



RESEARCH ARTICLE

Biomethanation of Acid Pretreated Biofuel Process Stillage for Enhanced Energy

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ABSTRACT

The development of sustainable energy systems based on renewable biomass feedstock is now a global effort. Fuels produced from biomass have the potential to reduce reliance on petroleum resources and reduce greenhouse gas emissions. Biofuels obtained from coproduced residues remains one of the major challenges in full-scale operations. This study investigated the potential of an anaerobic digestion system to convert the stillage obtained from pearl millet-based lignocellulosic biofuel production. Biomethanation experiment study was conducted in CSTR using acid pretreated pearl millet-based biofuel stillage up to 30 days. Using SAS software, the multi-collinearity was identified among different parameters of pH, TS, VS, and VFA profile were analyzed for identifying the major factor impacting biogas production. The initial parameters of stillage were pH 6.8, VS 978 mg.l⁻¹, TS 988 mg.l⁻¹ and VFA 802.44 mg.l⁻¹. The maximum biogas production 0.17 L was obtained at neutral pH of 7.4 on 16th day with respect to reducing the VFA 9.45 mmol.l⁻¹ acetate, VS 954 mg.l⁻¹ and TS 973 mg.l⁻¹, after which there was a reduction in the gas production due to alkaline pH even though TS, VS, and VFA were reduced.

Keywords: *lignocellulosic biomass; Acid pretreatment; Stillage; Anaerobic digestion; Continuous Stirred Tank Reactor; Biomethanation*

INTRODUCTION

The hunt for alternative sources of energy generation that are inexpensive, eco-friendly, renewable, and can replace fossil fuels is on, owing to the increasing demands of energy. One approach in this direction is the conversion of plant residues into biofuels wherein lignocellulose, which forms the structural framework of plants consisting of cellulose, hemicellulose and lignin, is first broken down and hydrolyzed into simple fermentable sugars, which upon fermentation form biofuels such as ethanol (Morales *et al.*, 2015) Methane is an energy-rich component that is formed as the end product during the anaerobic decomposition of organic matter, such as domestic slurries and residues coming from food processing manufactories. Among many different materials that can be used for biogas production, lignocellulose-rich materials, such as plant wastes, and protein-rich materials, such as animal manure, are highly promising due to their high methane potential (Divya *et al.*, 2015) It is estimated that the world lignocellulosic biomass fixes tenfold the solar energy amount per year compared to the total yearly energy demand of all humans. This study investigated the potential of using anaerobic digestion to convert acid pretreated stillage into biogas.

MATERIAL AND METHODS

Sample preparation

The pretreatment was carried out using TS 7.5 % (w/v) of pearl millet biomass immersed in a 12 % orthophosphoric acid solution. The hydrothermal treatment was done in the reactor at a 6 bar pressure with a temperature of 170 °C for 40 min. The resulting solid fraction was dried at 40 °C in an oven. Enzymatic saccharification was done using Cellulase enzyme at 40 FPU/g of cellulose (Mishra *et al.*, 2011). Fermentation was carried out by *Saccharomyces cerevisiae* and ethanol was quantified in a GC. The stillage obtained after ethanologenic fermentation was used as a substrate for biogas production.

Biomethanation of CHP fermented stillage in CSTR

CSTR studies

Biomethanation was conducted in a continuous stirred tank reactor (CSTR) Lark MOD53, for 30 days. A CSTR (stainless steel, 200l, 500 mm in diameter) was, equipped with a mechanical mixer, feeding pump, and monitoring and control systems, including programmable logic controller (PLC) and supervisory control and data acquisition system.

