

Effect of NaOH Pretreatment of Bagasse on Its Biodegradation through Anaerobic Digestion

Maninder Kaur, Yajvender Pal Verma, Sanchita Chauhan

Abstract: Bagasse the by-product of sugarcane crop, mainly utilized by the sugar industry itself in cogeneration power plant to produce power through cogeneration to full-fill their energy needs and export the excess power generated to the grid. The present study was conducted to analyze the effect of (2%, 4% and 6%) NaOH pretreatment of bagasse at room temperature for 24 hours on the biogas production through anaerobic digestion. NaOH pretreated and untreated bagasse co-digested with cow manure was assessed to optimize the NaOH concentration for enhanced biogas production in batch mode experiments. Analytical techniques such as Field Effect Scanning Electron Microscope (FESEM), Fourier Transform Infra-Red (FTIR) and X-ray diffractometer (XRD) were used to investigate the changes on physical and chemical structures of pretreated bagasse. The 4% NaOH pretreatment resulted in highest biogas production which is 55.3% higher than untreated bagasse.

Keywords: Anaerobic Digestion; Bagasse; Lignocellulosic; Pretreatment.

I. INTRODUCTION

Energy demands tend to increase rapidly, due to a rise in urbanization, industrialization, and development of infrastructure. The larger dependency on fossil fuels for energy needs globally harms the environment. Biomass, most abundant source of renewable energy resource throughout the world is contributing nearly 10% of energy supply, which is likely to increase to 30% by 2050. India, being an agrarian country with 100% collection efficiency, has the energy potential of 7236.27 PJ from crop residues [1]. Sugarcane, one of the major crops produced globally in which India's contribution amounts to be 348.44 MT, having a yield of 703935 hg/ha (FAO, 2016). Around 3-5% of bagasse which remains unutilized also openly burnt in fields, adding environmental pollution. Bagasse based cogeneration power in sugar mill industry was estimated to be 7260 MW as of March 2017, which contributes to 26% of the total estimated potential of renewable resources.

Bagasse, like other lignocellulosic material rich in organic matter, composed of three main components: hemicelluloses, cellulose, and lignin [2]. It can be utilized

sustainably by converting it into green energy in the form of biogas through anaerobic digestion. Anaerobic digestion is an effective biochemical process used to convert the organic materials present in biomass into biogas mainly involves four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [3]. However, the recalcitrance posed by the lignocellulosic nature of bagasse slows down its decomposition through anaerobic digestion. Thus, resulting in longer Hydraulic Retention Time (HRT) and lower biogas yields directly affecting the profitability of biogas plants [4].

Pretreatment of biomass before anaerobic digestion is one of the useful tools to overcome the recalcitrance posed by chemical compositions of lignocellulosic materials [5]. Various pretreatment methods are available in the literature, which helps to alter the physical structure and chemical compositions of lignocellulosic materials, thus making the digestibility of biodegradable materials faster and efficient. The alkaline pretreatment of bagasse was observed to be most effective in glucose production among the combined hydrothermal-alkaline, alkaline-acid and alkaline-hydrogen peroxide pretreatment of bagasse [6]. Chemical pre-treatment through alkaline solution along with physical pretreatment has been proven to be auspicious one to be followed by anaerobic digestion for biogas production as it is more compatible in comparison with other pretreatment methods [7]. Most of the studies concentrated on the pretreatment of lignocellulose at a high temperature for disrupting the LCC's (Lignin-Carbohydrate Complex's) [8]. The critical parameters such as reaction temperature, pretreatment time, and concentration of alkali play a significant role which needs to be optimized to maximize the effect alkaline pretreatment [9].

Therefore, the study aims to assess the effect of NaOH pretreatment of bagasse at an ambient temperature of 25⁰C for 24 hours to find the optimal concentration of NaOH to have enhanced biogas production. The analytical techniques FESEM, FTIR Spectroscopy, and XRD diffractometer were being used to have a more comprehensive view of conditioned bagasse on its chemical structure and crystallinity. Further, change in lignocelluloses matrix involving lignin, cellulose, and hemicelluloses were analyzed individually and conditioned bagasse co digested with cow dung was submitted to batch type anaerobic digester to study its effect on biogas production.

Revised Manuscript Received on January 15, 2020

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II. MATERIAL AND METHODS

2.1. Substrate and inoculums

Bagasse for study collected from fields of the village of District S.A.S. Mohali, Punjab (India) which was kept by the farmers for the heating purpose of making jaggery after extracting juice from it. It was sun-dried for a week and then grounded to tiny size particles less than 1mm through grinding machine and stored in an airtight container. Cow dung required for co-digestion collected from cattle shed of Village Dhanas, Chandigarh.

