Enhancing Biogas Production through Anaerobic Codigestion of Biologically Pretreated Crop Residues and Cattle Manure

Reckson Kamusoko^{*1}, and Patrick Mukumba²

Abstract—Anaerobic codigestion (AcoD) is an attractive route for management and valorization of agricultural waste into biogas. The aim of this study was to determine the effect of codigestion of biologically pretreated crop residues, such as wheat straw (WS), maize stover (MS) and soybean straw (SS) with cattle manure (CM) on biogas production. Hydrolysates of crop residues pretreated using a hot spring cellulolytic microbial consortium (HSCMC) were used as substrates for the study. This study was performed in batch-fed reactors at laboratory scale using a mixture ratio of 1:1. Results of batch tests revealed higher daily methane production in the range of 144**-**152 mL from AcoD of pretreated crop residues and cattle manure compared to unpretreated groups. Cumulative methane yields for the pre-treated groups of 2 722 mL (pretreated MS:CM), 2 916 mL (pretreated WS:CM) and 2 815 mL (pSS:CM) were 13.3%, 18.4% and 25.1%, respectively, higher than the non-pretreated experiments. It is shown that AcoD of cattle manure and crop residues pretreated with a consortium of bacteria could be an innovative solution for efficient biogas production.

*Keywords***—** anaerobic digestion, biogas, codigestion, microbial consortium, pretreatment.

I. INTRODUCTION

Anaerobic codigestion (AcoD) is a promising technology that can transform biomass waste into biogas [1],[2]. Biogas can substitute fossil fuels in heat and electricity generation, and as a vehicular fuel. AcoD has been shown to supplement nutrients, remove toxins and buffer the digester medium [1],[3]. In general, it enhances the break down of complex molecules and methane yield due to combined effect of microorganisms in the biodigester [2],[4].

Crop residues contain large amounts of fermentable sugars and their proximate composition is suitable for microbial growth and biogas production [5],[6]. More so, the anaerobic digestion (AD) of crop residues is restrained by high carbon to nitrogen (C/N) ratio and reduced microbial action. This elevated C/N ratio may deprive methanogens of nitrogen that is required for cell proliferation [7]. An ideal C/N ratio for optimum performance of an AD system falls within the range of 20-30 [5]. Simultaneous AD of crop residues with low carbon waste, such as animal manure is a viable method to achieve an optimal C/N ratio [1],[2]. Besides, animal manure contains an inoculum that may help to speed up the AD reaction [4].

Many studies have paid much attention on AcoD of unpretreated crop residues with animal manure [5],[8]. However, there is little interest on pretreatment of crop residues to enhance biogas production from AcoD with animal manure. This study focused on AcoD of biologically pretreated crop residues and cattle manure. The objective was to improve biomethane production of crop residues that are hard to digest feedstocks.

II. MATERIALS AND METHODS

A. Statistical Design

A complete randomized design with duplicate experiments was used in this study. Three batch setups were designed for AcoD studies. Two treatment combinations were assigned to each experimental set up: (a) a mixture of cattle manure and pretreated crop residue; (b) a mixture of cattle manure and untreated crop residue. The untreated groups were wheat straw and cattle manure (WS:CM), maize stover and cattle manure (MS:CM), soybean straw and cattle manure (SS:CM), while the pretreated groups were wheat straw and cattle manure (pWS:CM), maize stover and cattle manure (pMS:CM), and soybean straw and cattle manure (pSS:CM).

B.Feedstock and Inoculum

Crop residues, including maize stover, wheat straw and soybean straw were collected from farms located in Chinhoyi, Makonde District, Zimbabwe. Initial characterization of the raw crop residues was performed by reference [6]. Crop residues were subjected to biological pretreatment using the hot springs cellulolytic microbial consortium (HSCMC). HSCMC was prepared using three bacteria strains isolated from hot spring water. The strains were members of motile bacillus i.e. *Bacillus subtilis*, *Bacillus* sp., and *B. licheniformis*. Maximum cellulase activity of the strains was found after 24 hr at pH 7.0 and 40 $^{\circ}$ C, whilst using 1% carboxymethyl cellulose as a carbon source and 1% yeast extract as a nitrogen source [9]. Rumen solid waste of cattle acquired from a local abattoir was used as inoculum for this study. Chemical composition of pretreated crop residues and inoculum were determined as reported by reference [9]. Hydrolysates of crop residues pretreated with the HSCMC were used as main feedstocks for AcoD experiments. Cattle manure sourced from a livestock farm located in Chinhoyi was prepared as a cosubstrate for AcoD. Manure was ground to 1 mm, dried at room temperature and passed 41st Cape Town interactions and the state of the conference on the conference of the conference on the conference of the conference of the conference

