v) every 6 days and pH adjustment to 6.5. The cellulase activity can be used as an indicator to the hydrolysis process.

(Keywords: thermos-chemical pretreatment, hydrolysis, acidogenesis, anaerobic digestion, biogas)

INTRODUCTION

There are many types of substrates that can be used in biogas plants to obtain biogas. They all have different biogas production potentials. However, arable land in the world is limited. Therefore, selection of the substrate should be made carefully [1].

Across the globe, anaerobic digestion (AD) of farm waste has been practiced for many years, as it provides a way to treat raw, organic waste material, improves nutrient recovery and generated a renewable energy source known as biogas [3]. Anaerobic treatment comprises of decomposition of organic material in the absence of free oxygen and production of methane, carbon dioxide, ammonia and traces of other gases and organic acids of low molecular weight.

Dry matter of Maize Leaves (ML) comprises up to 75% cellulose, hemi-cellulose, and lignin. The crystalline structure of cellulose and the non-water soluble nature of lignin are barriers to the penetration of microbes and enzymes during anaerobic digestion [4]. Hydrolysis is often assumed to be a rate-limiting step in anaerobic digestion particularly with materials like ML that contain high structural carbohydrates [5]. Treating ligno-cellulosic biomass prior to anaerobic digestion can accelerate hydrolysis and improve biogas yields. Treatments include physical, biological, thermal, and chemical processes [6].

Physical pre-treatment aimed at reducing the particle size and crystallinity of lingo-cellulosic, such as milling processes (ball milling, two-roll milling, hammer milling, colloid milling and vibro-energy milling) and high-energy radiation (such as microwave irradiation at different power-levels), is expensive [7]. Biological pre-treatment with lignin-degrading micro-organisms is energy-saving (fossil fuel saving compared with physical and thermal pre-treatment) but it takes a long time to enrich sufficient lignin-degradation microorganisms; additionally, the cellulose and hemi-cellulose are partially consumed during pre-treatment [8].

Chemical pre-treatment with alkali at ambient temperature is readily achievable and may have commercial potential. Sodium hydroxide (NaOH) is typically used in alkaline pre-treatment. Lin et al. [9] investigated NaOH pre-treatment of pulp and paper sludge prior to anaerobic digestion and found that the methane productivity was improved by up to 83.5% at a NaOH loading rate of 8% by weight of volatile solids (VS) in pulp and paper sludge. Zhu et al. [10] applied NaOH pre-treatment to corn stover at an alkaline VS loading rate of 5% by weight of VS in corn stover and found enhanced biogas production by up to 37% compared with that without pre-treatment.

Thermal pre-treatment with steam or hot water is also effective in hydrolysis of crops and crop by-products. Thermal pre-treatment at temperatures of 160 ^{o}C or above results in the solubilization of hemicellulose and lignin. However, phenolic compounds can be produced, which have inhibitory or toxic effects on methanogenic bacteria [11]. Gossett et al and Chang et al. [12] proposed thermal pre-treatment in combination with alkaline pre-treatment at temperatures of between 100-150 ^{o}C to improve the pre-treatment efficiency for switch grass, corn stover and poplar wood.

Wu et al. [13] found that CH_4 production potential of meat and bone meal was increased from 389 to 503 ml CH_4/ g, after alkaline (NaOH loading of 25% by weight of VS in meat and bone meal) and heat (55 °C) pre-treatments. Rafique et al. [14] achieved an increase of 60% in methane production from dewatered pig manure after pre-treatment at 70^{o}C and a calcium hydroxide loading rate of 5% by weight of VS in pig manure.

There have been few studies conducted on thermal alkali pre-treatment of ML in order to improve subsequent methane production [15]. Therefore, the objective of this study was to examine (1) the effects of combined thermal (ambient, 70^{o}C, 110 ^{o}C and 160 ^{o}C temperatures) and alkali (NaOH loading rates of 2%, 3.5%, 6% and 8.5% by weight of VS in pre-treatment ML), on the hydrolysis yield, kinetics of hydrolysis process and degradation of ligno-cellulosic materials, and (2) the effects of pre-treatment on the methane production potential of ML.

