Enhanced Biogas and Biofertilizer Production from Anaerobic Codigestion of Harvest Residues and Goat Manure

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors UUNN and UAO designed the study, developed the protocol, performed statistical analysis and wrote the first draft of the manuscript. Authors NUA and VME managed literature searches and performed laboratory investigations and curated data obtained in the study. All authors read and approved the final manuscript.

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ABSTRACT

Comparative assays were conducted to assess the biogas and biofertilizer production efficiency from anaerobically co-digested goat manure (GM) and harvest residues: corn stover (CS) and rice straw (RS). All digesters were operated simultaneously under mesophilic temperature of 40°C and notable phosphate solubilizing and nitrogen fixing bacterial populations indicated qualitative biofertilizer quality of the digestates. Codigestion of the substrates significantly increased biogas yield (p < 0.05) compared to monodigestion, and the highest cumulative yield of 573 ml/g VS was obtained from co-digested rice straw (RS) and goat manure (GM). With a significant decimation in number of pathogens (p < 0.05), a 2 – 3 fold increase in populations of plant growth promoting bacteria (Bacillus and Pseudomonas species) was observed in digestate from codigestion assays when compared to monodigestion (control) and were identified as Clostridium sp., Bacillus subtilis, Bacillus megaterium, Lactobacillus sp., Pseudomonas fluorescens including methanogens: Methanothrix sp., Methanobacterium sp. and Methanosarcina sp. On the average, codigestion

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assays resulted in enhanced biogas yield and biofertilizer quality that was 2.3 – 4.1 times higher than single substrate digestion and generally improved the efficiency of biogas and biofertilizer production.

Keywords: Biogas; biofertilizer; codigestion; harvest residue; goat manure.

1. INTRODUCTION

Annually, large-scale quantities of harvest residues (HRs) are generated in Nigeria from agricultural practices with no adequate means of disposal or management thus contributing to environmental pollution [1]. The high carbon dioxide emissions due to the burning of fossil fuels and HRs has contributed to serious environmental and health hazards [2]. The increased use of inorganic fertilizer has also resulted in poor soil structure and fertility loss. To overcome these challenges, scientists have intensified efforts in transforming organic wastes (including harvest residues) into useful resources of energy and biomanure/biofertilizer so as to provide solution to these daunting environmental problems [3,4].

Anaerobic digestion (AD) is a complex metabolic process in which microbial consortia, in the absence of oxygen transform organic matter into energy. A combination of gases called biogas is usually produced during this AD process [5]. Biogas production during anaerobic digestion process is thus a complex multistep yet sequential order of events involving substrate hydrolysis, acidogenesis, acetogenesis and methanogenesis [6]. The first stage which is usually considered a rate limiting step involves hydrolysis of complex polymers of carbohydrate, protein and fat into usable monomeric substances and precursors for uptake and conversion to methane by fermentative bacteria [7]. Then acid-forming acidogens produce short chain organic acids (acetic acid and propionic acid) with the analogous release of CO$_2$, creating an anaerobic condition that favours methane-forming methanogens [8]. Sometimes through the process of acetogenesis, by-products of acidogenesis are not directly converted to methane by methanogens but are converted to volatile fatty acids (VFAs) and alcohols [9,10]. During methanogenesis, methanogens more readily utilize hydrogen, CO$_2$ and acetic acid to form methane and CO$_2$. Thus anaerobic digestion process is influenced and limited by several factors such as temperature, pH, carbon-nitrogen ratio, nutrient concentration, pre-treatment, presence of toxic compounds and single or co-substrate digestion [6].

In this study, the harvest residues (corn stover and rice straw) which are essential sources of energy for biofuel production are among the greatly underutilized agricultural wastes [11]. Several studies have shown that codigestion of agricultural wastes and animal manure can increase biogas production [12–15]. Zhang et al. [15] demonstrated that codigestion of goat manure with three crop residues significantly increased biogas production than monodigestion. Also, Zhang et al. [16] reported maximum methane yield from codigestion of chicken manure and cereal residues. Codigestion therefore improves the limitations of monodigestion which are imbalanced nutrients and build-up of toxic metabolites or recalcitrant compounds in the feedstock [17].

Apart from biogas, anaerobic digestion process also results in the production of a digestion residue and waste stream called digestate. The digestate usually consists of different microbial communities and readily available nutrients [18]. Both organic and inorganic matter contained in the substrates determines the quality of the digestate. The microbial communities degrade most of the organic matter during the anaerobic digestion process while converting some to inorganic compounds. For example, the available nitrogen in the digester is converted to ammonium and nitrates which remain in the digester until the end of the anaerobic digestion process [19]. Besides being imbued with nutrients such as nitrogen, phosphorus and potassium, the digestate from biogas plants/digesters has been shown to be rich in populations plant growth promoting bacteria (including Bacillus and Pseudomonas species) with remarkable biofertilizer attributes hence its potential for use as biofertilizer [20,21]. Notwithstanding, the digestate is not entirely innocuous (as it may harbour some pathogens) thus requiring continuous monitoring and evaluation of its quality before use on arable land [22–26]. Some authors [11,13,14,15,27] have reported the biomethane potential of harvest residues when codigested with animal manure, however there is little or no information regarding the effect of codigestion on biofertilizer quality of the resultant digestate. Although their study was not conducted on harvest residues, only
Cestonaro et al. [28] revealed that codigestion of sheep bedding and ≥ 50% cattle manure enhanced biogas production and resulted in a high quality biofertilizer. The present study therefore intended to enhance biogas production through anaerobic codigestion of harvest residues and goat manure. The biofertilizer quality of the resultant digestate was also examined for the presence of indicator bacteria, potential pathogens and plant growth promoting bacterial groups.