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through a 1 mm sieve to remove coarse materials. Triplicate samples of cattle manure were characterized for pH, ash, total solids (TS) and volatile solids (VS) using American Public Health Association (APHA) standard methods [10].

C. Anaerobic Codigestion Experiments

Crop residues (untreated and pretreated) were mixed with cattle manure in a 1:1 ratio. The use of a 1:1 ratio was based on previous research work, in which it was reported to be optimal for AcoD experiments [11]. The slurry was prepared by adding distilled water in a 1:10 ratio. AcoD of crop residues and cattle manure was carried out in duplicate in a batch set up at ambient temperature for 30 d. Plastic bottles (500 mL) with a working volume of 350 mL were used as biodigesters for this study. Biodigesters were purged with N_2 gas for 5 min to remove oxygen. The pH was maintained at 7.0 using NaHCO₃. The digesters were linked via silicon tubing to plastic bottles filled with 2% NaOH solution.

A control experiment containing rumen waste mixed with distilled water (1:10) was designed to assess the BMP of inoculum. The digesters were manually shaken once per day to ensure adequate mixing. Liquid displacement method was used to quantify the daily methane production.

D. Data Analysis

Experimental data were presented as means and standard deviations for the two measurements of the variables analyzed. Significant differences between variables were tested at $p \leq$ 0.05 by one way ANOVA using OriginPro Version 8.5 software package. Graphs were constructed using Microsoft Excel.

III. RESULTS AND DISCUSSION

A. Characteristics of Cattle Manure

The pH, ash, VS and TS content of cattle manure were 7.12, 15.10%, 47.20% and 51.34%, respectively (Table 1). The study demonstrated that cattle manure is an appropriate cosubstrate for AcoD with crop residues. Cattle manure can possibly reduce the inhibitory effect of free ammonia nitrogen during AcoD with crop residues. The pH of cattle manure falls in the range of 6.8-7.2 that was suggested to be suitable for an efficient AD [12]. High TS content of cattle manure can promote degradation of VS leading to enhanced biogas production [12]. A model cosubstrate for AcoD with crop residues should be able to supply basic nutrients required for higher methane yield and a steady bioreactor operation [13].

*Values are mean \pm standard deviation (n = 3)

B. Effect of Codigesting Crop Residues and Cattle Manure on Biomethane Production

This study infers that synergistic effect of codigesting pretreated crop residues and cattle manure is capable of improving methane production. Overall, AcoD indicated higher methane yield compared to monodigestion of untreated and pretreated crop residues reported by reference [9]. It was found that pretreated groups (pWS:CM, pMS:CM and pSS:CM) significantly ($p \leq 0.05$) enhanced methane production compared to untreated groups (WS:CM, MS:CM and SS:CM) during codigestion. This may have been due to pretreatment which conferred accessibility of more holocellulose to microbial attack during AD [14]. Biological pretreatment is based on natural processes, and it is considered to be a more economically viable, sustainable and effective in terms of capital and energy needs, equipment use, and environmental impact [15]. Animal manure has been reported to increase buffering capacity and supplement nutrients, whilst plant biomass stabilizes the C/N ratio and suppress ammonia inhibition during codigestion [16],[17]. 41.2. Although the Conference of Conference on The Conference on The Conference of Conference on Chemical and Conference on the Conference of Conference on Conference on Chemical and Conference of Conference of Conferenc

Profiles of daily methane production and cumulative methane yields obtained after AcoD of untreated and pretreated maize stover with cattle manure are shown in Fig. 1. The highest daily methane production (152 mL) was achieved after 10 d of codigesting pretreated maize stover and cattle manure (pMS:CM). Thus, pretreated maize stover supplemented with cattle manure can release more fermentable sugars within a shorter residence period of AD compared to unpretreated groups. The cumulative methane yield of pretreated groups of maize stover (pMS:CM) was 13.3% higher than non**-**treated groups (pMS:CM).