MATERIALS AND METHODS

Maize Leaves

MLs were obtained from the Crop Science Department, University of Nigeria, Nsukka. The ML was harvested in May, 2015 from a Maize farm in the Department. The herbage was field-wilted for 24 hours before being baled and wrapped with 6 layers of polythene stretch film. 2 kg of the baled ML was obtained from each of 5 bales and mixed in a plastic bag after 5 weeks of ensilage. After transport to the laboratory, it was oven dried at 60°C until no further weight loss and sliced in a blender (Philips HR2000/50 Silver Food Blender) to an average length of approximately 10 mm. In order to prevent biological decomposition, the dried and sliced ML was stored in an air tight container at 4°C until one day before it was used; and then it was transferred to the laboratory at an ambient temperature of about 25 °C. The characteristics of fresh ML are given in Table 1.

Table 1: Characteristics of Fresh Maize Leaves.

Characteristics	Values
C/N ratio	26
VS (%)	89.3
TS (%)	16.2
COD (mg/l)	1200
Cellulose (%TS)	32.7
Hemi-Cellulose (%TS)	25.6
Lignin (%TS)	7.5

Alkali-Thermal Pre-Treatment of ML

The pre-treatment experiment was carried out in 50 ml digestion vials. In each vial, 2g VS of Dried ML (2.1 g DML) and 8 ml of NaOH solution were added. The NaOH solutions had NaOH concentrations of 2.5 g/l, 6.25 g/l, 12.5 g/l and 18.75 g/l, corresponding to NaOH loading rates of 1 g per 100 g VS from Dried ML (2%), 2.5 g per 100 g VS from Dried ML (3.5%), 5.0 g per 100 g VS from Dried ML (6%) and 7.5 g per 100g VS from Dried ML (8.5%), respectively. The vials were sealed with caps and placed in a temperature controlled oven where the temperature was set at 30°C, 70°C, 110°C, or 160°C. Under each pre-treatment condition, 12 parallel digestion vials were used.

Two vials were used as controls, in which only 2 g VS of Dried ML and 8 ml of de-ionized water were added. The vials were taken out of the oven at intervals depending on the time to reach equilibrium which varied from 4 - 48 hours. After cooling, samples were taken from the vials for measurement of soluble COD in the liquid phase, and cellulose, hemi-cellulose and lignin contents in the biomass. The results from the pre-treatments were obtained after subtracting the results from the control treatments. The experiment was conducted in duplicate.

Measurement of Biological Methane Production Potential of Untreated and Pre-treated ML Samples

The biological methane production potentials (BMPs) of the untreated and pre-treated dried ML were tested in 1-litre batch glass digesters. Each digester had two ports on the lid, one for liquid sampling and the other for gas sampling. Liquid samples were taken using a 5-ml syringe and gas samples were taken using a 50-ml syringe.

First, 20 g VS Dried ML (untreated Dried ML or Dried ML pre-treated at 100°C and NaOH VS loading rates of 2%, 3.5%, 6% and 8.5% by weight of VS in Dried ML) was added to each digester. Second, 600 ml of inoculum (33.3 g VS/l) was added, which was taken from laboratory-scale continuously stirred digesters treating mixtures of pig manure and ML.

The VS-based feedstock to inoculum ratio was 1:1. For the control digesters, only 600 ml of inoculum was added. Thirdly, tap water was added to each digester to make a final working volume of 800 ml so as to obtain a Dried ML-inoculum mixture with a solid concentration suitable for anaerobic digestion and to reduce the headspace of the digesters. Finally, the digester headspaces were flushed with N_2, and then the digesters were sealed with caps and placed in a shaker incubator at 35°C.

The experiment was carried out in duplicate. The methane content in the headspace and the biogas volume produced from each digester were measured daily. The biological methane production potential of each sample, in liters was calculated by dividing the total volume of methane produced up to the point when anaerobic degradation was complete by the total mass of VS initially added. During the experiment no additional nutrients were added to the digesters.

RESULT AND DISCUSION

Hydrolysis Yield and Kinetics

The effect of several inhibitors (oxygen, chloroform, halogenated organics, heavy metals, etc.) has been studied by several investigators and discovered to have negative effect on anaerobic digestion [16]. That anaerobic digestion is a gradual process involving degradation of organic matter by anaerobic organism [17]. Ugwuoke et al., reported that more degradation of lignocelluloses gives higher biogas yield [18]. The thermal-chemical pre-treatment resulted in the solubilization of ligno-cellulosic materials and the release of sCOD [19]. The increase in sCOD under each pre-treatment condition, referred to the total COD of fresh maize leaves, is shown in Figure 1.