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Mixing was applied for 20 sec within every 2 minutes (30 rpm) to get stable biogas flow from the reactor. The temperature of the reactor was set to 37°C. The fermentor of 2 l capacity containing 1.5 l working volume was provided with 10 % inoculum of cow dung slurry given as startup inoculums and 0.5 l as headspace for this reactor. The headspace was filled with N₂ at a flow rate of approximately 0.5 -1 min⁻¹ for 15 min. (Wang *et al.*, 2012) Biogas production was measured on alternate days using the water displacement method; the daily biogas produced from CSTR treatment was measured by the water displacement method. The evolved gas was collected in a graduated measuring jar kept on a platform in an inverted position inside a tub of water. The gas outlet of the experimental bottle is connected to the measuring jar. When the cock of the biogas bottle is released, the evolved biogas gets filled in the measuring jar displacing the equal volume of water in the tub. The volume of water displaced is equal to the amount of biogas produced. Methane content quantified using GC- FID poropak-Q column. The parameters viz, TS, VS, and VFA were measured with respect to gas production. The

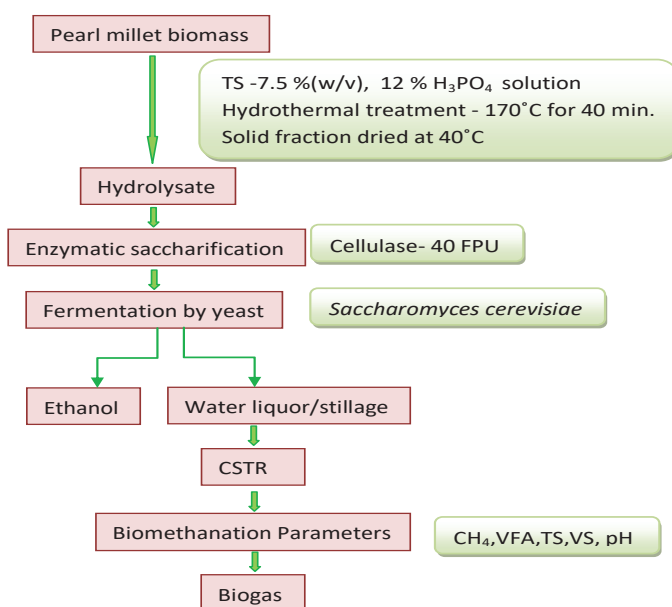
reactor pH was probe sensor. Statistical analysis system (SAS)-9.4 version software monitored with linear regression analysis, and the major factors influencing the biogas production was determined. High *F* and *R*² values and low *P* values for biogas yield indicated the model predictability (Ye *et al.*, 2013).

FT-IR analysis

FT-IR (Fourier Transform Infrared) analysis of the tested samples were studied using an FT-IR (FTIR-6800 JASCO, Japan). Absorbance spectra were recorded between 4000 to 400 cm⁻¹ wavenumbers with a spectral resolution of 4 cm⁻¹ and 64 scans per sample.

Scanning Electron Microscope of Acid pretreated biomethanated stillage

The sludge samples were collected from the CSTR reactor by scanning electron microscopy (SEM: Quanta 250, FEI, Hillsboro, OR, USA) using an ETD detector. The SEM was operated in a vacuum, 10 kV, with a spot size of 4 and pressure of 60 Pa. The SEM images of tested samples were captured at x4000 magnification.



RESULTS AND DISCUSSION

The initial parameters of stillage were as, pH 6.8, VS 978 mg.l⁻¹, TS 988 mg.l⁻¹ and VFA 802.44 mg.l⁻¹. The maximum biogas production 172 ml was obtained at neutral pH of 7.4 on 16th day with respect to reducing the VFA 9.45 m.mol.l⁻¹ acetate, VS 954 mg.l⁻¹ and TS 973 mg.l⁻¹ after which there was a reduction in the gas production due to alkaline pH even though TS, VS and VFA were reduced.

During the first 10 days of methanation process, the gas production in the digester slightly increased

to about 0.25 -0.63 l biogas. The pH ranged between 6.8–7.2, and the VS, TS, and VFA contents decreased gradually. The gas production reached a maximum (0.17 l) on 16th day with a pH of 7.4. As the process progresses, the volatile fatty acids were metabolized and the pH gradually increased to the sufficient buffering capacity (neutral pH) necessary for the production of biogas. Moreover, both acidogenic and methanogenic microorganisms have their optimal pH for metabolism, but the methanogens are highly pH-sensitive and thrive optimally within the pH range of 6.8-7.6. This explains the high flammability rate

of the biogas in this study, at pH 7.4 on the 16th day of the digestion process owing to the increase in methanogenic activity of the digester system (Babae et al., 2015).