There is insufficient information in literature to support the hypothesis on AcoD of biologically pretreated maize stover with animal manure. However, positive results were observed from AcoD of NaOH-pretreated maize stover and cattle manure [18]. Reference [19] observed higher methane yields from AcoD of pig manure and maize stover pretreated with NaOH and CaO. Results from this study are lower than those of reference [20] who obtained 45% more methane yield after treating a mixture of rice straw and pig manure with cellulolytic flora.

(a)

Fig. 1. Daily methane production (a) and cumulative methane yield (b) from codigestion of pretreated and untreated maize stover with cattle manure

Fig. 2 shows daily methane production and cumulative methane yields obtained after AcoD of untreated and pretreated wheat straw with cattle manure. Pretreated wheat straw attained the highest daily methane production of 149 mL after 10 d of AcoD with cattle manure. This value was 16% higher than the unpretreated combination. Cumulative methane yield from AcoD of cattle manure with pretreated wheat straw (pWS:CM) was 2 916 mL, which was 13.3% higher than untreated group (WS:CM).

Authors mostly report on codigestion of chemically pretreated wheat straw with cattle manure. For example, enhanced methane production was achieved through simultaneous digestion of cattle manure and wheat straw pretreated with 10% Ca(OH)₂ [11]. Reference [21] reported a higher methane yield from AcoD of wheat straw pretreated with 3% H2O² and cattle manure compared to untreated group.

Fig. 2. Daily methane production (a) and cumulative methane yield (b) from codigestion of pretreated and untreated wheat straw with cattle manure

Daily methane production and cumulative methane yields obtained after AcoD of untreated and pretreated soybean straw with cattle manure is shown in Fig. 3. Pretreatment of soybean straw before AcoD with cattle manure resulted in maximum daily methane production of 144 mL after 11 d of digestion. Comparatively, the untreated group (SS:CM) yielded relatively lower maximum daily methane (126 mL) after a longer period of 13 d. Codigestion of pretreated soybean straw with cattle manure resulted in 25.1% increase in cumulative methane production compared to unpretreated combination.

Enhancement of biogas production through AcoD of biologically treated soybean straw and cattle manure is not widely reported in literature. However, reference [22] found 62% more methane production from codigestion of thermochemically treated soybean straw and farm wastewater. Codigestion of untreated soybean straw with activated sludge increased methane production by 2.3 times over monodigestion experiments [23].

Fig. 3. Daily methane production (a) and cumulative methane yield (b) from codigestion of pretreated and untreated soybean straw with cattle manure

IV. CONCLUSIONS

The study successfully confirmed that biomethane production could be enhanced by codigestion of biologically pretreated crop residues and cattle manure. The pH, ash, VS and TS content demonstrated that cattle manure is suitable substrate for AcoD with crop residues. Higher daily methane production in the range of 144 **-** 152 mL was recorded from AcoD of crop residues pretreated using a consortium of bacteria with cattle manure compared to unpretreated groups. Maximum cumulative methane yields for pretreated groups (pMS:CM, pWS:CM and pSS:CM) were significantly higher than the nontreated experiments. It is shown that AcoD of cattle manure and crop residues pretreated with bacteria consortium could be one of the viable options for efficient biomethane production. However, it is critical to optimize the mixing ratio and reactor conditions, conduct cost-benefit analysis and design a prototype digester based on the laboratory-scale findings towards fullscale valorization. Scaling up this process could offer an alternative route for integrated waste management systems in an environmentally sustainable, economically viable and socially acceptable way.

AUTHOR CONTRIBUTIONS

Conceptualization, R.K. and P.M.; methodology, R.K. and P.M; formal analysis, R.K.; investigation, R.K; resources, R.K; data curation, R.K.; writing-original draft preparation, R.K.;

writing-review and editing, R.K and P.M.; visualization, R.K. and P.M. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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