At a constant temperature and pH, the hydrolysis rate followed a first-order equation for the conversion of particulate biomass to soluble substrates by chemicals Rajan et al., [20]. The hydrolysis of dried ML by thermo-chemical pre-treatment can therefore be described with a first-order equation [20].

As shown in Figure 1, increasing both the pre-treatment temperature and the NaOH loading rate increased the release of sCOD at the equilibrium state. However, temperature had a much greater effect than NaOH concentration [21]. For example, increasing temperature from 30^{o}C to 160^{o}C resulted in 8.3, 7.7, 7.2, and 6.2 fold increases in sCOD at NaOH loading rates of 2%, 3.5%, 6%, and 8.5%, respectively. In contrast, increasing the NaOH loading rate from 2% to 8.5% increased sCOD by 90%, 38%, 42%, and 36%, respectively, at pre-treatment temperatures of 30^{o}C, 70^{o}C, 110^{o}C, and 160^{o}C.

Degradation of Lingo-Cellulosic Materials

In biomass containing ligno-cellulose, such as Maize leaves, cellulose and hemicellulose are densely packed by layers of lignin, which protects them against enzymatic hydrolysis; therefore, the digestibility of biomass mainly depends on the degree of solublization of lignin [22]. Alkali treatment breaks alkali-labile linkages between lignin monomers, or between lignin and polysaccharides, because alkaline solution can ionize the carboxylic and phenolic groups, increase the solubility of individual fragments or induce the swelling of the cell wall [23].

Increasing the NaOH loading rate increased the reduction of the lignin content in Dried ML, causing the solubilisation of lignin from Dried ML to the liquid phase. In each pre-treatment condition, the reduction percentage of lignin in Dried ML was the highest among the three ligno-cellulosic materials: lignin, cellulose and hemicellulose. At each temperature, increasing the NaOH loading rate resulted in the increased lignin reduction.