2.2. NaOH Pre-treatment

The NaOH solution of 2%, 4%, and 6% was prepared using pellets dissolved in distilled water in weight by volume ratio to prepare the required concentration. The bagasse was then soaked in different concentration of NaOH (2%, 4% & 6% w/v) solution in one-liter beakers at ambient room temperature for 24 hours. After 24 hours, the wet bagasse thoroughly washed with running tap water to neutralize the pH effect of alkaline pretreatment and filtered through the cotton cloth. Wet bagasse is then dried at room temperature till it becomes moisture free and again stored in an airtight container for further use.

2.3. Experimental set-up

The untreated and NaOH pretreated bagasse was digested in batch anaerobic digesters of one-liter capacity. Each digester seeded with the slurry made up from mixing of bagasse and cow dung in ratio 1:2, the total solid is mixed with water in ratio 1:3 as to maintain the carbon to nitrogen ratio between optimum range of ratio between 25:1 to 30:1 [9]. The one side opening fitted with rubber cork to hold thermometer and note down the pH of slurry from time to time. Another side opening fitted with a pipe through which biogas so formed passes through to other ends of the pipe entering an inverted cylinder filled with water placed alongside the digester to measure the biogas through well-known water displacement method [10]. The process operated at mesophilic temperature of (35± 2⁰C) by placing the digesters in the water bath to maintain the temperature constant.

2.4 Characterization of Bagasse

2.4.1 Analytical Methods

The proximate analysis comprised of total solids, volatile solids, and moisture content has great significance on the biogas production capacity of biomass. Therefore, total solid content and moisture content of bagasse understudy was calculated using ASTM E1756-08 method and Volatile solid content of the sample was calculated using ASTM E872-82 method. The 2gm of samples were dried in the oven at 105 ± 3⁰ C for 3 hours to calculate the total solids (TS) and moisture, and 1gm of the sample was oven-dried at 950 ± 20⁰ C to calculate volatile solids (VS) of untreated and pretreated bagasse [11].

2.4.2 FESEM & FTIR Spectroscopy

FESEM was used to observe the NaOH pretreatment effect on the morphological surface of bagasse. Different samples of bagasse were mounted on metal stubs by double-faced tape in

a Scanning Electron Microscopy (SU 8000 Series, Hitachi make, Japan) to take SEM images at accelerating voltage of 5KV. The FTIR spectrum of powdered samples recorded by FTIR Spectrophotometer using KBr technology (Model RZX, Perkin Elmer, USA) in transmittance mode with a wavelength range from 4000-450 cm⁻¹.

2.4.3 X-ray Diffractometer

The X-ray diffraction profiles of untreated and pretreated bagasse collected to check the crystallinity of bagasse. X-ray diffractometer system (Philips X'Pert Pro) using Cu Kα radiation at the wavelength of 0.1541nm and 40 kV, 40 mA intensity used to scan the samples of bagasse over an angular range of 2θ from 10-60⁰ C. Crystallinity Index (CrI) of cellulose was calculated using Segal empirical Method as follows [12].

$$CrI (%) = \frac{I_T - I_{Am}}{I_T} \times 100$$

Where I_T represents the maximum intensity of the (0 0 2) lattice diffraction at 2θ angle lies between 22⁰ to 23⁰ and I_{Am} is the intensity diffraction of amorphous material between 18⁰ to 19⁰, 2θ diffraction angle where intensity is minimum.

III. RESULTS & DISCUSSION

3.1 Sample analysis and pretreatment effect on the lignocellulosic composition

Bagasse understudy has enough total solid greater than 91 % (Table 1) of which volatile solid observed to be greater than 75 % having moisture less than 9% representing enough organic matter to get converted into biogas. C: N ratio of bagasse in the present study varies between 59.61 to 93.12% which was adjusted by mixing the bagasse with cow dung in ratio 1:2 to bring this into the optimum range for efficient biogas production.

Table 1 Characterization of bagasse through proximate analysis

Bagasse	Total Solid (%)	Volatile Solid (%)	Moisture (%)
Untreated	94.79	84.31	5.21
2% NaOH	92.71	82.13	7.29
4% NaOH	91.51	76.31	8.49
6% NaOH	93.86	79.39	6.14

3.2 Effect of NaOH pretreatment on morphological structure of bagasse

The Field Effect Scanning Electron Microscopy (FESEM) is one the effective analytical technique used to study the morphological structure of bagasse. As seen from FESEM images of the bagasse, the NaOH pretreatment has a significant effect on the fibrous structure of bagasse.

