CO-DIGESTION OF CASSAVA MILL EFFLUENT PRETREATED RICE HUSK LIGNOCELLULOSIC BIOMASS WITH POULTRY MANURE AND EFFECT ON BIOGAS PRODUCTION

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ABSTRACT

The effect of fresh cassava (*Mannihot esculanta*) mill effluent (CaME) with pH 3.6 as wetting and mild hydrolytic agent compared to water (control) in anaerobic co-digestion of rice husk was evaluated using a modified biomethane potential assay for 45 days at 37 ± 2 ^{0}C. Mean biogas volume in the control ranged between 14 ± 2 to 23 ± 5 mL gVS^{-1} and 27.3 ± 5 to 48 ± 9 mL gVS^{-1} in the treatment. The difference in the mean biogas volume from the CaME treated digestate was significant at $p = 0.05$ and 1.95 to 2.1 times higher than the biogas volume of the control. The mean volatile solids in the control ranged between 8 ± 0.8 to 9 ± 0.8 mg kg^{-1}, whereas in the CaME pretreated digestate, the values were 4 ± 0.2 to 7.5 ± 0.1 $mgkg^{-1}$.The volatile solid concentration in the CaME treated digestate was 1.2 to 2 times lower than the control. Anaerobic microbial counts ranged from 5.8 to 6.8 ± 0.1 Log_{10}CFU g^{-1} in the control and from 6.26 to 6.34 ± 0.2 Log_{10}CFU g^{-1} in the CaME treated digestate. The microbial counts were 1.03 to 1.08 times higher than the values recorded for the control. Similarly there were significant differences in volatile solid concentration and microbial counts between the CaME treated digestate and control at $p = 0.05$. The methanogens in the digestate associated with biogas production were members of the genera *Methanothrix*, *Methanosarcina*, *Methanobrevibacter* and *Methanocorpusculum*. The results indicate that CaME enhanced hydrolysis of the rice husk lignocellulosic biomass for efficient microbial conversion, reactor performance and improved biogas production.

INTRODUCTION

Energy is an essential need required for optimum use of available resources and includes those from wind, water, nuclear, sun, fossil and renewable sources (Sambo *et al*., 2015). Despite the importance of energy to man, studies have shown that most modern services rely mainly on fossil fuel as the primary source of energy (Ezekoye *et al.,* 2014., Asere and Aliyu, 1992). It is common knowledge that non-renewable energy from fossil fuel poses a serious challenge because of the negative ecological effects, concerns of depletion and contribution to the global warming and climate change phenomenon. In contrast, renewable energy such as bioethanol and biogas as alternatives to non-renewable fossil fuels are eco-friendly (Sambo *et al.*, 2015; Sweeten, 2004). The production of biogas from waste material minimizes environmental pollution associated with indiscriminate disposal and management challenges (Sambo *et al.,* 2015).

Biogas production is mediated by diverse group of microorganisms through anaerobic digestion of biodegradable organic materials in sewage, municipal and domestic wastes, and agricultural wastes. The anaerobic process involves complex synergistic and syntrophic microbial community during hydrolytic, acidogenics, acetogenic and methanogenic phases (Appels *et al.,* 2008 and Wagner *et al.,* 2010). In addition, different optimal conditions are required by the microorganisms to grow, metabolize and are influenced by factors such as pH, temperature, concentrations of volatile fatty acids, ammonia and hydrogen sulfide. Thus, an efficient process requires a delicate balance of microbial community activity and optimum reactor operation which is a challenge because of the inherent substrate composition, conversion and accumulation of metabolites (Wagner *et al*., 2010).

The use of rice husk for biogas production has been reported (Elijah *et al.,* 2009; Iyagba *et al.,* 2009), although efficient conversion of the substrate is still a challenge. Notably, the high content of lignin in lignocellulosic biomass hinder hydrolysis, reduce the rate of microbial and enzyme catalysis because few organisms can elaborate ligninases (Habeed and Mahmud, 2010; Sambo *et al*., 2015). The resistance of lignocellulosic biomass to hydrolysis can be mitigated through pretreatment to modify the biomass for efficient bioconversionor co-digested for optimum biogas production (Eduok *et al.,* 2018). In practical terms, pretreatment can alter lignin structure and reduce crystalinity of cellulosic material for easy bioconversion (Singh *et al.,* 2014; Liu and Wyman, 2005). However, pretreatment approaches such as acid and alkali hydrolysis generate downstream products that inhibit the microbial communities involved in the bioconversion (Bolado-Rodriquez *et al*., 2016). The search for simple, readily available organic, cost effective and ecofriendly pretreatment approach for efficient bioconversion of the abundant lignocellulosic biomass becomes imperative.