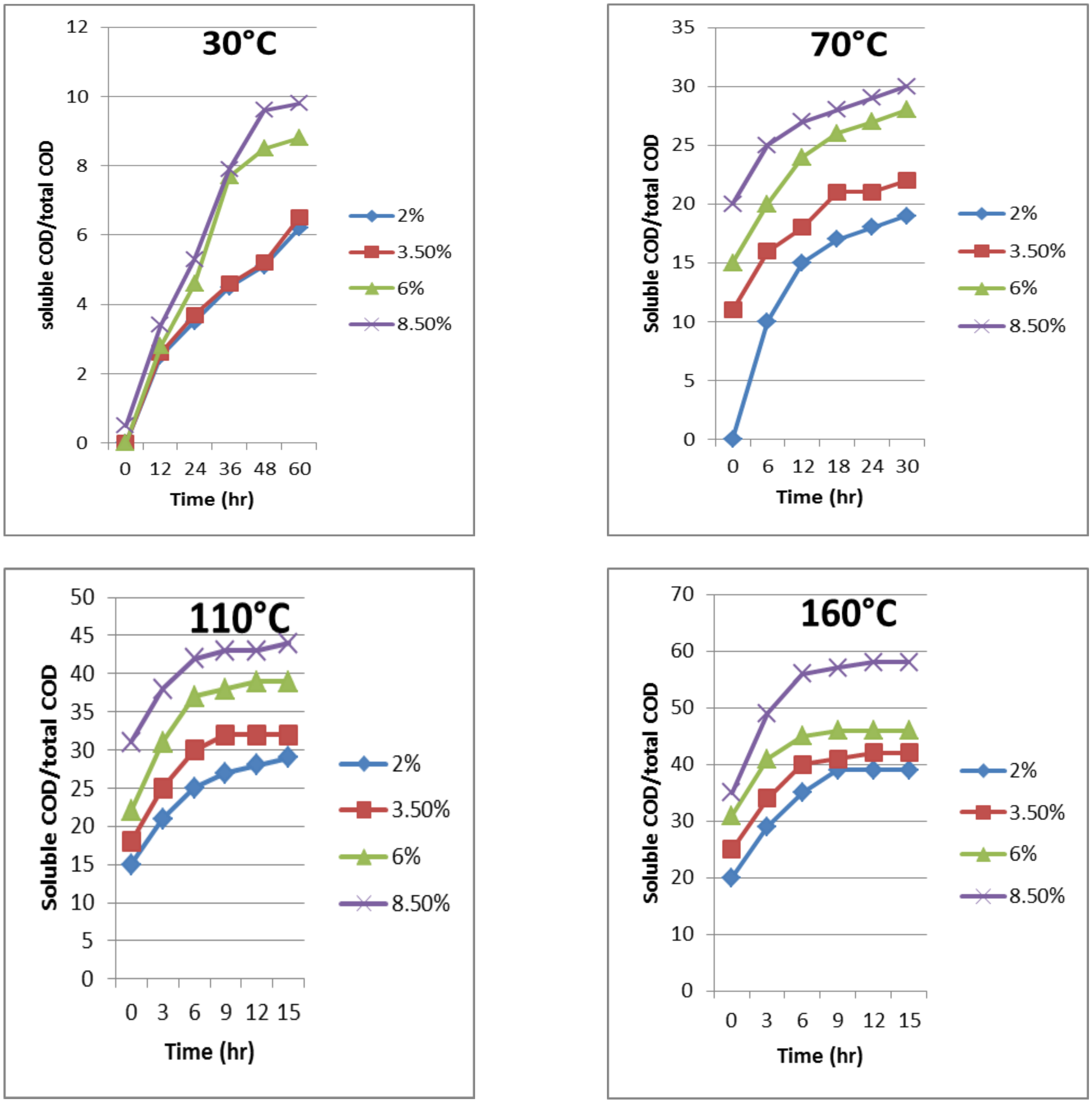

Figure 1: Profiles of Soluble COD Release during Maize Leaves Pretreatment.

This result is confirmed by Zhu et al. [24], who found that increasing the NaOH loading rate from 2% to 8.5% resulted in increased lignin reduction in corn stover from 9.1% to 46.2% at ambient temperature.

The reduction of the hemicellulose content in the dried ML biomass was lower than that of lignin, but higher than that of cellulose. Xiao et al. [25] attribute solubilization of hemicellulose by alkaline solution to the disruption and breaking of hydrogen bonds; they have observed that nearly all of the ester-linked structures of hemicellulose can be cleaved by alkali. The reduction of hemicelluloses increased as the NaOH loading rate and temperature increased (Figure 2). The reduction of the cellulose content in Dried ML was much lower than that of lignin and hemicellulose.