However, after 16 days of operation, the efficiency of the process deteriorated with a considerable decrease in gas production, indicating process instability. Initially, the pH was maintained around 7.4 but continued to increase after 20 days of operation, and then kept varying between 7.58 and 8.12. Because of the alkaline pH the methane content of the gas decreased to about 50%. So it was evident that though the TS, VS and VFA contents reduced constantly, the pH kept increasing, thus negatively affecting the biogas production.

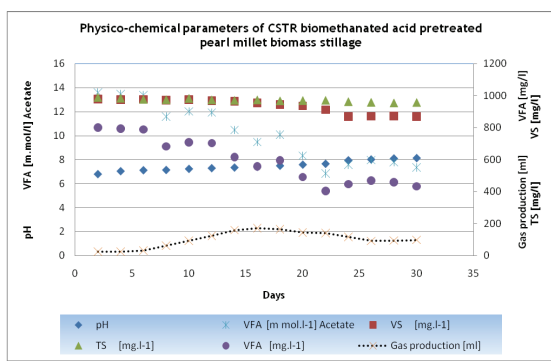


Figure 1. Physico-chemical parameters of CSTR biomethanated acid pretreated pearl millet biomass stillage

SAS Analysis of biomethanated pretreated pearl millet biomass stillage in CSTR

Influence of pH on biogas production:

The polynomial cubic shows the best fit for observed data, and the following equation provided the best fit to predict the different days of pH change on biogas production. The quadratic equation provided a higher r^2 value of 0.7475 and p-value < 0.1 compared to the quadratic, polynomial, or bilinear equations (data not shown) for the influence of pH on biogas production. (Fig. 2a)

$$Y = 78090.12 - 33424.39x + 4738.78x^2 - 223.3781x^3 \dots\dots\dots(1.1)$$

The pH reached 8.2 on 30th day and biogas production declined to 98 ml.

Influence of VS on biogas production:

The polynomial cubic shows the best fit for experimental data, and the following equation provided the best fit to predict the influence of VS on biogas production. The highest r^2 value was 0.8019 and p value < 0.1 was obtained for the following equation as compared to that of the quadratic, polynomial or bilinear equations for the experimental

data used for the analysis for the influence of VS on biogas production.

$$Y = 329377.36 - 1094.34x + 1.21x^2 - 0.004x^3 \dots\dots\dots(1.2)$$

The initial and final values of VS content of selected feedstock recorded on the first and 30th days of the experiment were 978 and 870 mg.l⁻¹. From the graph, it was observed that biogas production increased with a decrease in VS content and attained maximum biogas production of 170 ml on 18th day. After the 18th day the biogas production and VS content were found to decrease in trend up to 30th days of the experiment period. (Fig. 2b)

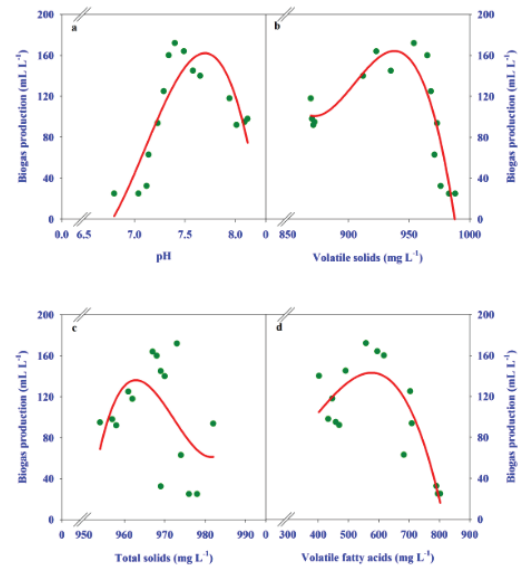


Figure 2. Linear regression analysis of biogas production (a) pH (b) VS (c) Total solids (d) Volatile fatty acids

Influence of TS on biogas production:

The polynomial cubic shows the best fit for the observed data and the following equation found as the best fit to predict the influence of VS on biogas production. The highest r^2 value was 0.2550 and p value > 0.1 was obtained for the following equation as compared to that of the quadratic, polynomial or bilinear equations for the experimental data used for the analysis for the influence of TS on biogas production.

