INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 4, p. 101-110, 2021

RESEARCH PAPER OPEN ACCESS

Effects of sequential pretreatments on selected agricultural biomass feedstock for bio methane production

Antonio-Abdu Sami M. Magomnang*, Dianne Mae M. Asiñero

Mechanical Engineering Department, University of Science and Technology of Southern Philippines - CDO Campus, CM Recto Avenue, Lapasan, Cagayan de Oro City, 9000, Philippines

Key words: Agricultural biomass, bio-methane, pretreatment.

http://dx.doi.org/10.12692/ijb/18.4.101-110 Article published on April 29, 2021

Abstract

Agricultural Biomass are abundant and potential alternative for fossil fuels in Mindanao, Philippines. However, biomass contains lignocellulose that is recalcitrant to enzymatic hydrolysis because of its structural complexity. As a solution, pH controlled liquid hot water (LHW) and Ultrasonic pretreatment of cellulosic feedstock is employed in order to improve its enzymatic digestibility and making the cellulose more accessible to cellulase enzymes. The LHW pretreatment is carried out by cooking the feedstock using autoclave at temperatures between 160 and 190 degrees C and at a pH of 4-7. An additional 3-4% w/v Sodium Hydroxide solution is deployed to further improve its enzymatic digestibility. This resulted in an increase of methane production up to 300% more due to the pretreatment of rice straw and coconut shells. Further, the pretreated coconut shell subjected to Ultrasonication with 3% NaOH and Liquid Hot water has the best effect among the pretreatment of biomass feedstocks of rice straw and coconut shell at certain NaOH concentrations. This would give a viable estimate on the possible methane production from the co-digestion of these resources. Also, the enhancement of the biogas yield was mainly attributed to the improvement of biodegradability of rice straw and coconut shells through these pretreatments. The changes in chemical compositions, chemical structures, and physical characteristics made rice straw and coconut shell feedstocks become more available and biodegradable and thus were responsible for the enhancement of the biogas yield. These results are contributing to develop a feasible biogas production from rice straw and coconut shell.

* **Corresponding Author:** Antonio-Abdu Sami M. Magomnang ✉ a_magomnang@yahoo.com

Introduction

Biogas is a renewable, high-quality fuel that can be produced from various organic raw materials and used for various energy services. Biogas technology has been developed and widely used over the world because it has several advantages – reduction of the dependence on non-renewable resources, high energy-efficiency, environmental benefits, available and cheap resources to feedstock, relatively easy and cheap technology for production, and extra values of digestate as a fertilizer (Bagher *et al.*, 2015). However, the current status of biogas production and utilization varies largely among continents. To overcome the issues related to the utilization of lignocellulosic materials like rice straw and coconut shell for biogas production, new pretreatment strategies have been evaluated and developed. One of these strategies is the combination of sodium hydroxide hydrolysis, ultrasonication, and liquid hot water pretreatment. Typically, the ultrasonication and liquid hot water pretreatment were used to modify the lignin and cellulose structure of the biomass to generate ethanol (Placido *et al.*, 2013); but for this case, said pretreatments were done to improve biogas yield. In a study by He *et al.* (2008), sodium hydroxide hydrolysis was found to substantially increase the biogas yield of rice straw.

Currently, there are researches on the pretreatment of biomass wastes similar to paddy straw using sodium hydroxide-microwave pretreatment of Kaur and Phutela (2016), it was reported that 4% NaOH-30 min microwave was found to be the best pretreatment which resulted in a 65.0% decrease in lignin content and 88.7% reduction in silica content that resulted in 54.7% increase in biogas production (Kaur and Phutela, 2016). Moreover, a mechanical disruption of algae using ultrasound homogenizer and thermo-alkaline pretreatment enzymatic hydrolysis demonstrated the best biogas yield than other pre-treatment which reached 626.5 mL/g COD with 62.65% of biodegradability (Karray *et al.*, 2015). Furthermore, liquid hot water pretreatment of giant reed in a Parr reactor and alkaline pretreatment was studied using the various NaOH concentrations which improve glucose yield that is significantly increased the cumulative methane yield by 63% than of the untreated biomass (Jiang *et al.*, 2016). Also, the ultrasonication of Cotton Gin Trash (CGT) followed by a liquid hot water pretreatment and ligninolytic enzyme pretreatment combination generated high amounts of sugar that increased delignification and modified the cellulose structure of the CGT as confirmed by the FT-IR Spectrum (Placido *et al.*, 2013).