In Nigeria, agricultural wastes from major food crops such as rice and effluent from cassava processing contributes to the increased environmental waste load, cause public nuisance and reduce aesthetics. Cassava is an important raw material for the production of starchy staple foods such as g*arri*, *lafun* and *fufu* (Oguntoyinbo, 2011). The waste stream from cassava processing mills disposed into surface water and soil is potentially injurious to ecosystem health because of the bio-physicochemical composition of the cassava mill effluent (CaME). The CaME harbors lactic acid bacteria (LAB) associated with acidity and decreased pH of the wastewater (Freire *et al.,* 2014).Based on the nature and composition of the wastes, we evaluated the production of biogas by anaerobic digestion of rice husk lignocellulosic biomass pretreated with fresh CaME and co-digested with poultry manure.

MATERIALS AND METHOD

Sample Collection

Shredded rice husk from a local rice processing plant in Ini Local Government Area of Akwa Ibom State, poultry manure and cow dung from the Animal Husbandry Unit, University of Uyo were collected separately into air tight containers. Cassava mill effluent (CaME) was collected from a cassava processing centre in Uyo Metropolis into sterile plastic containers.The samples were transported to the laboratory and stored at 4 ^{o}C until required for use.

Experimental Design

A modified approach of Eduok *et al.,(*2018) in which the lignocellulosic waste was subjected to solid state fermentation with a near absence of free moisture from water or cassava mill effluent was adopted. Precisely 800 g of the milled rice husk in two wooden troughs were pretreated by separate exposures to water and cassava mill effluent as wetting agent for 21 days. The substrate were separately moistened with 20 mL of distilled water (control) or cassava mill effluent (CaME), mixed daily and allowed to stand at ambient temperature (29 ± 3^{0}C). The biomethane (BMP) assay involved the use of 100 mL amber serum bottles (Gerresheimer 61020G, USA) and 20 mm closure size aluminium crimp seal with central moulded PTFE/Butyl septum (W224224, Wheaton) as reactor. 15 g subsample of the pre-treated substrate retrieved at day 7, 14 and 21 were mixed with 10g poultry manure and inoculated with 5 g VS^{-1} aged-cow dung and fed into the reactor bottles. Prior to inoculation, the fresh cow dung was incubated at 45 ± 0.2 ^{0}C for 25 days until no biogas was detected.

The wetting and buffering agent used in the BMP assay was 35 mL of distilled water and CaME added to the pretreated substrate with a total reaction volume of 65mL. The substrate and inoculum in the reactor bottles were allowed to equilibrate for 1 hour at 45 ± 0.2 °C and sealed using standard hand operated crimper, 20 mm cap size (JG Finneran 9300-20, USA).

Triplicate reactors used for each treatment were incubated for 45 days in a thermostat-regulated water bath at 45 ± 0.2 °C with 15 min manual agitation every 12 h. For comparison, microorganisms in the cow dung (inoculum) were exposed to untreated rice husk lignocellulosic biomass and poultry manure (control). The biogas was measured at interval of three days using the volumetric method (Esposito *et al*., 2012) based on the principle of liquid/water displacement as described elsewhere (Eduok *et al*., 2017).

Determination of pH and Volatile Solids
The pH and volatile solids of the substrate and digestate were determined in triplicates according to the methods of AOAC (2012).

Isolation and Enumeration of Anaerobic Bacteria
A ten-fold serial dilution of the digestate was carried out according to standard method. Aliquots (1mL) of 10^{-3} and 10^{-4} dilutions were inoculated using pour plate technique onto reinforced clostridial agar. The plates were incubated in anaerobic jar with inserted gaspak for 3 to 5 days. Thereafter, discrete colonies that developed were enumerated and purified by repeated sub-culturing (Cappuccino and Sherman, 2002).