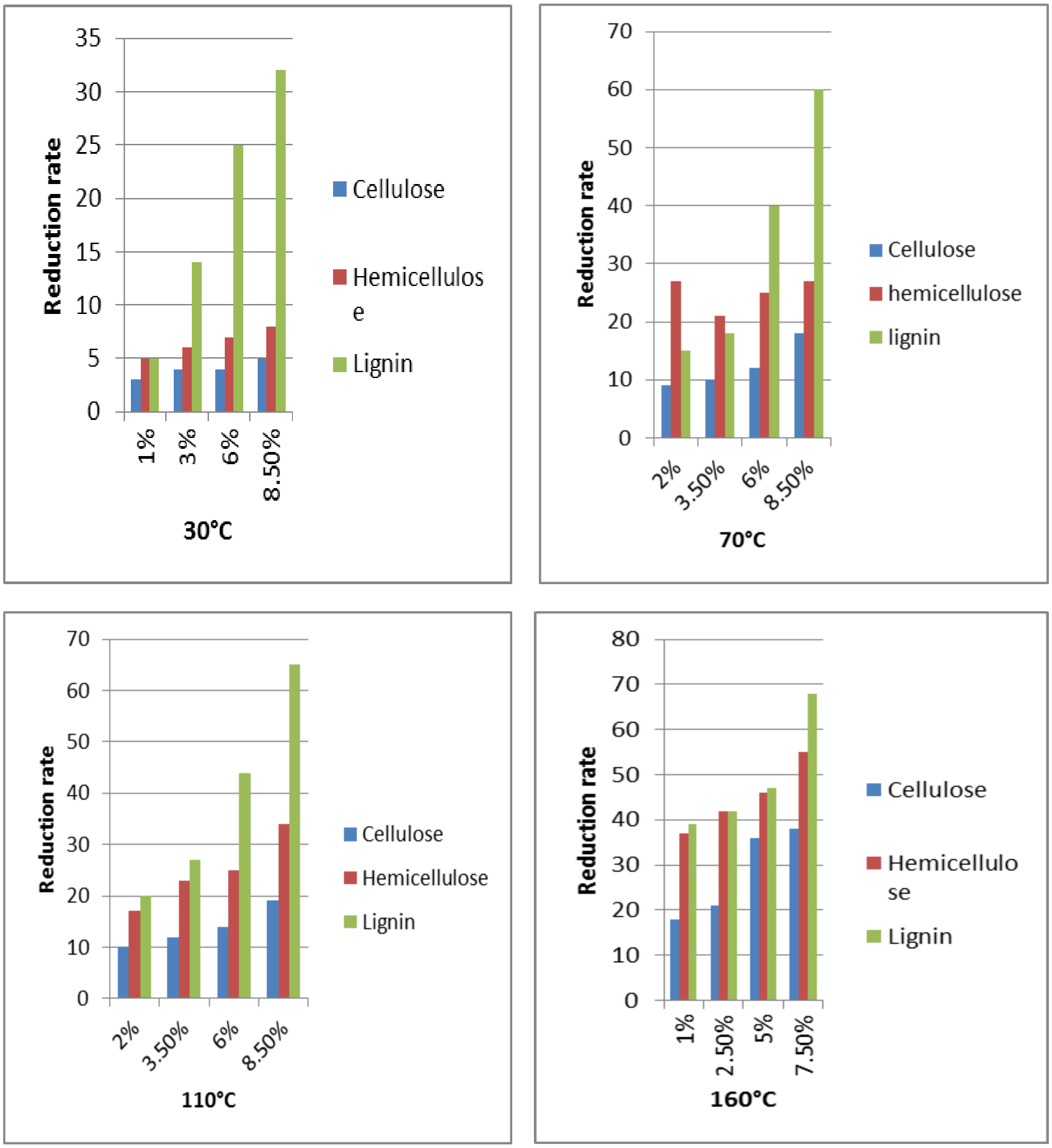

Figure 2: Profiles of Reduction of Lignocellulosic Materials with Thermo-Chemical Pre-treatment.

At 30 ^{o}C, 60 ^{o}C, 100 ^{o}C and 150 ^{o}C, with the increase of NaOH loading rate from 2% to 8.5%, the reduction of cellulose increased from 3.6% to 5.4%, from 9.6% to 18.9%, from 10.3% to 21.2%, and from 17.6% to 35.2%, respectively. The relatively low cellulose reduction was possibly due to (1) the physical protection afforded by lignin and hemicellulose, and (2) the modification of cellulose crystallinity during alkaline treatment, rather than direct solubilization [26].

Temperature proved to be a crucial factor in the pre-treatment of ligno-cellulosic biomass. Increasing temperature can result in a higher reduction rate of cellulose, hemicellulose and lignin in DML. However, high pre-treatment temperatures need increased energy input, increase the complexity of the process operation and may generate possible inhibitors (such as phenolic compounds) to enzymatic hydrolysis and fermentation [27].

As mentioned previously, the solubilization of lignin is critical to the digestibility of ML. Zhu et al. reported the solublization of lignin needed to be at least 31% to allow corn stover to be efficiently digested. In addition, the methane production rate and the methane yield from anaerobic digestion depend on the hydrolysis process in terms of concentrations of sCOD Xie et al.

At 30°C, the solublization of lignin was less than 31%. At 70°C, the solublization of lignin was 38% and 57% at the NaOH loading of 6% and 8.5% by the weight of VS in ML. When the temperature was increased to 100 °C, the reduction of lignin at NaOH loading rates of 6% and 8.5% was improved by 13%-16% in comparison with that at 60 °C. In addition, the sCOD concentration in the equilibrium state was 50% - 54% higher. When the temperature increased from 110^{o}C to 160^{o}C, the solublization of lignin was more than 31% at all NaOH loading rates. The sCOD production was 25%-38% higher than that at 100°C; however, the solubilization of lignin only increased by 4.0% at the NaOH loading rate of 8.5% compared to that at 100^{o}C. Therefore, 100^{o}C is considered to be an appropriate pre-treatment temperature at these perspectives. However, further research on energy balancing, in consideration of the energy input due to thermo-chemical pretreatment and the energy output due to the enhanced biogas yield, should be carried out.