However, a combination of these pretreatments (ultra-sonication, liquid hot water, and NaOH hydrolysis), has not been tested in rice straw (RS) and coconut shell (CS) biomass wastes. Thus, the synergic effect of these technologies may raise de-lignification, cellulose conversion, and biogas yield from RS & CS. The effect of these pretreatments over the RS & CS structure can be determined using the Fourier transform infrared (FT-IR) spectrum. In principle, FT-IR analysis is applied to study qualitatively the modifications in the structure and is not utilized for quantitative analysis. However, it can be used to identify the modification in biomass structure after different pretreatments than the conventional structural examination.

From a broad perspective, the requirements of the pretreatment are to (1) improve the accessibility of the enzymes to the cellulose and hemicelluloses and thereby the degradability, (2) avoid degradation or loss of the carbohydrates, (3) avoid the production of potential process inhibitors, (4) be cost and energy-efficient, and (5) have a low negative impact as much as possible on the environment (Philippoussis *et al.*, 2001).

Anaerobic co-digestion experiments were performed in this study to provide information on the long-term effects, such as eventual inhibition during the digestion of a lignocellulosic substrate. A co-digestion is often beneficial, since it supplies the system with more nutrients, leading to a better balance in the C/N ratio and the pH. It also improves the stability of the process and increases methane yield due to positive synergistic effects thereby increasing the economic value of the biogas.

This study aims to determine the effects of various pretreatment methods (alkalinity using NaOH, ultrasonication, liquid hot water using autoclave) on the biomass properties and biogas yield in the anaerobic co-digestion of agricultural biomass waste utilizing cattle (cow) manure co-digested with different biomass feedstocks such as rice straw, coconut shell, and sewage sludge as inoculum for power generation applications.

Materials and methods

Preparation of co-digestion mixtures

Rice straws were obtained from the Texas A&M AgriLife Research Center in Beaumont, Texas. Coconuts, on the other hand, were bought from the local supermarket in College Station, Texas. The coconut meat was removed and the shells were set aside for further processing. The rice straw and coconut shell samples were ground in a Wiley mill to achieve an average particle size of approximately 1 mm in diameter.

Pretreatment of selected agricultural biomass wastes

The experiment followed the sequence of pretreatments such as ultrasonication with alkaline treatment through NaOH simultaneously; liquid hot water. Furthermore, rice straw and coconut shell samples were subjected to mechanical disruption using the ultrasonication process. A solution containing 10% w/v solid biomass was put in an ultrasonicator (Hielscher Ultrasonic Processors, Ringwood, NJ, USA) with operating conditions set at the highest value of amplitude (100%) and cycle (1). Inside the reactor, ultrasonic waves create cavitation in the liquid medium (NaOH) which contains 250 grams of rice straw sample. Samples in 3,000 ml Erlenmeyer flask were manually stirred, then sonicated for 1 hr. After the ultrasonication process, the biomass was not washed before hot water treatment. The liquid hot water pretreatment was performed in an autoclave for 1 hr at a temperature of 121 ^{o}C and working pressure of 15 psi. The slurry was drained off. The remaining solids were washed with deionized water until the washings were clean, colorless and the pH was neutral. The rice straw was then dried overnight in an oven at 105 ^{o}C. Dried rice straw and coconut shell were stored in polyethylene bags and were used for proximate analysis (total solids, volatile solids, and ash), chemical analysis (cellulose, hemicellulose, lignin) using FT-IR. Untreated rice straw and coconut shell were also subjected to analysis to determine the extent of their degradation.