As seen from Fig. 1(a) the bagasse is composed of long, attached bundles of fibers in which the carbohydrates are bounded together with plant cell wall known as lignin. The mild pretreatment of bagasse with 2% NaOH results in distortion of outer boundary wall as seen from Fig. 1(b) which may be attributed to starting of cleavage of bonds present in complex lignin structure.

The 4% NaOH pretreatment of bagasse has observed to effective in reducing the degree of polymerization as seen from Fig. 1(c). The digestibility of organic matter takes place more rapidly by methanogenic bacteria with separation of linkages between carbohydrates and lignin resulting in the distortion of fiber bundles [13]. The 6% NaOH pretreatment of bagasse shows not only the cleavage of different bonding presents in bagasse but also represents the solubility of organic matter, resulting in decreased matter available for microorganism attack. Thus, due to NaOH pretreatment of bagasse, Saponification of esters bonds takes place resulting in the swelling of lignocellulosic material, thus increasing the internal surface area for the enzymatic attack [14].

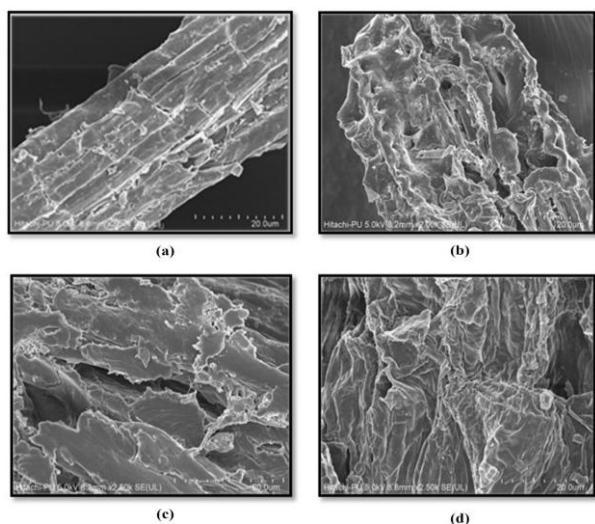


Fig. 1 FSEM images: (a) Untreated (b) 2% NaOH (c) 4% NaOH (d) 6% NaOH Pretreated Bagasse

3.3 Effect of NaOH pretreatment on chemical structure

In the chemical structure of bagasse, the cellulose, hemicelluloses and lignin are linked to each other with different types of bonds within the individual component (intrapolymer linkages) and connects with different components (interpolymer linkages) to form complex structure constituting the lignocellulosic material. Lignin, most complex structure, constituted of carbon-to-carbon bonds, ether bonds and associated with carbohydrates (cellulose, hemicelluloses) by ester bonds, α -ether bonds, phenyl glycosidic linkages [14]. The LCC's are the main obstacle for the conversion of organic matter into biogas. The different concentration of NaOH pretreatment has a significant effect on the chemical structure of bagasse as analyzed from FTIR spectra that saponification takes place in the pretreated bagasse resulting in damaged ester bonds linkages between lignin and carbohydrates thus making more cellulose available for microorganism attack in anaerobic digestion process [15].

The peak at 1244 cm^{-1} attributed to the presence of aryl group in lignin associated with untreated bagasse has reduced after pretreatment with 2% NaOH and absent in the 4% NaOH and 6% NaOH pretreated bagasse. The bend at 1730 cm^{-1} due to C=O stretching vibration of acetyl and esters linkages in lignin appeared only in untreated in bagasse and disappeared after pretreatment indicating that side chains of lignin broken down. Aromatic skeletal rings present in lignin assigned to band 1514 cm^{-1} also disappeared after 4% NaOH pre-treatment [16]. The peak at 1635 cm^{-1} existing in all the four spectra attributed to C=C stretching vibration in aromatic skeletal of lignin however its intensity gets reduced in 4% NaOH and 6 % NaOH pretreated bagasse as observed from Fig. 2. The stretching of the band at 1323 cm^{-1} is attributed to phenol hydroxyl functional group stretching.

