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Characterization of Rice Straw and Biogas Production under Mesophilic Conditions

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ABSTRACT

In this study, the impact of alkali and acid pre-treatments on the structure and composition of rice straw (RS), along with the biogas and methane production potential of the pre-treated substrates was investigated. XRD and Fourier Transform Infrared Spectroscopy demonstrated the partial decomposition of rice straw and alteration of lignin structure as an outcome of pre-treatment by un-bonding the ether chain inside lignin molecules. After pre-treatment with NaOH, hemicellulose and lignin significantly reduced up to 89.45 and 93%, respectively. Cumulative methane yield after 40 days of anaerobic digestion (AD) of RS pre-treated with 1, 2, 3 and 4% NaOH was 28.97, 55.78, 95.08 and 55.64%, respectively, higher than the untreated sample. The acid pre-treatment was not very effective in the enhancement of biogas and methane production. The findings of this study indicated that 3% NaOH pre-treatment had the potential to be an effective way to increase the viability of producing methane from rice straw.

Key words: Pre-treatment, rice straw, anaerobic digestion, biogas, FTIR, XRD

INTRODUCTION

The global population is increasing every day and is expected to cross 9.8 billion by the year 2050 with India surpassing China around 2024 (Mona *et al.,* 2021). As a consequence, the world's energy demand is also rising sharply with a growth rate of 1.5% annually (Sawatdeenarunat *et al.,* 2015) and the consumption is expected to increase up to 26.7 billion tonnes of oil (BTO) by 2050 (Lyon *et al.,* 2018). The increasing global population also increases waste generation in every sector i.e. municipal solid waste (MSW), lignocellulosic waste, dairy and animal waste and others. There has been a paradigm shift towards a waste-to-energy (WTE) approach to solve these problems in the form of biogas production (Bhatt and Tao, 2020). The biogas production through the anaerobic digestion (AD) process which utilizes a variety of waste generated and provides a renewable and environmentally-friendly source of energy (Zhu *et al.,* 2022; Dhull *et al.,* 2023). AD is defined as a biological process in which organic materials are converted into methane and other gases in the absence of oxygen with the help of various anaerobic microorganisms (Vijin Prabhu *et al.,* 2020). It has been regarded as a more intriguing and viable strategy that is more cost-effective, has fewer negative environmental effects and provides high energy recovery linked to the process (Tao *et al.,* 2020; Truong *et al.,* 2020). The process comprises four steps i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis carried out by specific microbes (Rocha-Meneses *et al.,* 2022). To improve the proficiency of the AD process, several pre-treatment methods are employed. They improve the solubility and biodegradability of the available feedstock (Dhull *et al.,* 2023). Moreover, the pre-treatment methods are required as a result of the hindrance originating during the degradation of the feedstock, generation of toxic and inhibitory compounds, slow microbial activities, etc. (Paul and Dutta, 2018).

A competent pre-treatment process should

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preserve the organic components in the feedstock, produce minimal hazardous or inhibitory compounds, enhance the surface area for improved microbial and enzymatic activity and maximize the biogas yield (Mirmohamadsadeghi *et al.,* 2021). The pre-treatment reaction severity and chemistry greatly influence the extent of conversion of feedstock structures into soluble forms which is further dependent on the temperature, pH, chemical dosage levels and residence time (Kim and Karthikeyan, 2021). Physical and chemical pre-treatment are simple and cost-effective ways of treating the feedstock and modifying their structure to increase the biogas yield (Khanh Nguyen *et al.,* 2021). The chemical pre-treatment including acid and alkaline can disintegrate sludge and lead to fatty acid production. It is an intriguing method to improve the feedstock and makes it biodegradable by reducing the amount of lignin as well as hemicellulose in it through treatment with acids, ionic liquids, oxidative treatments, alkalis, gases, etc. The most commonly used acids are HNO_2, HNO_3, H_3PO_4, H_2SO_4, HCl and organic acids either in concentrated or dilute forms. The most commonly used alkalis in the chemical pre-treatments are KOH, uronic acid, NaOH, $Mg(OH)_2$, Na_2CO_3, ammonia, $Ca(OH)_2$, acetyl group and urea. These degrade the crosslink between cellulose, hemicellulose, lignin and polysaccharides; therefore, helps in increasing porosity and accessibility to microbes (Wagner *et al.,* 2018). Further alkaline pre-treatment destroys the cell wall through saponification and hydrolyses the extracellular biopolymers; thereby, increasing the biodegradability of the feedstock (Zheng *et al.,* 2021).