Experimental set-up

For each batch trial, reactors were filled with the inoculum, which is cattle manure, and sewage sludge; and co-digested simultaneously with various combinations of biomass like rice straw and coconut shell. Control reactors containing only cattle manure, and cattle manure with sewage sludge were maintained during each batch trial. Feed compositions used for the batch trials are given in Table 1. Each reactor had a total working volume of approximately 4.25 L, and duplicate reactors were used for each feed composition. The trials were performed in an environmental chamber maintained at 35°C, as shown in Fig. 1. The digesters were mixed daily by turning them upside-down and shaking for about 20 seconds.

Biogas volumes were recorded daily. The gas collectors were marked to provide a direct reading of volume. Biogas was discharged from the gas collectors by lifting the overflow carboy above the collectors to refill the collectors with water and opening the valves on top of the digesters to allow the gas to exit.

Analytical methods for determining the pretreatment effects

To determine the structural composition of the rice straw biomass before and after the pretreatments, the analytical protocols developed by the National Renewable Energy Laboratory (NREL) of the US Department of Energy were followed. This entails the determination of the total solids in biomass and total dissolved solids in liquid process samples (Sluiter *et al.*, 2011); the past protocols were developed using dried biomass. Fourier Transform InfraRed (FT-IR) spectroscopy (Shimadzu, IR Affinity–1 with a MIRacle universal sampling accessory) was used to evaluate the structural properties of the rice straw and coconut shell with and without pretreatments. The infrared spectra collected range was 4000 to 700 cm^{-1} with a resolution of 4 cm^{-1}. Further, the functional groups and their respective wavelengths on IR spectra are shown in Table 2.

Gas analysis

Gas sampling was done through the use of a tee connection located between the gas collector and digester which was sealed with a rubber stopper. The stopper was removed and a syringe was inserted into the tee to collect a gas sample. While gas was flowing out of the gas collector, the syringe was gradually opened to withdraw a sample. Approximately 60 mL was collected for each biogas sample which was analyzed immediately using a gas chromatography unit (SRI Gas Chromatograph). Gas samples were collected daily during the batch trials.

Results and discussion

Characterization of biomass

Based on the characterization results as shown in Table 3, the coconut shell and rice straw potentially be used as a substrate for the biogas production since it has lower nitrogen content (0.81% and 0.86%), which is a factor to consider to avoid ammonia inhibition of the digestion process. Aside from that, the digested cow manure and cow manure with rice straw from the previous anaerobic co-digestion experiments are used as inoculums produce a biogas production of 1,100 mL from the mixture of cattle manure with 3% NaOH pretreated rice straw was observed on the 1st day.

It indicates that reactors inoculated with CM+RS were efficient as well as the digested manures since the inoculum substrates are already acclimatized to the environmental conditions of the digester. This effect can be shown on the values of the carbon to nitrogen ratio of the digested manures.

Table 1. The pretreatment employed for the experiment.

Pretreatments	Rice straw	Coconut Shell	Cow Manure	Sludge
Liquid Hot Water (LHW)	250 ml	-	3,000 ml	1,000 ml
	-	250 ml	3,000 ml	1,000 ml
Ultrasonication (U)	250 ml	-	3,000 ml	1,000 ml
	-	250 ml	3,000 ml	1,000 ml
LHW + U	250 ml	-	3,000 ml	1,000 ml
	-	250 ml	3,000 ml	1,000 ml
Control (NO treatment)	250 ml	-	3,000 ml	1,000 ml
		250 ml	3,000 ml	1,000 ml
Cow Manure + Sludge	-	-	3,000 ml	1,000 ml
Cow Manure	-	-	3,000 ml	-
Sludge	-	-	-	1,000 ml

The analysis shows a high volatile solids content (VCM) in a coconut shell (92.42%) and followed by rice straw (66.56%), sewage sludge (70.38), and cattle manure (48.54) is significantly lower, demonstrating the spent microbial activity from the original process. Cattle manure (77.57%) showed a disparity of moisture contents, with rice straw (7.20%) having a low content level.