$$Y = -21317194.59 + 65793.86x - 87.68x^2 + 0.023x^3 \dots\dots\dots(1.3)$$

The initial and final values of TS content of selected feedstock recorded on the first and 30th days of the experiment were 988 and 957 mg.l⁻¹. From the graph, it was observed that biogas production increased with decrease in VS content and attained a maximum biogas production of 170 ml on 18th day. After 18th day the biogas production and VS content were found to decrease in trend up to 30th days of the experiment period. (Fig. 2c)

Influence of VFA on biogas production:

The polynomial cubic shows the best fit for the recorded data, and the following equation provided the best fit to predict the influence of VFA on biogas production. The highest r^2 value was 0.7386, and p -value < 0.1 was obtained for the following equation as compared to that of the quadratic, polynomial, or bilinear equations for the experimental data used for the analysis of the influence of VFA on biogas production.

$$Y = 119.58 - 0.91x - 0.003x^2 - 0.00000299x^3 \dots\dots(1.4)$$

The initial and final values of VFA content of selected feedstock recorded on the first and 30th days of the experiment were 802.44 and 433.03 mg.l^{-1} . From the graph, it was observed that biogas production increased with decrease in VS content and attained a maximum biogas production of 170 ml on 18th day. After the 18th day the biogas production and VFA content were found to decrease in trend up to 30th days of the experiment period. (Fig. 2d)

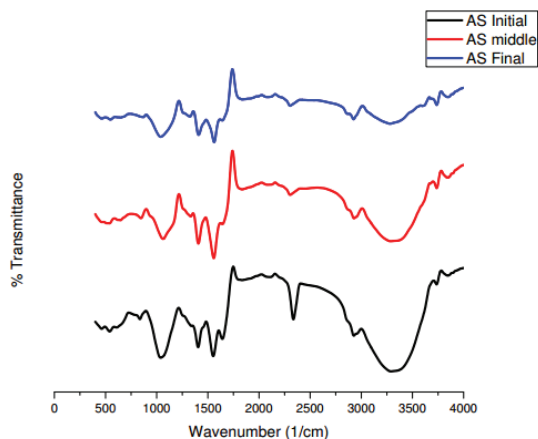


Figure 3. FTIR- ATR spectrum of acid pretreated pearl millet biodigested stillage

The biomethanation study was conducted and the different physico-chemical properties were measured. Initially, the pH was maintained around 7.4 continued to increase after 20 days of operation, and then varying between 7.58 and 8.12. Because of the alkaline pH the methane content of the gas decreased to about 50 per cent. So it was evident that though the TS, VS and VFA contents reduced constantly, the pH kept increasing, thus negatively affecting the biogas production. As the process progresses, the volatile fatty acids were metabolized and the pH gradually increased to the sufficient buffering capacity (neutral pH) necessary for the production of biogas. Moreover, both acidogenic and methanogenic microorganisms have their optimal pH for metabolism, but the methanogens are highly pH-sensitive and thrive optimally within the pH range of 6.8-7.6. This explains the high flammability rate

of the biogas in this study, at pH 7.4 on the 16th day of the digestion process due to the increase in the digester system (Babae et al. 2013).

The characteristics of the substrate and the presence of alkaline pH affect the microbial succession of anaerobic degradation and subsequent methane production. During methane production of organic material, four main metabolic groups have been identified: hydrolytic bacteria, fermentative bacteria, acetogenic bacteria and methanogens. The activity of methanogens and acetogens, which perform the last degradation steps, is essential for an efficient and stable process. Inhibitory compounds can negatively affect the growth and activity of the microorganisms performing the last degradation steps, resulting in the accumulation of volatile fatty acids (VFA) and possibilities of the subsequent increase in pH, which inhibits the entire degradation and may reduce the efficiency of biomethanation (Wang et al. 2009). Acetic acid was found to be the most abundant contributor to the total concentration of VOAs. Butyric acid was the second most abundant and its concentration was about half of that of acetic acid. The concentration of all other VOAs was negligible.