Characterization and Identification of Methanogens
Characterization and identification of methanogens was done based on their cultural, morphological and biochemical characteristics (Holt *et al.,*1994). Catabolic substrates test was carried out using basal medium for selective enrichment and growth of methanogens (Zeikus,1977; Zinder *et al.,*1984;Manimegalai *et al.,*2014).

RESULTS AND DISCUSSION

The use of lignocellulosic waste such as rice husk in anaerobic digestion for biogas production pose some challenges because of the complex nature of the substrate. However, improvement of the substrate characteristics can be achieved through the pretreatment process. We pretreated the substrate with cassava mill effluent to enhance the synergistic roles of the microbial consortia involved in the digestion process. The result of the BMP assay indicates that the rice husk lignocellulosic biomass pretreated for 7, 14 and 21 days produced a mean biogas volume of 27.3, 37.6 and 48.6 mL gVS^{-1}(Figure 1) and a cumulative biogas volume of 437, 601 and 778 mL gVS^{-1} respectively. The difference in the cumulative biogas volume of the pretreated substrate was1.9, 2.03 and 2.10 times higher than the respective controls at 7, 14 and 21 days and significant at $p = 0.05$.The volume of biogas generated from the biomass suggests that the pretreatment was effective and influenced by the exposure time.

The biogas production exhibited an initial, plateau and decline phase in all the digesters (Fig. 1) with the control characterized by very low volume of biogas. The results indicate that maximum biogas yield was obtained from pretreated rice husk within 3 to 27 days. The cumulative biogas yield was in the order: $Treatment_{21}$> $Treatment_{14}$ > $Treatment_{7}$ > Control in all digesters (Fig. 2). The reduced biogas yield in the control is attributed to the low availability of easily degradable organic constituent of the rice husk lignocellulosic biomass. Whereas, the improved biogas yield from substrates pretreated with cassava mill effluent indicated available degradable material as a result of the combined action of the pH, microflora associated with cassava mill effluent and poultry manure. Microorganisms associated with CaME have been implicated in the breakdown of complex organic materials (Freire *et al.,* 2014).

Further to this, the results indicate that CaME served as dilute acid pretreatment to hydrolyze lignocellulosic biomass and increased available cellulose for microbial conversion. On the other hand, poultry manure provided the complimentary LAB and ammonium (with pH of 6.4 ± 0.2) that neutralized the residual effect of CaME acidity for enhanced methanogenic activity and biogas production. In addition, the ammonium concentration maintained pH balance in the reactors and similar to ammonia fiber explosion (Bals *et al.,* 2010), contributed to the disruption of the lignocellulosic structure.

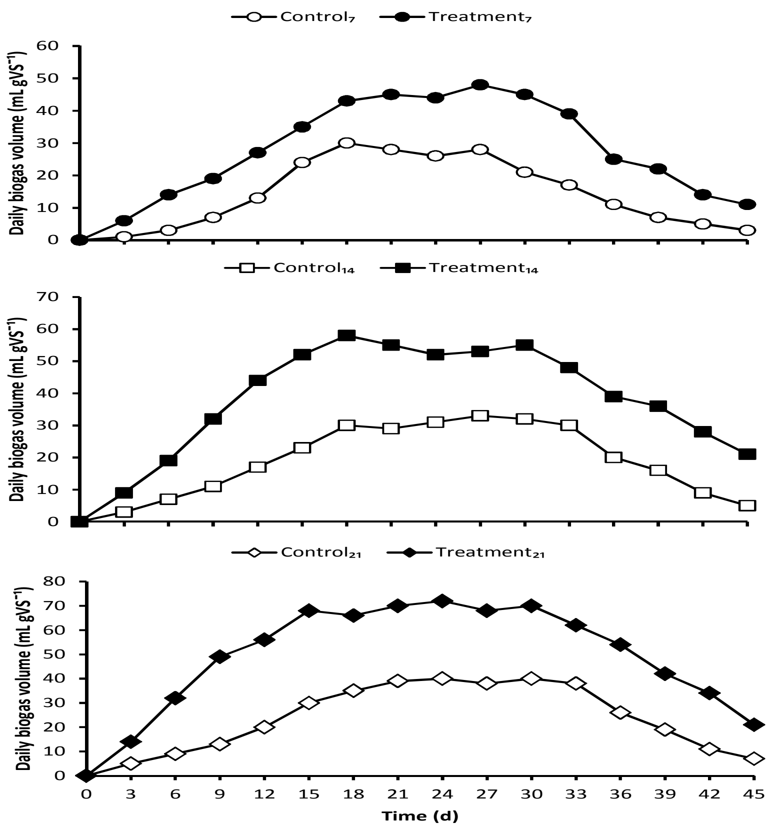

Figure 1: Influence of cassava mill effluent pretreated rice husk lignocellulosic biomass on biogas production.