The analyzed peaks as shown in Fig. 2 were seen in the control and the pretreated samples of rice straw and coconut shell spectra. The highest peaks in all the samples are the ones related to cellulose (1320 cm^{-1}) for rice straw, which also have the highest variation among the pretreatments. The principal variation in the cellulose peaks is presented in the (1320 and 1645 cm^{-1}) for rice straw and (1240 and 1730 cm^{-1}) for coconut shell. These pretreatment peaks increased the strength of the signal compared with the control rice straw and coconut shell. The NaOH-ultrasonication and liquid hot water pretreatments showed the highest differences in signals compared with the biomass that was not subjected to any pretreatment.

Table 2. Functional group and their respective wavelengths on FTIR spectra.

Functional Group	Wavenumber (cm^{-1})
O-H bond stretch, Hydrogen bond hydroxyl (Alcohol/Phenol)	3335-3340
C-H stretch, Alkane (alkyl)	2930-2940
C=O Stretch, Conjugated carbonyl (amide)	1645-1730
C-H plane, bending of cellulose I&II	1320
C=O stretch, Ester Carbonyls,	1240
β-D- cellulose	898

The reduction in the lignin signals of pretreated rice straw was principally observed in the 1645 cm^{-1} spectra. This reduction is related to the modification of the lignin aromatic skeletal structure generated in the pretreatments mixture. Additionally, the coconut shell pretreatments made an important reduction in the bands near 1,500 cm^{-1}, where the band is associated with the aromatic C-H region of lignin.

Table 3. Composition of feedstock for anaerobic co-digestion process.

Composition	Cow Manure	Rice Straw	Coconut Shell	Sludge
Moisture (%)	77.57	7.20	22.29	96.43
Carbon (%)	21.64	33.71	45.21	36.17
Hydrogen (%)	2.83	4.73	5.93	5.13
Nitrogen (%)	0.84	0.81	0.86	6.32
Sulfur (%)	1.18	0.20	0.17	1.02
C/N	25.86	41.88	52.57	5.73
VCM (%TS)	48.54	66.56	92.42	70.38

The said results are consistent with the results reported in Zhang *et al.* (2015) Teghammar *et al.* (2012); He *et al.* (2008), wherein the NaOH-treated rice straw increased the biogas production by 27.3 – 64.5%; and this was attributed to the improvement of the degradability of the rice straw through NaOH pretreatments since lignin-carbohydrate complexes (LCC) was destroyed throughout the hydrolysis reaction, releasing more cellulose for biogas production (He, *et al.*, 2008). Similarly, a decrease in the crystallinity of cellulose fibers increases the biodegradability of rice straw (Teghammar *et al.*, 2012). Likewise, the association of lignin with holocelluolose decreased; and the cellulose and hemicellulose were degraded with NaOH treatment that enabled the rice straw to be easily accessed, attacked, and digested by anaerobic bacteria and enhanced the biodegradability of the rice straw and methane production (Zhang *et al.*, 2015).

The hemicellulose signals showed the most variable behavior. In 1240 cm^{-1}, and 1730 cm^{-1} signals, the U+NaOH+LHW pretreatments using coconut shell biomass present an increment in the absorbance. After the pretreatments, it shows a reduction in the signal of 1730 cm^{-1}, and any effect over the 1240 cm^{-1}. Likewise, the pretreated rice straw has not shown the wavelength near the 1730 cm^{-1} region.

Table 4. Production of Methane from selected agricultural biomass subject to different pretreatments.

Pretreatments	C/N Ratio	Biogas Yield (L)	Methane Yield (L)	CH_4 Conc. (%)
Rice Straw (3% NaOH U+LHW)	27.05	18.18	9.88	53.80
Rice Straw (4% NaOH U+LHW)	27.03	12.54	6.96	53.00
Coconut Shell (3% NaOH U+LHW)	24.49	23.12	13.84	55.90
Coconut Shell (4% NaOH U+LHW)	24.79	8.44	4.77	54.50

The variation in the signal intensity of 1730 cm^{-1} can be related to major exposure of the hemicellulose in the coconut shell biomass, which increased the absorption in the spectrum. This major exposure can be explained as the pretreatments resulted in modification of the hemicellulose structure or disrupted some of the linkages between the hemicellulose and cellulose or lignin. Also, the reduction in this signal can be related to the inversion of sugars from the hemicellulose in the liquid medium and propitiate a reduction of this signal in the biomass. This kind of reduction in hemicellulose was also found in acid hydrolysis of cotton stalks (Silverstein *et al*., 2007).