FT-IR analysis of biodigester test samples

A strong broad absorption at 3280 - 3290 cm^{-1} was observed related to the stretching of H-bonded OH groups Table 4.12. In addition, there are many well-defined peaks in the fingerprint region between 1060 and 1800 cm^{-1} . This indicates that there were structural changes of cellulose after pretreatment. Major changes were broadening of band at 3200 - 3300 cm^{-1} which was associated with the O-H stretching of the hydrogen bonds and the structural features of lignin. Bands at 1000 - 1100 cm^{-1} represented the structural features of cellulose and hemicelluloses. The enhancement of absorption peaks at 1000 - 1100 cm^{-1} after pretreatment indicates the increase in cellulose content in the solid residue. The peaks at 3300 cm^{-1} and 2300 cm^{-1} are the distinguished features of cellulose. (Fig. 3)

FT-IR spectroscopy is frequently used to investigate the structure of constituents and the chemical changes in lignocellulosic biomass during pretreatment. A strong, broad absorption at 3280 - 3290 cm^{-1} is observed which is related to the stretching of H-bonded OH groups. In addition, there are many well-defined peaks in the fingerprint region between 1060 and 1800 cm^{-1} . This indicates that there were structural changes of cellulose after pretreatment. Major changes were broadening of band at 3200 - 3300 cm^{-1} which was associated with the O-H stretching of the hydrogen bonds. Bands at 1000 - 1100 cm^{-1}

were related to structural features of cellulose and hemicelluloses. The enhancement of absorption peaks at 1000 - 1100 cm^{-1} after pretreatment indicate the increase in cellulose content in the solid residue. The peak of O-H stretching at 3300 cm^{-1} and the peak of $-\text{CH}_2$ stretching near 2300 cm^{-1} are the distinguished features of cellulose (Sun and cheng, 2005). The pretreated samples displayed significant decreases in band intensities at 1450 (C-H deformation), 1508 (aromatic skeletal vibrations), and 1640 cm^{-1} (conjugated C=O stretch).

This indicates that the pretreatment causes a breakdown of the aromatic structure of lignin.

Sun *et al.*, (2003) reported that the band at 3353 cm^{-1} is depicting the stretching of hydroxyl group in treated bagasse. The absorption at 2335 cm^{-1} in treated sugarcane bagasse arises from C-H stretching, moreover, the absorbance at 1315 1561 and 1032 cm^{-1} corresponds to the aromatic skeleton vibration, ring breathing in the C-O stretching in lignin.

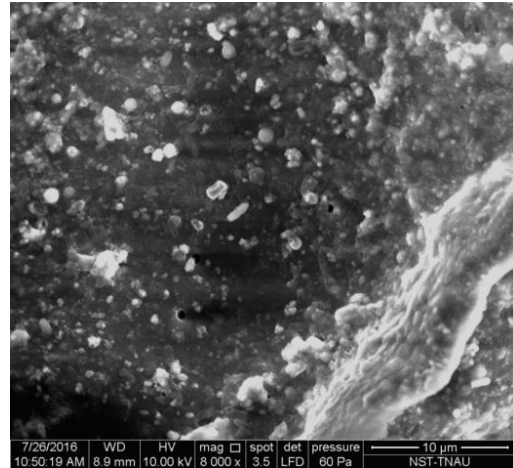
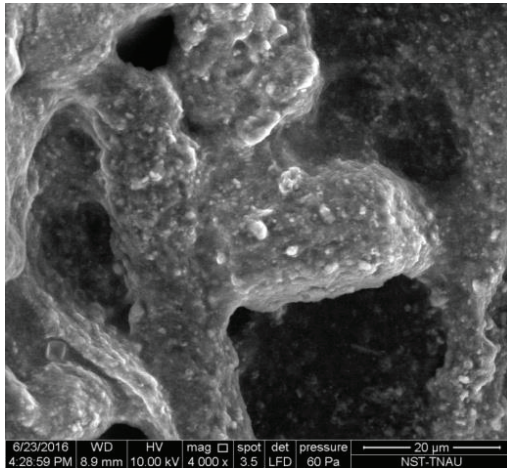


Figure 4. SEM analysis of acid pretreated pearl millet stillage in CSTR

Fig. 4 shows SEM pictures of the anaerobic sludge (AS) sample used as inoculum and sludge samples obtained from ACHP at the end of the experiment. 4000 \times pictures gave us a general overview of the different sludges developed in the CSTR.