In the present study, the outcome of physical and chemical pre-treatment on the characterization of RS has been evaluated on a batch mode scale. Two types of pre-treatment methods i.e. acid and alkali pre-treatment were employed in different concentrations. The effect of both types of pre-treatments on biogas production was also evaluated.

MATERIALS AND METHODS

The RS was collected from the nearby fields of Hisar, Haryana, India during the harvesting season. It was air-dried and cut down into sizes less than 2 mm and homogenized using a mixer grinder. The crushed rice straw (RS) sample was stored at room temperature for further laboratory work. The inoculum used was biogas plant slurry collected from a household biogas plant using cattle dung as substrate.

The RS sample was subjected to two types of pre-treatments i.e. alkali and acid treatment at different concentrations to understand the degradation of RS. The alkali (NaOH) concentration taken was 1, 2, 3 and 4%, while the concentration of acid (HCl) used was 0.5, 1, 1.5 and 2% for 24 h at 45°C temperature in a temperature-controlled incubator. The other factors were kept constant. Different concentrations of alkali and acid were added to 100 g of oven-dried RS. All the experiments were performed in triplicates. After treatment, Whatman filter paper was used to filter mixed solutions, and the residual solids were taken out and processed with deionized water. The wet solid was then used for further physico-chemical analysis.

The untreated and pre-treated RS samples were evaluated for various physico-chemical parameters. Standard techniques were employed in order to determine the contents of total solids (TS), cellulose, hemicellulose, lignin and volatile solids (VS). All the values were reported in triplicates. The lignocellulosic analysis of the feedstocks neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was measured. The hemicellulose and cellulose amounts were determined using two distinct equations:

$$\text{Hemicellulose} = \text{NDF} - \text{ADF} \text{ and}$$
$$\text{Cellulose} = \text{ADF} - \text{ADL}$$

The standard methodologies were used for estimating TS and VS contents. For the TS, the samples were heated up at 105°C for 2 h, then cooled down and weighed; for the VS, the samples were subjected to heating for 30 min at 550°C, then allowed to cool before being weighed to determine the amount of fixed solid (FS) and VS was calculated according to the following:

$$\text{VS} = \text{TS} - \text{FS}$$

Fourier transform infrared (FT-IR) spectra were analyzed in the 400 to 4000/cm wavenumber range using Spectrum Two, PerkinElmer, to identify the chemical interactions and functional group structure of the RS sample. To determine the structural phase and calculate particle size, the spectra of the RS samples were recorded using an X-ray diffractometer. The model used for the analysis was the Rigaku Miniflex- II diffractometer, Japan.

The untreated and pre-treated samples were subjected to the process of AD in a 1 l glass bottle (Glassco GL0620134) set up as batch reactors for biogas production. The bottles were sealed with rubber cork having two holes for sample collection and to transfer the gas which was connected with rubber and glass tubes. The leakage was prevented by wrapping the bottles with the help of sealing wax. AD process was carried out in the first bottle, while in the middle bottle, water was added. The water was displaced by gas pressure into the third bottle. The dry matter on the TS basis was kept at 5% in the reactors. The inoculum used was 10% of the working volume of the reactor. The pH was maintained at 7 using NaOH and HCl solution. The reactors were kept at 38°C (mesophilic conditions) in an incubator. The reactors were agitated manually two to three times a day to maintain homogeneity. Before the fermentation, nitrogen gas was passed from the headspace to sustain an anaerobic condition by removing oxygen. Each run was done in triplicates and the mean values were obtained. The 1 l tedler bags were used for the collection of gas. The water displacement technique was used for collecting the biogas. At intervals of 24 h, the total volume of biogas was calculated. The experiments were performed for 40 days. Gas chromatography (GC) equipped with a thermal conductivity detector (TCD) was used to determine the composition of the biogas. The column used was a 1.5 m stainless steel packed column with a molecular sieve and a Porapak-Q packed column. The injector, oven and detector temperatures were 70, 70 and 150°C, respectively. With a flow rate of 30 ml/min, argon was used as the carrier gas.