Fig. 1. Experimental set-up for the Anaerobic Digestion of biomass.

Biogas production: Effects on production and methane concentration

Fig. 3 shows the gas production for the different treatments (i.e. CM, CM+RS, etc.) conditions. Under the mesophilic conditions, the production of gases for the pretreatments was higher than the untreated ones. Compared with the study of Linhua *et al.* (2010), the mesophilic AD processes produced more methane rather than digesting at ambient temperature conditions. Based on that, it can be proved that temperature plays an important role in digesting rice straw from a mixture of cow manure (Linhua *et al.*, 2010). Applying alkaline-ultrasonication and liquid hot water pre-treatments for the rice straw and coconut shell transforms these biomasses into digestible carbon with minimal nitrogen component. This confirms the results of Quiroga *et al.* (2014), that using pretreatments (i.e. sonication) can lead to the best results for both biogas and methane production.

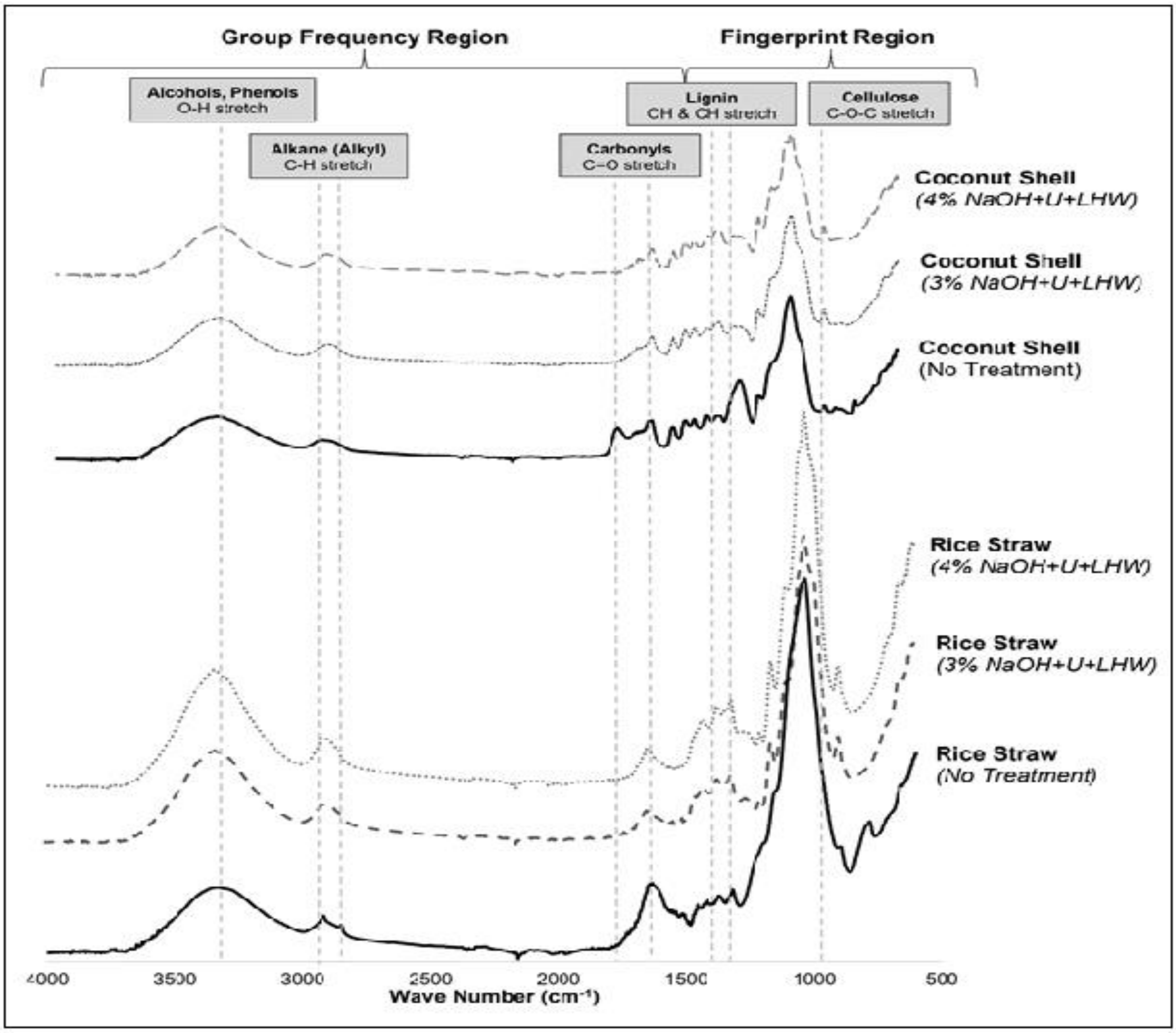

Fig. 2. FTIR spectra of biogas produced from the digestion of Rice Straw and Coconut Shell at varying pretreatments.

The CH_4 content in all the treatments including the control was relatively stable even though it reached the respective peak levels. Although the pretreated rice straw and coconut shell (at 4% NaOH and Liquid Hot Water) produced better results in terms of percent CH_4 in the first five sampling days, there was little statistical difference in average CH_4 volume produced between these two treatments over the experimental period because rice straw had higher biogas productivity in the early sampling days than untreated ones. Generally, both rice straw and coconut shells worked equally well in terms of increasing the CH_4 content given the C/N ratio of 25 and 27 (Table 2).

However, in the last four sampling days (Day 34-37), the CH_4 content in biogas generated from the 4% NaOH U+LHW pretreated coconut shell was consistently higher than that from the digester treated with rice straw (57.31% and 57.19% versus 52.69% and 53.35%), implying that using 4% NaOH U+LHW pretreatment of rice straw and coconut shell as a co-digestion substrate with cow manure could increase the methane content in the biogas by roughly 7% as opposed to using rice straw, and 350% for the untreated coconut shell which was significant. It was also observed that unlike the scenario in the C/N ratio of 24.79/1 and 27.03/1 and the NaOH concentration of 3% and 4% with U+LHW pretreatment, has no statistical difference in CH_4 content among all the treatments (a clear advantage of adding pretreated rice straw and coconut shell over untreated ones which virtually different than the control) to cow manure anaerobic digestion at a C/N of 25.