Changes of Structure of and Functional Groups in Pre-Treated Dried ML

Changes of the physical structure of dried ML before and after thermo-chemical pre-treatment were imaged by a scanning electron microscope SEM at the magnification of 100 and 2500X. The texture of untreated dried ML was compact and smooth, which is due to the crystalline structure of untreated lignin and cellulose. After thermo-chemical treatment, the overall structure of the dried ML samples was still relatively intact, whereas dried ML treated at 100^{o}C and the NaOH loading rates of 6% and 8.5% became thinner.

The surface of all treated dried ML samples became rough with small particulates attached, which resulted from partial breakdown of the lignin and cellulose structure. With increasing the NaOH loading rate up to 8.5%, it was observed that more particulates were attached to the surfaces.

Methane Production Potential of Pre-Treated Dried Maize Leave

The daily methane yield and cumulative methane yield of untreated dried ML and dried ML pre-treated at NaOH loading rates of 2%, 3.5%, 6%, and 8.5% at 100^{o}C are presented in Figure 3.

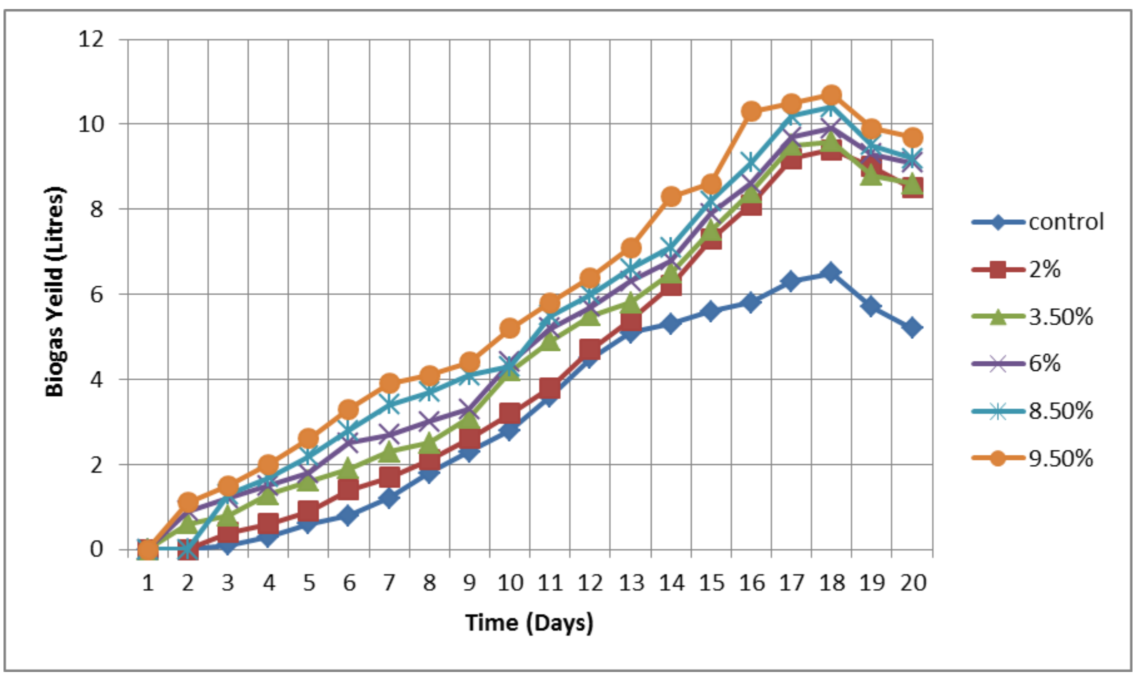

Figure 3: Daily Biogas Yield (Liters) versus Time (Days).