RESULTS AND DISCUSSION

The different physico-chemical characteristics of the untreated RS and pre-treated RS were studied (Table 1). Untreated RS contained 89.31% (w/w) total solids (TS) and 77.89% volatile solids (VS). The cellulose, hemicellulose and lignin content of the untreated RS were found to be 35.65, 29.86 and 3.88%, respectively. It was found to be suitable to be utilized in the AD process and biogas production. Different pre-treatments were applied to degrade the RS and enhance the biogas production (Table 1). It was observed that the alkaline conditions highly degraded the lignin than cellulose as compared to acidic pre-treatment. It can be due to the crystalline nature of the cellulose which makes it difficult to be degraded. As the concentration of alkaline pre-treatment increased, the degradation of the cellulose was increased. The degradation of lignin and hemicellulose could have enabled the degradation of the cellulose more easily. The maximum loss of lignin was achieved at 3% of alkaline pre-treatment. The delignification is necessary to increase the biogas production. The -OH bond present in NaOH weakens the hydrogen bonds between cellulose and hemicellulose while also weakening the ester and ether bonds between lignin and polysaccharides, which facilitates the dissociation and disintegration of cellulose, hemicellulose and lignin. Also, the lignin is transformed into soluble hydroxyl lignin and is easily accessible for microbial degradation during AD. In acidic pre-treatment, the cellulose and lignin pre-treatment increased which can be because of the decrease in hemicellulose. At 2.5% concentration of the acidic pre-treatment, the maximum decrease in the hemicellulose was achieved. The results of the current study are in accordance

Table 1. Physico-chemical characterization of rice straw (RS) and pre-treated rice straw

Parameters	Rice straw (RS)	Alkali pre-treated rice straw				Acid pre-treated rice straw			
		1%	2%	3%	4%	1%	1.5%	2%	2.5%
Cellulose (%)	35.65	38.98	30.21	30.75	17.36	43.40	42.56	41.14	42.85
Hemicellulose (%)	29.86	20.98	6.94	5.61	3.15	8.26	7.83	7.64	7.29
Lignin (%)	3.88	4.53	1.03	0.27	0.43	17.11	18.47	17.99	17.50
Total solids (TS) (%)	89.31	-	-	-	-	-	-	-	-
Volatile solids (VS) (%)	77.89	-	-	-	-	-	-	-	-

with the literature (Kim *et al.,* 2018). The acidic pre-treatment works best in the degradation of hemicellulose. The primary function of HCl is to degrade hemicellulose and disrupt the crystalline structures of lignin. The lignin surrounding cellulose gets exposed and is easily degraded by microbes. The dissolved lignin cannot be further degraded because of rapid coagulation and precipitation under acidic conditions. Hence, the mild or dilute acid pre-treatment is recommended. To better understand the structural changes in the rice straw after pre-treatment, FTIR and XRD were compared for untreated RS and pre-treated RS. Fig. 1 depicts the structural changes of the