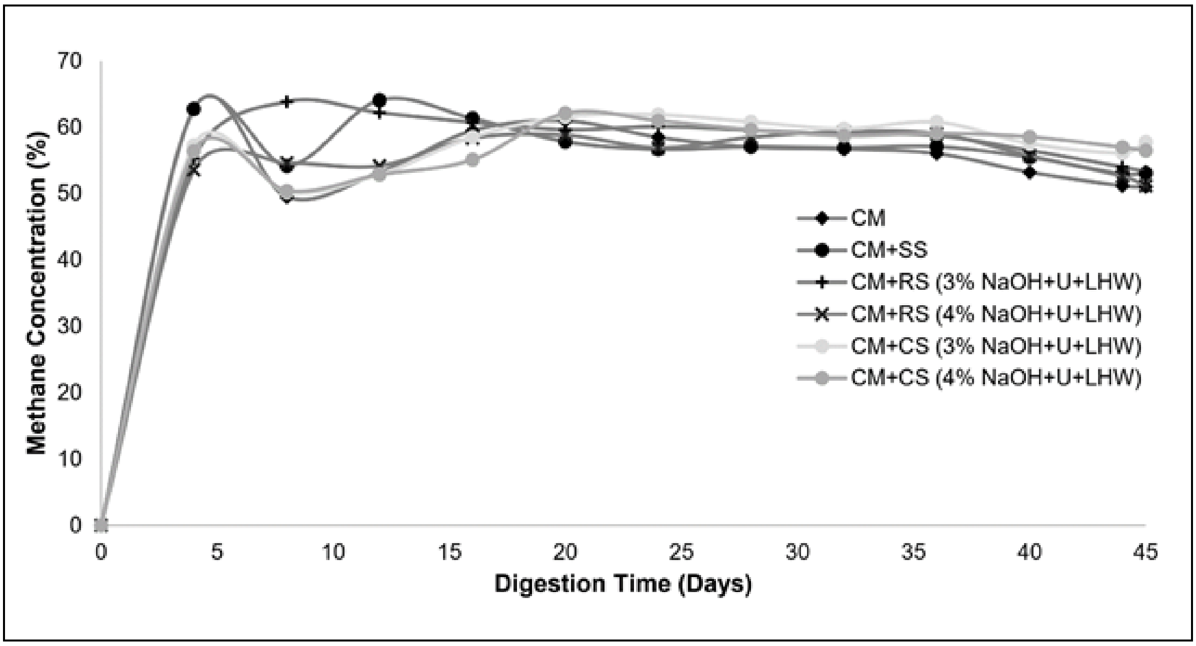

Fig. 3. Methane Concentration from the digestion of selected biomass subject with different pretreatments.

Substrate composition

As shown in Fig. 4 the pretreatment (using alkaline-ultrasonication and Liquid Hot water using autoclave) of rice straw and coconut shell with 3% w/v NaOH concentration (3% NaOH+U+LHW RS and 3% NaOH+U+LHW CS), yielded the highest heating value, followed by the 4% NaOH (4% NaOH+U+LHW RS and 3% NaOH+U+LHW CS). An additional examination as shown in Table 4, was conducted on the analysis for the untreated and pretreated rice straw and coconut as well as the sewage sludge and cattle manure.

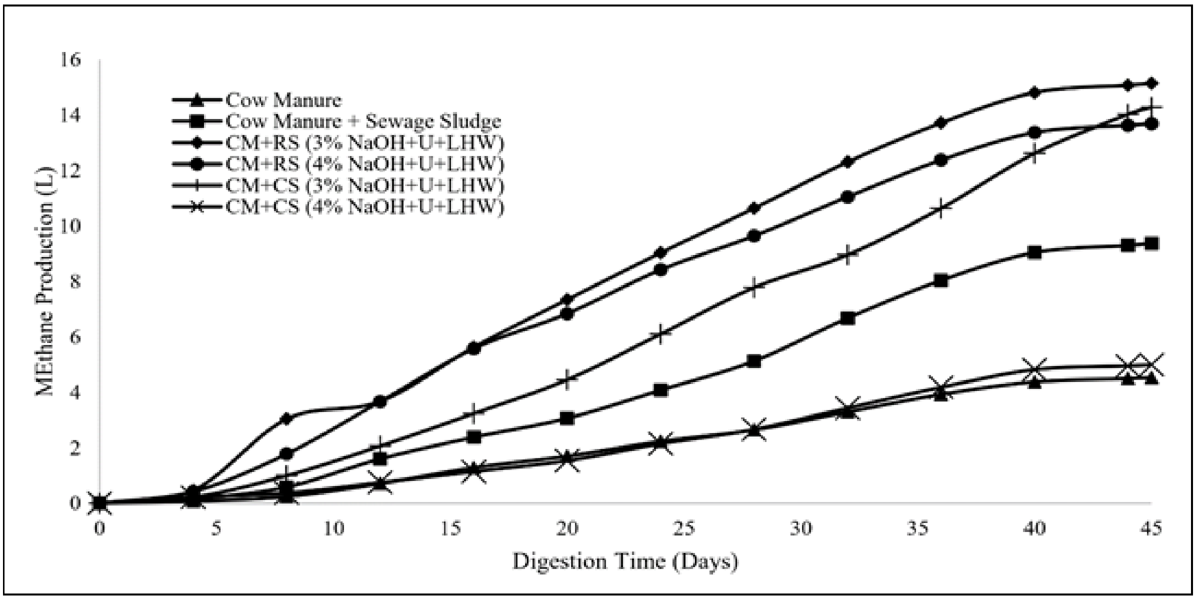

Fig. 4. Biogas Production from the digestion of selected biomass subject with different pretreatments.