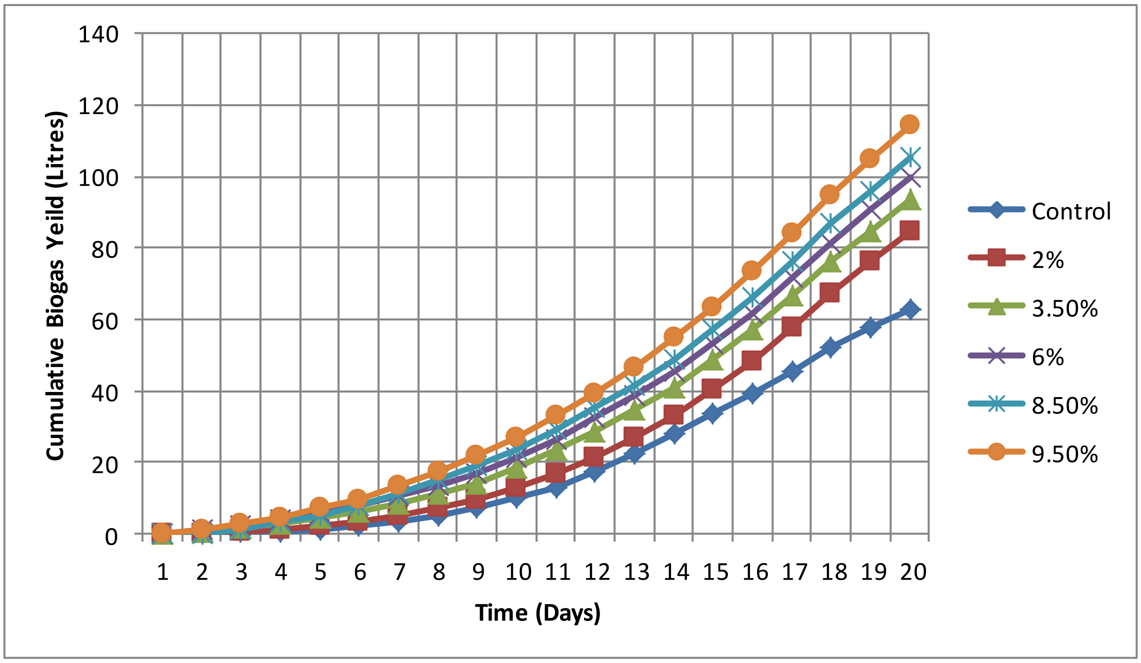

Figure 4: Cumulative Biogas yield (Liters) versus Time (Days).

Results show that there were significant differences ($P<0.01$) between treated dried ML samples and untreated dried ML samples and among dried ML samples treated at the four NaOH loading rates. Similar trends were observed for the daily methane yield of dried ML pre-treated at NaOH loading rates of 2%, 3.5% and 6%, but the trend for Dried ML treated at the NaOH loading rate of 8.5% was different. The main differences included: (1) the peak value of the daily methane yield; (2) duration of the lag phase; and (3) the duration of the effective biogas production period. The daily methane yield peaked on Day 8 (1.8L/day), Day 9 (2.3L/day), Day 12 (4.5/day), Day 21 (5.2L/day) and Day 17 (6.3L/day) for Dried ML treated at NaOH loading rates of 2%, 3.5%, 6% and 8.5%, and untreated Dried ML, respectively.

The cumulative methane yields were 7.1, 14.1, 26.5, 105.3 and 39.2 L for dried ML treated at the NaOH loading rates of 2%, 3.5%, 6% and 8.5% and untreated dried ML, respectively. The results show that thermal-alkali pre-treatment resulted in higher biological methane production potentials. At the NaOH loading rates of 2%, 3.5%, 6% and 8.5%, in comparison with untreated Dried ML, the increase in the VS was 10%, 23%, 38% and 39%, respectively. The higher VS of pre-treated Dried ML was attributed to the opening of the 'acetyl valve' and partial opening of the 'lignin valve', making the substrate more accessible for hydrolysis. According to, lime (calcium hydroxide) pre-treatment (with heating from 100 to 140 ^{o}C) increased the digestibility of low-lignin containing biomass, such as corn stover.

CONCLUSION

The study revealed further that Maize leaves as biomass waste has great potentials for generation of biogas and its use should be encourage due to its early retention time and high volume of biogas yields. Also in this study, it has been found that temperature variation, pH, and concentration of total solid, etc., are some of the factors that affected the volume yield of biogas production. The maximum atmospheric temperature recorded in the experiment was 39°C.

Pre-treatment at increasing temperature and NaOH loading rates enhanced the solubilization and the biodegradability of dried ML biomass and thereafter, increased the biological methane production potential, which were increased by 10%-38.9%. However, pre-treatment at the NaOH loading rate of 8.5% resulted in the longest lag phase. The VS removals achieved were in the range of 76.9%-96.7%. It is recommended that pre-treatment condition of 100^oC and the NaOH loading rate of 6% be used.

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