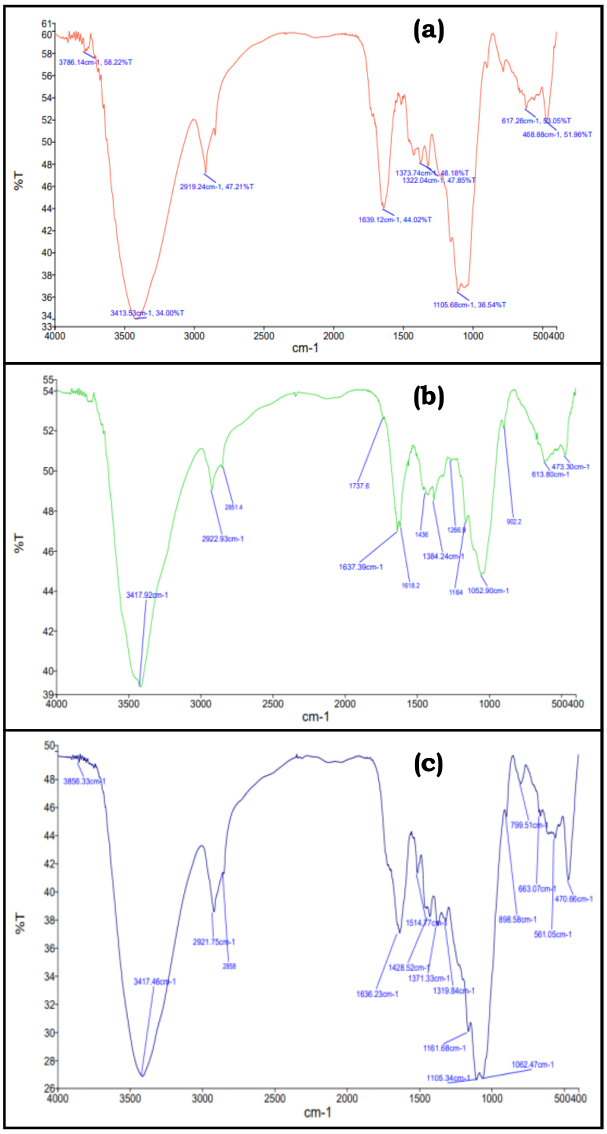

Fig. 1. FTIR analysis of (a) untreated rice straw, (b) alkali pre-treated rice straw and (c) acid pre-treated rice straw.

untreated and both types of pre-treated RS at maximum lignin degradation i.e. 3% alkaline and 1% acidic pre-treatments.

The alterations in the structure can be seen. The FTIR spectrum of all samples demonstrates the differences in band intensities that were seen as a result of various functional groups being impacted by the pre-treatments. In the pre-treated RS, it turned out that several band positions were shifted and some bands had disappeared entirely. The broad vibrations at 3400-3500/cm are due to the hydrogen bond compounds, especially hydroxyl (-OH) bonds in aliphatic and phenolic compounds (Singh *et al.,* 2023). The vibrations around 2900/cm were because of the C-H vibrations in aromatic methylene and methoxyl groups, a characteristic feature of cellulose. The changes in shape and intensity of these bands were observed more in alkaline pre-treatment indicating rupture in cellulose. During the alkaline pre-treatment, the lignin structure exhibited a substantial change that is correlated with unaltered carbonyl/carboxyl stretching, as indicated by an absorbance band at 1737.6/cm. Similar results have been found in the study of (De *et al.,* 2020). The peaks 1053 and 1062/cm in Fig. 1b and Fig. 1c demonstrate the C-O stretching due to cellulose, hemicellulose and lignin. The absorption band at 1639/cm is related to the C=C of the aromatic ring present in lignin. It is reduced to 1637 and 1636/cm in alkaline and acidic pre-treatment, respectively. The results are in accordance with Ashoor and Sukumaran (2020). The absorption bands present between 133-1000/cm represent C-C, C-O and C=O groups present in cellulose, hemicellulose and lignin. The changes in the intensity of the above-mentioned bands indicate structure modifications in the cellulose, hemicellulose and lignin in the RS before and after pre-treatment. Fig. 2 represents the XRD pattern of untreated RS and pre-treated RS with clear indication by showing changes in the peaks and their intensities.

XRD spectrum analysis is an essential means to study the structure of substances in our sample. In Fig. 2a, the presence of quartz in the form of SiO_2 is represented by the sharp and crystalline peak at $2\theta = 26.56°$ in the untreated RS which is commonly present in RS. This peak shows clear changes in both pre-