Note: The volume of biogas produced in the treatments was 1.95 to 2.1 times higher than the control in relation to the exposure time.

Several studies indicate that pretreatment of substrate can enhance biogas production (Ariunbaatar *et al.*, 2014; Carlson *et al.*, 2012; Mussoline*et al.,*2012). However, the cumulative biogas yield of the rice husk pretreated with cassava mill effluent correlated positively with increased the exposure duration. This indicates the retention time for the rice husk during pretreatment with cassava mill effluent was a key factor for the substrate conversion into easily degradable components. Thus, the duration of substrate exposure influenced the degradation of the lignocellulosic biomass into simple sugars for microbial conversion.

The mean volatile solids in the control ranged between 8 ± 0.8 to 9 ± 0.8 mg kg^{-1}, whereas in the CaME pretreated digestate, the values were 4 ± 0.2 to 7.5 ± 0.1 mg kg^{-1} (Fig. 3).The volatile solid concentration in the CaME treated digestate was 1.2 to 2 times lower than the control and significant at p = 0.05. The results indicate the role of digestion process in converting the substrate to biogas, with a reduced volatile solid content of the digestate.

The counts of anaerobic bacteria in the control digestate ranged between 5.8 Log_{10}CFU g^{-1} to 6.8 ± 0.1 Log_{10}CFU g^{-1} and 6.26 Log_{10}CFU g^{-1} to 6.34 ± 0.2 Log_{10}CFU g^{-1} in the CaME pretreated digestate (Figure 4). The difference in bacterial counts of the pretreated digestate was 1.03 to 1.08 times higher than the control and significant at p = 0.05.The variation in bacterial loads is attributed to the availability of degradable materials in the various digesters for optimum use by bacteria .

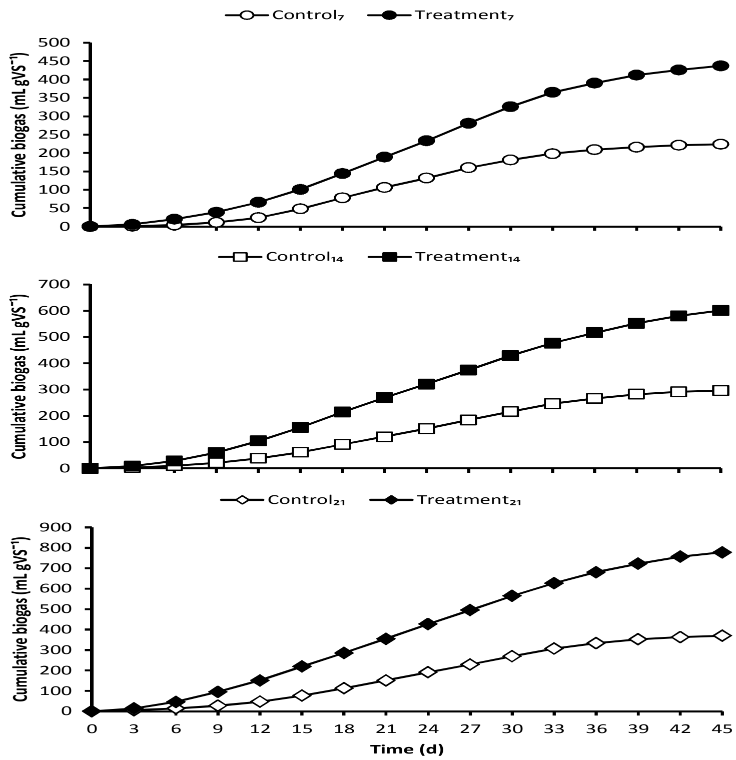

Figure 2: Cumulative biogas production from rice husk lignocellulosic biomass pretreated with cassava mill effluent.

Note: The biogas generated from the pretreated lignocellulosic biomass correlated positively with exposure time.