SEM analysis of pretreated pearl millet stillage in CSTR

SEM pictures of the anaerobic sludge (AS) sample used as inoculum and sludge samples obtained from ACHP at the end of the experiment. 4000 \times pictures gave us a general overview of the different sludges developed in the reactors. AS sample presented a homogenous appearance. Anaerobic bacteria are not able to degrade lignin (Robbins *et al.*, 1979), which can even reduce cellulose bioavailability by reducing the surface area available for enzymatic penetration anaerobic sludge by SEM.

Acid-catalyzed thermochemical pretreatment of lignocellulosic feedstocks has several advantages: it is a simple and inexpensive approach for pretreatment that efficiently improves the susceptibility to cellulolytic enzymes, even for more recalcitrant types of lignocellulose. A drawback is the formation of by-products that inhibit enzymes

and microorganisms in subsequent biocatalytic conversion steps. However, rapid progress in several areas, such as conditioning or detoxification of slurries and hydrolysates, fermentation technology, and microbial resistance to inhibitors, makes acid pretreatment a highly competitive future alternative in the bioconversion of lignocellulosic feedstocks. Management of inhibition problems is likely to become more important in a development that favors flexibility with respect to feedstocks, processes based on high dry matter content and high product concentrations and recirculation of process water.

CONCLUSION

Biomass is a versatile and abundant resource, which can be used to produce energy via different routes, including fermentation and anaerobic digestion. The above study investigated the major factors influencing the biogas production from the acid pretreated stillage. It was observed that the accumulated fatty acids were quickly degraded, but the biogas production decreased when the reactor pH continued to increase beyond 7.4. Future studies aimed at optimizing the reactor parameters need to be conducted to attain maximum conversion of stillage into biogas production by biomethanation.

REFERENCES

- A. Babae and J. Shayegan. Anaerobic slurry co-digestion of poultry manure and straw: effect of bioethanol: Environmental impacts and energy balance. *Renew. Sustain. Energy Rev.* **42**:1349–1361. (2015).
- D.Divya, L.R.Gopinath, C. P.Merlin. A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renew. Sustain. Energy Rev.*, **42**: 690–699. (2015).
- J.E. Robbins, M.T. Arnold and S.L. Lacher. Methane production from cattle and straw. *Applied Environmental Microbiology.* **38**: 175–8. (1979).
- J.X.Sun, X.F. Sun, R.C. Sun, P. Fowler and M.S. Baird. Inhomogeneities in the chemical structure of sugarcane bagasse lignin. *Journal of Agriculture and Food chemistry.* **51**: 6719-6725. (2003).
- M.Morales, J.Quintero, R.Conejeros, G.Aroca. Life cycle assessment of lignocellulosic bioethanol: Environmental impacts and energy balance. *Renew. Sustain. Energy Rev.* **42**: 1349–1361. (2015).
- M.S.Mishra, B.Chandrashekhar, T.Chatterjee, K.Singh. Production of bioethanol from Jatropha oilseed cakes via dilute acid hydrolysis and fermentation by *Saccharomyces cerevisiae*. *International Journal of Biotechnology Applications.* **3(1)**: 41-47. (2011).
- J.Q. Ye, D. Li, Y.M. Sun, G.H. Wang, Z.H. Yuan, F. Zhen and Y. Wang. Improved biogas production from rice straw by codigestion with kitchen waste and pig manure. *Waste management.* **33(12)**: 2653-2658. (2013).
- X.Wang, G. Yang, Y. Feng, G. Ren and X. Han. Optimizing feeding composition and carbonnitrogen ratios for improved methane yield during anaerobic codigestion of dairy, chicken manure and wheat straw. *Bioresource Technology.* **120**:78–83. (2012).
- Y.Sun, and J.J. Cheng. Dilute acid pretreatment of rye straw and Bermuda grass for ethanol production. *Bioresource Technology.* **96**: 1599–606. (2005).
- Y.Wang, Y.Zhang, J.Wang, L.Meng. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass Bioenergy.* **33**: 848–853. (2009).