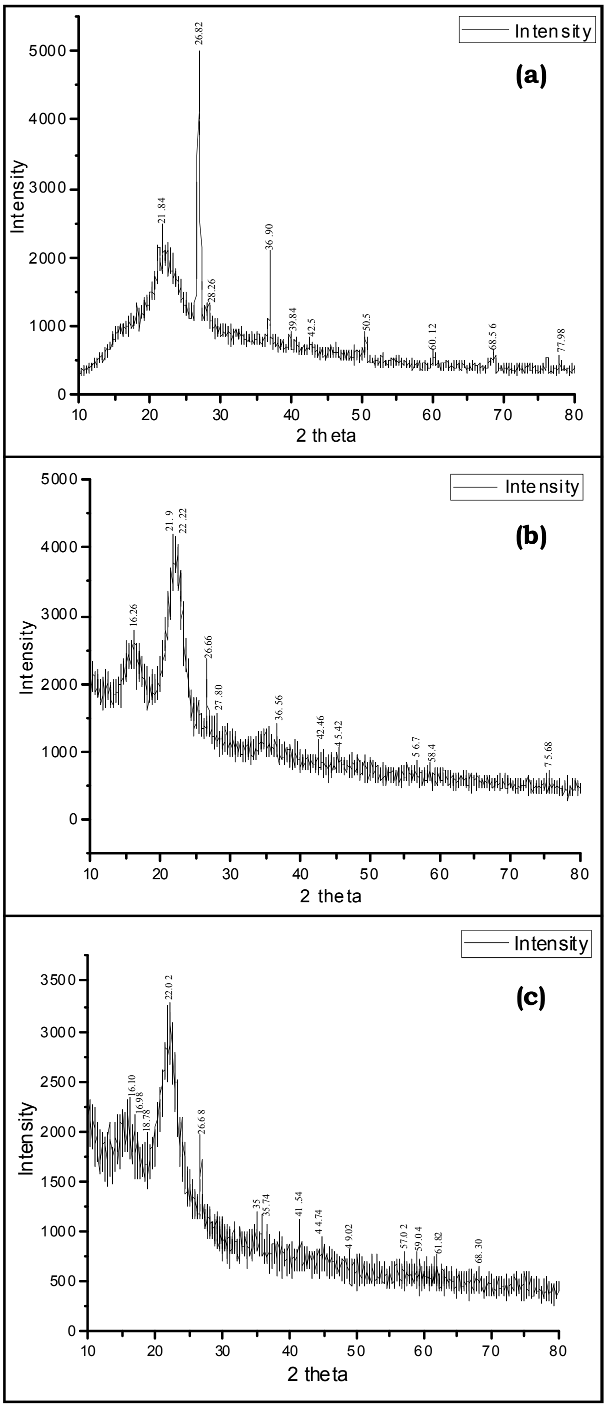

Fig. 2. XRD analysis of (a) untreated rice straw, (b) alkali pre-treated rice straw and (c) acid pre-treated rice straw.

treated samples of RS. In Fig. 2b, the peak has broadened and divided into 2θ = 21.9° and 2θ = 22.2°, while in Fig. 2c, the peak changes to 2θ = 22.02° indicating the structural change in RS. Higher levels of amorphous particles are represented by the weaker and wider peaks and increase their digestibility by the microbes during the AD process. After pre-treatment, the intensity peak around 2θ = 16.0° became more prominent with 2θ = 16.26° after alkaline pre-treatment 2θ = 16.10° and 2θ = 16.98° after acidic pre-treatment. This result is in accordance with Ningthoujam *et al.* (2023). As shown in Fig. 2, untreated RS's crystallinity has been changed into amorphous structure upon pre-treatments. Additionally, the peak region of 2θ = 10° and 30° suggests the existence of amorphous aromatic carbon particles, which are created as a result of the parallel stacking of sheets in small domains (Esmaeili *et al.,* 2023). Therefore, the XRD spectra of both untreated RS and pre-treated RS confirm the changes in the structure of the RS.

Batch AD experiments were conducted for 40 days with untreated RS and pre-treated RS to study the methane and biogas potential (Table 2). Fig. 3 demonstrates the daily methane and biogas potential of pre-treated RS and untreated RS considered as control, whereas Fig. 4 demonstrates the cumulative methane and biogas potential of untreated RS and pre-treated RS. It is important to ensure that there is an abundance of all the microbial communities required to facilitate the bio-methanation of lignocellulosic material. These microbes consist of hydrolytic, acidogenic, acetogenic bacteria and methanogens (Shetty *et al.,* 2017). A steady temperature is required to avoid detrimental impacts on biogas production. Therefore, to maximize the yield of biogas, the temperature in this particular study was maintained at 38°C (as it corresponds to the average temperature in the majority of regions of India). The rate of generation of gas is a significant measure of performance for biogas production. When the volumetric gas production rate is high, more methane can be produced in the same volume of reactor space. This means that biogas production has the potential to be achieved by using a smaller-scale AD plant in the project's conceptualization and implementation, which significantly reduces the amount of site space needed for construction and the associated construction costs.