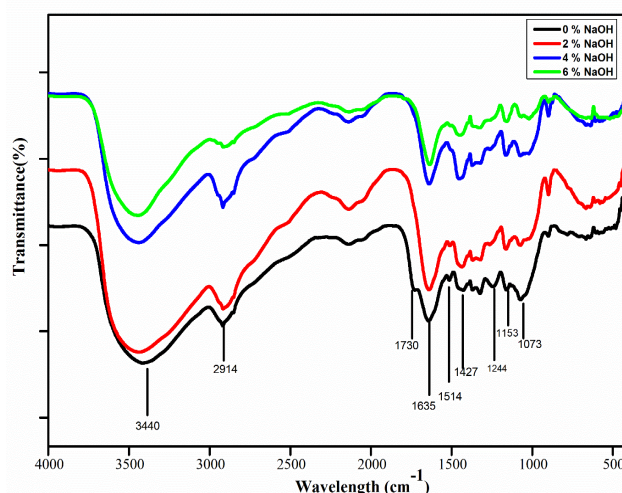


Fig. 2 FTIR spectrum of untreated and NaOH pretreated Bagasse

The wideband between 3300 cm^{-1} and 3450 cm^{-1} attributed to a hydroxyl group (OH) presents in cellulose and hemicellulose, which forms the backbone of carbohydrates. The intense peaks at 3440 cm^{-1} in the spectrum, as shown in Fig. 2 represents that NaOH pretreatment partially affected hydrogen bonds of cellulose, leading to enhance the accessibility of cellulose. The band at 2914 cm^{-1} attributed to the stretching of C-H bond represents some rupture in the methyl and methylene of cellulose [17].

The band at 1427 cm^{-1} representing scissoring of CH_2 and CH_3 in cellulose has been observed maximum intense in 4% NaOH pretreated bagasse as compared with other three spectrums of bagasse. The stretching of the band between $1157\text{--}1163\text{ cm}^{-1}$ attributed to the asymmetrical stretching of C-O-C bonds which are characteristics of cellulose and hemicelluloses. The region between band $1100\text{--}1200\text{ cm}^{-1}$ represents the main contribution of cellulose and hemicelluloses which exhibits maximum value at 1073 cm^{-1} due to C-O and C-C stretching The intermolecular changes observed at band 899 cm^{-1} attributed to the characteristics of β -1, 4-glycosidic bond linkages present in cellulose and cleavage of intermolecular hydrogen bonds.

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From FTIR spectrum study it has been observed that intensity of peak at band 899 cm^{-1} is higher in 2% NaOH pretreated, and 4 % NaOH pretreated bagasse in comparison with untreated bagasse whereas it is deficient in 6% NaOH pretreated bagasse this may be attributed to the fact the maximum cellulose content has solubilized.

It has observed from FTIR spectroscopy that both intra-molecular and inter-molecular changes occurred in the chemical structure of bagasse after pretreatment with NaOH. However, the 4% NaOH pretreatment seems to be most effective as represented by the removal of the prominent peak at 1244 cm^{-1} indicating the cleavage of intermolecular bonds present in lignin-hemicelluloses. More intense peaks observed at 1427 cm^{-1} and 899 cm^{-1} in 4% NaOH pretreated bagasse indicates that sufficient cellulose made accessible to the microorganism for degradation to convert it into biogas in the anaerobic digestion process.

3.4 Effect of NaOH pretreatment on crystallinity

Cellulose is a complex polymer constituted of crystalline and amorphous material. The inner microstructure of cellulose can be studied through X-ray diffractometer as the crystalline structure of the material can diffract X-rays resulting in specific patterns. The peak observed, Fig. 3 at $2\theta = 18^\circ$ corresponds to (0 0 1) crystallographic plane represents the characteristic of cellulose I, as its intensity very low for untreated bagasse and starts increasing after NaOH pretreatment [18]. The shift in crystalline peak from the less intense peak at 18° to most intense peak at 22.49° may be attributed to release more cellulose from hemicelluloses and lignin linkages after pretreatment with different concentration of NaOH in the (0 0 2) plane.