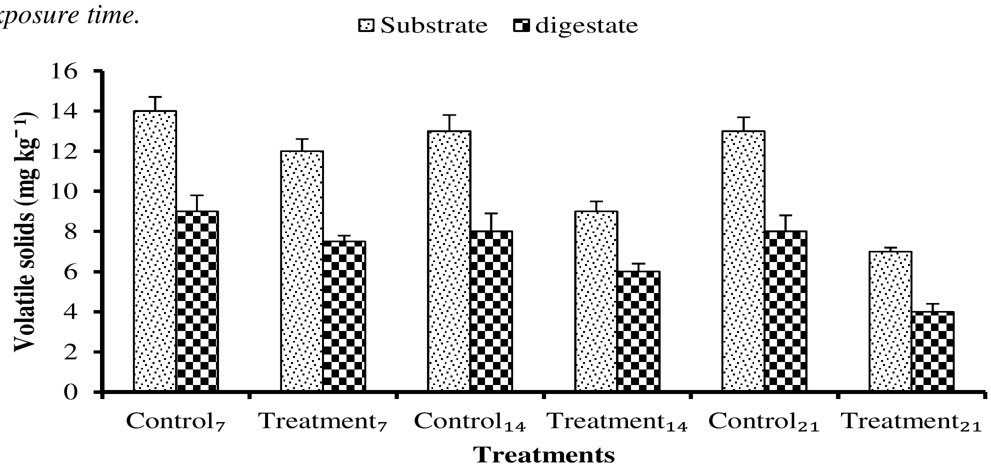

Figure 3: Effect of cassava mill effluent pretreatment on the volatile solids concentration in the substrate and digestate.

The result indicates that the pretreatment was effective in the saccharification of rice husk lignocellulosic biomass for enhanced bacterial growth and biogas yield.The probable organisms associated with the anaerobic co-digestion process were species of the genera *Methanosarcina, Methanobacterium*, *Methanocorpusculum*, *Methanobrevibacter* and *Methanothrix*. The presence of these methanogens indicates they played vital role in the anaerobic digestion of lignocellulosic material. Overall, the pretreatment of rice husk lignocellulosic biomass with CaME, co-digested with poultry manure, similar to a combined acid treatment and ammonia fiber explosion accelerated substrate hydrolysis for optimum AD performance and biogas production.

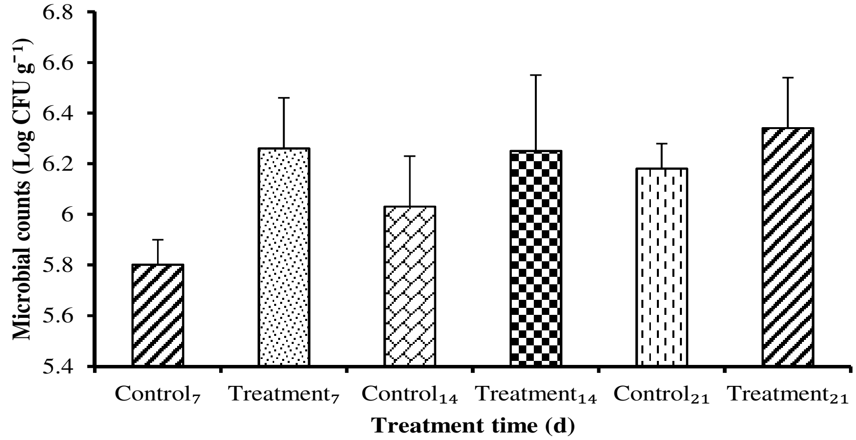

Figure 4: Effect of pretreatment on the anaerobic microbial community abundance in the digestate.

CONCLUSION AND RECOMMENDATION

Breakdown of lignocellulosic biomass to release available sugars for optimum biogas production requires an efficient pretreatment process that is organic and environment friendly. The use of CaME as a pretreatment agent, similar to the mild acid treatment and was effective to alter lignocellulosic structure, reduce cellulose crystalinity and enhance microbial activities for biogas production. The volume of biogas produced correlated positively with exposure time of the lignocellulosic biomass to the cassava mill effluent. Thus, the duration of substrate exposure influenced the degradation of the lignocellulosic biomass into metabolizable sugars for microbial conversion.

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