The cumulative biogas production of untreated RS was 203.33 ml/g VS. Cumulative biogas production of straw pre-treated with 1, 2, 3 and 4% NaOH for 24 h was 31.77, 43.63, 73.89 and 44.76%, respectively, higher than the control

Table 2. Methane and biogas potential of untreated and pre-treated RS

	Untreated	0.5% HCl	1% HCl	1.5% HCl	2% HCl	1% NaOH	2% NaOH	3% NaOH	4% NaOH
Cumulative biogas yield (ml/g VS)	203.33	213.07	241.28	183.84	155.64	267.94	292.05	353.58	294.35
% Biogas difference	0	4.79	18.69	09.56	23.45	31.77	43.63	73.89	44.76
Cumulative CH_4 yield (ml/g VS)	111.85	107.48	135.26	93.53	75.82	144.26	174.25	218.20	174.09
Cumulative CH4 (% difference)	0	03.90	20.92	16.37	32.21	28.97	55.78	95.08	55.64
Average CH_4 content (%)	45.38	48.62	52.72	49.57	47.98	50.32	54.61	57.92	54.56
CH4 content (% difference)	0	7.13	16.17	9.23	5.72	10.88	20.33	27.63	20.22

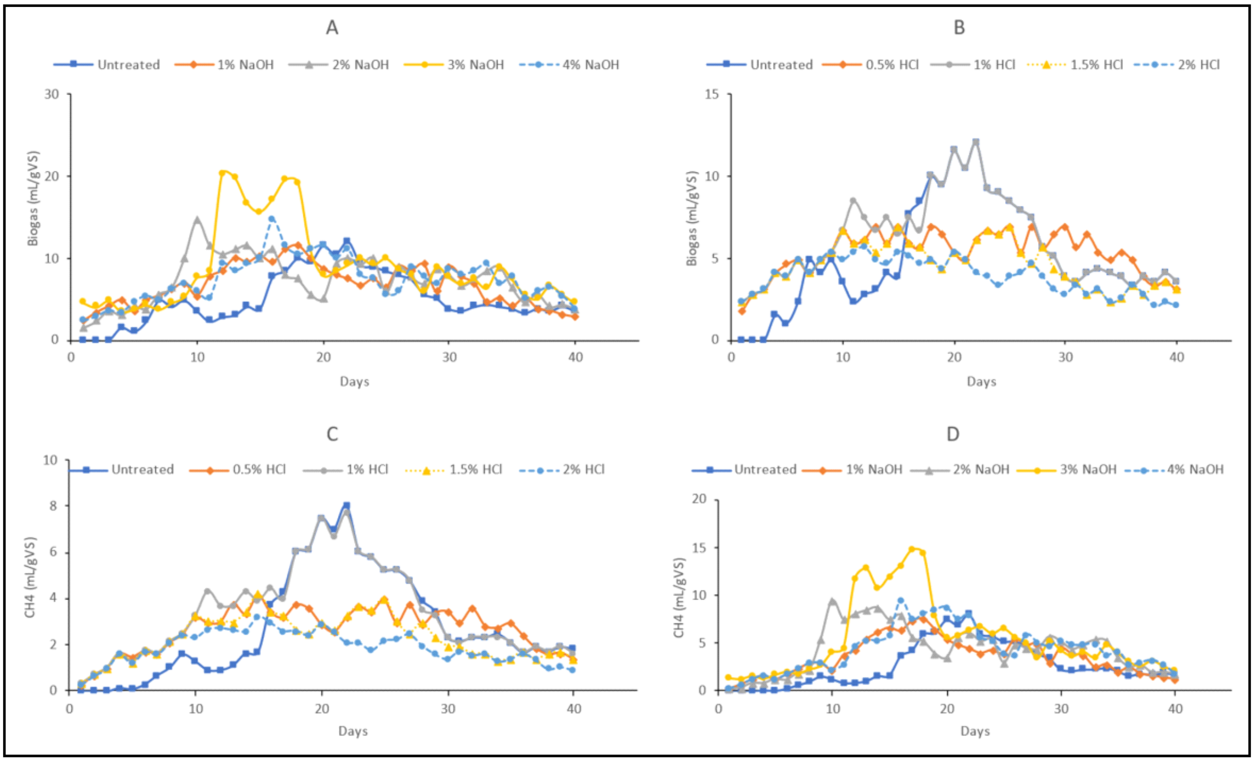

Fig. 3. Daily methane and biogas yield during batch digestion pretreated with different concentrations of NaOH and HCl (a) daily biogas yield with NaOH pre-treatment, (b) daily biogas yield with HCl pre-treatment, (c) daily CH4 yield with NaOH pre-treatment and (d) daily CH_4 yield with HCl pre-treatment.