Therefore, the said biomass samples are quite combustible, as compared with the untreated rice straw. The amount of Volatile Combustible Matter increases 30% more than the untreated ones. The said pretreatments can be interpreted that the lignin component of the rice straw is degraded during the pretreatments.

Conclusion

Physical pretreatment of biomass was applied to produce better-quality biogas. The alkali-ultrasonication-liquid hot water (NaOH+U+LHW) pretreatment improved the cellulose and lignocellulose conversion and resulted in methane yields increase of 140% & 290% from anaerobic co-digestion of rice straw and coconut shell with cow manure and sewage sludge as inoculum compared with untreated material. The use of FT-IR was effective as a tool to identify the variations in the signal of the cellulose, hemicellulose, and lignin from rice straw and coconut shell after subjecting to different pretreatments. We also reported the chemical structure of agricultural biomass with and without pretreatments. This study also shows how substrate composition, pretreatment methods, and operational parameters during anaerobic digestion affect the microbial consortia working in the digester.

References

Bagher AM, Fatemeh G, Saman M, Leili MM. 2015. Advantages and Disadvantages of Biogas Energy. Bulletin of Advanced Scientific Research, **1(5),** p 132-135.

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

Jiang D, Ge X, Zhang Q, Li Y. 2016. Comparison of liquid hot water and alkaline pretreatments of giant reed for improved enzymatic digestibility and biogas energy production. Bioresource technology **216,** p 60-68. https://doi.org/10.1016/j.biortech.2016.05.052

Kaur K, Phutela UG. 2016. Enhancement of paddy straw digestibility and biogas production by sodium hydroxide-microwave pretreatment. Renewable Energy **92,** p 178-184. https://doi.org/10.1016/j.renene.2016.01.083

Karray R, Hamza M, Sayadi S. 2015. Evaluation of ultrasonic, acid, thermo-alkaline and enzymatic pre-treatments on anaerobic digestion of Ulva rigida for biogas production. Bioresource technology **187,** p 205-213. https://doi.org/10.1016/j.biortech.2015.03.108

Lianhua L, Dong L, Yongming S, Longlong M, Zhenhong Y, Xiaoying K. 2010. Effect of temperature and solid concentration on anaerobic digestion of rice straw in South China. International journal of hydrogen energy **35(13),** p 7261-7266. https://doi.org/10.1016/j.ijhydene.2010.03.074

Plácido J, Imam T, Capareda S. 2013. Evaluation of ligninolytic enzymes, ultrasonication and liquid hot water as pretreatments for bioethanol production from cotton gin trash. Bioresource technology **139,** p 203-208. https://doi.org/10.1016/j.biortech.2013.04.012

Philippoussis A, Zervakis G, Diamantopoulou P. 2001. Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms Agrocybe aegerita, Volvariella volvacea and Pleurotus spp. World Journal of Microbiology and Biotechnology **17(2),** p 191-200. https://doi.org/10.1023/A:1016685530312

Quiroga G, Castrillón L, Fernández-Nava Y, Marañón E, Negral L, Rodríguez-Iglesias J, Ormaechea P. 2014. Effect of ultrasound pre-treatment in the anaerobic co-digestion of cattle manure with food waste and sludge. Bioresource technology **154,** p 74-79.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker DLAP. 2010. Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure, (TP-510-42618). https://doi.org/10.1016/j.biortech.2013.11.096

Silverstein RA, Chen Y, Sharma-Shivappa RR, Boyette MD, Osborne J. 2007. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresource technology **98(16),** p 3000-3011. https://doi.org/10.1016/j.biortech.2006.10.022

Teghammar A, Chandra R, Saddler JN, Taherzadeh MJ, Horváth IS. 2012. Substrate characteristic analysis for anaerobic digestion: A study on rice and triticale straw. BioResources **7(3),** p 3921-3934.

Zhang Y, Chen X, Gu Y, Zhou X. 2015. A physicochemical method for increasing methane production from rice straw: extrusion combined with alkali pretreatment. Applied energy **160,** p 39-48. https://doi.org/10.1016/j.apenergy.2015.09.011