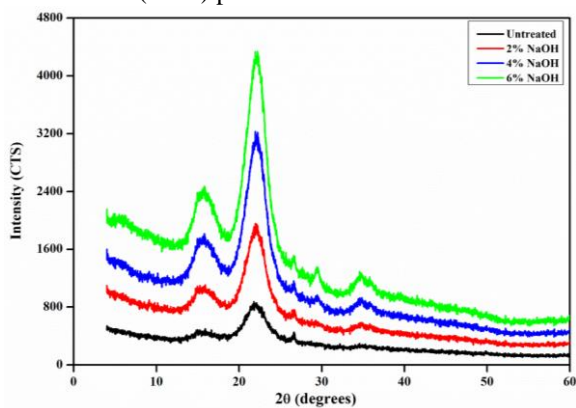


Fig. 3 XRD Pattern of untreated and NaOH pretreated Bagasse

It has observed that the 4% NaOH pretreated bagasse has maximum intensity owing to the most organized crystalline structure. The crystallinity index of cellulose increased from 61.9% for untreated bagasse to 66.67% for 4% NaOH pretreated bagasse, which may be attributed to the greater hydrolyzation of the amorphous area. Further, it has observed that crystalline index of 6% NaOH pretreated bagasse has reduced to 64.8% which may be attributed to the rearrangement of polysaccharide chains from cellulose I to Cellulose II, resulting in contraction of the crystalline part due to less ordered material. Thus, the maximum value of crystallinity index corresponds to 4% (w/v) NaOH pretreated bagasse which is in conformation with the observation given

by Oudiani [19] that the optimum concentration of NaOH corresponding to higher crystallinity index of fiber remains in order of 2-5% (w/v) NaOH. After 5% (w/v) NaOH cellulose II starts dominating cellulose I and crystallinity of fiber increases due to the coalescence of cellulose I and cellulose II.

3.5 Effect of pretreatment on Cumulative Biogas Production

The untreated and NaOH pretreated bagasse anaerobically digested in different digesters at $35 \pm 2^\circ\text{C}$ temperature. The daily biogas production from each digester was recorded for 45 days. The biogas production from untreated and pretreated bagasse almost started from first day and the first peak of biogas production was observed during 5th- 10th days. After that second peak of biogas production observed between fifteen to twenty days of Hydraulic Retention Time (HRT). The 4% NaOH pretreatment of bagasse is most effective in enhancement of biogas production as observed from Fig.4. The biogas production by 4% NaOH pretreatment is 55.3% higher than that of untreated bagasse, which is followed by 2 %NaOH resulting into enhancement of 27.34% higher than untreated bagasse.

It has observed that NaOH pre-treatment beyond 4% (w/v) was not effective in enhancing the biogas production from the bagasse. This may be attributed to the fact as observed from the FTIR analysis that carbohydrates content of bagasse also gets reduced by 6% NaOH pretreatment of bagasse may be due to their greater solubilization at higher concentration of NaOH pretreatment. Further, it has observed that after 25 days the biogas starts decreasing, which may be attributed to that maximum biodegradable material has been exhausted.

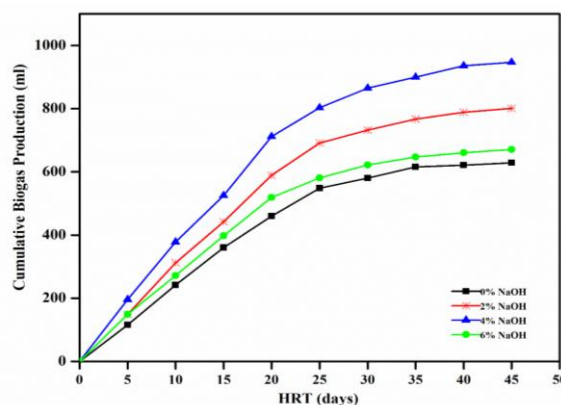


Fig. 4. Cumulative Biogas Production from untreated and NaOH pretreated Bagasse

IV. CONCLUSION

In the study, it has observed that 4% NaOH concentration was most effective in biogas production from the bagasse. The enhancement of biogas may be attributed to the distortion of intermolecular linkages and intermolecular linkages between cellulose, hemicelluloses, and lignin. The distortion of ester bonds of LCC (Lignin-Cellulose Complex) by hydrolysis reaction results in the release of more cellulose for degradation, thus enhancing the biogas production.

However, in 6% NaOH pretreated bagasse the more cellulose content seems to get dissolved therefore the lesser cellulose was available for degradation causing small biogas production near to untreated bagasse. The pretreatment of bagasse with NaOH shows that the crystallinity of cellulose has increased without damaging its crystal style as observed from the XRD pattern. The change of chemical structure, chemical compositions, and physical structure after pretreatment with NaOH the bagasse became more biodegradable and easily digestible thus enhanced biogas production.

ACKNOWLEDGMENT

The authors, are grateful to the PURSE Grant II, Panjab University, for providing financial support for the purchase of chemicals and glassware. The authors are also thankful to CIL, SAIF lab, and Purse lab/ Central Facility Lab of Dr. S. S. B. University Institute of Chemical Engineering & Technology, Panjab University, Chandigarh.

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