at the end of the AD process. The maximum increase in cumulative biogas yield was reported in the experimental setup having substrate pretreated with 3% NaOH (i.e. 353.58 ml/g VS), while in pre-treatments with 2 and 4% NaOH the biogas yield was 292.05 and 294.35 ml/g VS, respectively. However, the cumulative biogas yield from HCl-pretreated RS substrate was 213.07, 241.28, 183.84 and 155.64 ml/g VS for 0.5, 1, 1.5 and 2% HCl, respectively, after 40 days of HRT. In the case of acid pre-treatments, only one AD setup (i.e. 1% HCl pre-treated substrate) showed an increase of 20.92% in biogas yield when compared with the untreated RS setup. The lowest biogas yield was observed in the AD setup having substrate treated with 2% HCl followed by 1.5 and 0.5% pre-treatments with a total biogas yield of 155.64, 183.84 and 213.07 ml/g VS, respectively.

The average CH_4% production and cumulative methane yield of untreated RS after 40 days of digestion were 45.38% and 107.48 ml/g VS, respectively. Methane concentration fluctuations are able to provide an overview of the quality of biogas produced through the AD process of the RS after pre-treatment. The average methane content was increased in all AD setups pre-treated with both alkali and acid. There was an increase of 7.13, 16.17, 9.23 and 5.72% in average methane content after pre-treatment of RS with a concentration of 0.5, 1, 1.5 and 2% of HCl acid, respectively. The NaOH pre-treatment showed a higher increase in

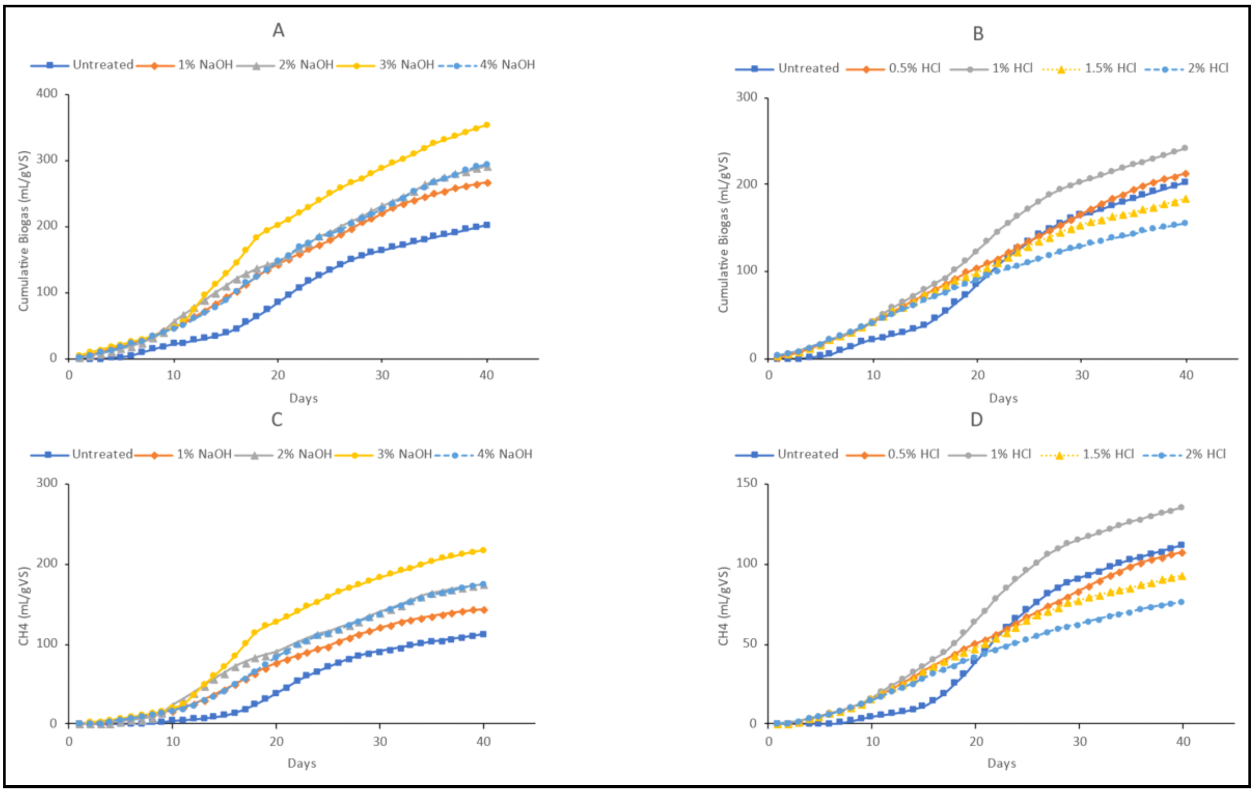

Fig. 4. Cumulative methane and biogas yield during batch digestion pre-treated with different concentrations of NaOH and HCl, (a) cumulative biogas yield with NaOH pre-treatment, (b) cumulative biogas yield with HCl pre-treatment, (c) cumulative CH4 yield with NaOH pre-treatment and (d) cumulative CH_4 yield with HCl pre-treatment.

average methane content compared to acid pre-treatments with an increase of 10.88, 20.33, 27.63 and 20.22% when treated with a concentration of 1, 2, 3 and 4% of NaOH, respectively.

The methane yield was increased in all alkali pre-treatments with a maximum increase of 95.08% in 3% NaOH pre-treatment followed by 2, 4 and 1% NaOH pre-treatments with an increase of 55.78, 55.64 and 28.97%, respectively, compared to the untreated RS setup. While in acid pre-treatments, a 20.92% increase in methane yield was reported in the setup pre-treated with 1% HCl acid, while a lower yield of 03.90, 16.37 and 32.21% was reported in setups pre-treated with 0.5, 1.5 and 2% HCl, respectively.

The anionic sulfuric acid component, or sulfate ion, is a more logical explanation for the decreased biogas and methane production subsequent to acid pre-treatment. The most significant methane precursors, hydrogen and acetic acid, are consumed by sulfate-reducing bacteria (SRB), which significantly decrease the quantity of methane produced (Kim *et al.*, 2018). Instead of the absolute sulfate concentration, the inhibitory action of sulfate on methanogenesis is primarily reliant on the molar ratio of organic feedstock and sulfate. Considering methane production potential, the degradability of lignocellulose and the probability of microbial inhibition among those studied, 3% NaOH pre-treatment is suggested to be the most effective.

CONCLUSION

The experiment on untreated and pre-treated rice straw revealed the impact of different pre-treatments on the composition of lignocellulosic compounds in rice straw. The process of pre-treatment might serve as an efficient way to enhance the biodegradability of rice straw and improve the effectiveness of its biochemical transformation into bioenergy. The lignin content was removed up to 93% by alkali pre-treatment, while hemicellulose content was removed up to 75.58% by the acid pre-treatment. Alkali pre-treatment increased the biogas and methane yield up to 73.89 and

95.08%, respectively, in comparison with untreated RS. On the contrary, acid pre-treatment was not very effective in improving the biogas and methane production potentials. Only one acid treatment (i.e. 1% HCl) was able to enhance the methane yield up to 20.92% in comparison to untreated RS.

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