2. MATERIALS AND METHODS

2.1 Collection and Sources of Samples

In this study, harvest residues (HRs) used as feedstock consisted of corn stover (CS) and rice straw (RS) collected from the National Cereal and Research Institute in Akwa Ibom State, Nigeria. Goat manure which served as co-substrate was obtained from a loafing shed at a local farm outbuilding and aged cow dung (inoculum) was obtained from Animal Science Department, University of Uyo. Before use, the feedstock were physically pretreated by mechanical reduction to particle sizes of about 1 – 2 mm diameter using manual grinder and cow dung was used as inoculum because of its associated methanogens [29]. To ensure a quick start-up of the anaerobic digestion process, the inoculum was acclimated at 45°C (to give an initial temperature for methanogens) and anaerobically degassed to exhaust background methane.

2.2 Experimental Design and Biomethane Potential Assay

The anaerobic codigestion experiment was carried out following the methods of Zhang et al. [15] with some modifications. Three (3) sets of 100 ml laboratory-scale reactors were used as described by Eduok et al. [30]. The digestion assay was conducted in a batch-type mode reactors where the mixture of feedstock and inoculum slurry was added once to the reactors with a working volume of 80 ml (to allow headspace for biogas accumulation). The reactors were crimped using a 20 mm capsule standard hand held crimper (JG Finneran 9300-20, USA). All reactors were maintained at mesophilic temperature (40 ± 2°C) for a hydraulic retention time of 35 days and manually agitated daily to mix. Daily biogas yield was measured by liquid displacement method in a graduated inverted cylinder. Based on volatile solids concentration, three different combinations at ratio 1:1 of harvest residue (HR) and goat manure (GM) were used and monodigestion of each harvest residue and goat manure was performed as control experiment (Table 1).

2.3 Analytical Methods

The anaerobic digestion process performance indicators [pH, total solids (TS), volatile solids (VS)] were measured before and after digestion. The pH of influent and effluent mixtures were measured using a hand-held pH meter (HI 98107 pHep). While TS was determined at 100°C, VS was determined after combustion at 550°C using the following mathematical expressions below according to the protocol of APHA [31].

\[
\text{TS} = \left( \frac{W_d}{W_w} \right) \times 100 \tag{1}
\]

Where,

- \( W_d \) = weight of oven-dried sample, g
- \( W_w \) = weight of wet sample, g

\[
\text{VS} = \left( \frac{W_d - W_a}{W_w} \right) \times 100 \tag{2}
\]

Where,

- \( W_d \) = weight of dry sample, g
- \( W_a \) = weight of dry ash remaining after igniting the sample in muffle furnace, g
- \( W_w \) = weight of wet sample, g

2.4 Biofertilizer Quality of Digestate

2.4.1 Screening for indicator Bacteria in digestate

The detection and enumeration of indicator bacteria which included total coliforms and faecal coliform (Esherichia coli) in the biofertilizer (digestion effluent) was performed using pour plate method. After serial dilution of digestate, one (1) ml of 10^{-6} dilution was plated on MacConkey agar and Eosin methylene blue agar for the isolation of total coliforms and faecal coliform respectively. For the specific isolation of E. coli O157: H7, the sample was plated on chromogenic HiCrome ESBL agar containing ceftazidime supplement [32].
Table 1. Anaerobic codigestion assay and experimental design

| Treatment               | GM:CS | GM:RS | GM:CS:RS |
|-------------------------|-------|-------|----------|
| Codigestion             | 50:50 | 50:50 | 50:50:50 |
| Monodigestion (Control) | 0:50  | 0:50  | 50:0:0   |

Key: GM = Goat manure; CS = Corn stover; RS = Rice straw

The plates were incubated for 24 hrs at 37°C and observed for colony growth expressed as colony forming units per millilitre (CFU/ml⁻¹).

2.4.2 Screening for potentially pathogenic bacteria in digestate

A ten (10) fold serial dilution was carried out and one (1) ml of the diluent was plated on Xylose Lysine Deoxycholate agar for the isolation of *Salmonella* species, *Campylobacter* agar base supplemented with defibrinated Sheep blood and Skirrow solution (Cefoperazone) for the isolation of *Campylobacter* species, Citrate Azide Enterococcus agar base supplemented with 1% TTC solution for the isolation of *Enterococcus* species and Thiosulphate nitrate citrate deficient medium for the cultivation of *Vibrio* species. All plates were observed for growth after 24 hrs of incubation at 37°C and only representative colonies that emerged were counted and reported as per CFU/ml⁻¹[21].

2.4.3 Biofertilizer properties of Digestate

As per their biofertilizer characteristics, digestate samples were drawn from the bioreactors and analysed for the concentration of known plant growth promoting bacteria (particularly *Pseudomonas* and *Bacillus* species). A 10-fold serial dilution was carried out and 0.1 ml of the aliquot inoculum was spread on BD BBL MYP agar (for the isolation and enumeration of *Bacillus*) and Difco Cetrimide agar (for *Pseudomonas*). After incubation, typical colonies were counted and calculated as CFU/ml [33]. The isolates were tested for plant growth promoting characteristics: phosphate solubilization and diazotrophic nitrogen fixation.

The isolates were screened for their potential to solubilize inorganic phosphate using Pikovskaya’s medium. Formation of visible halozones around the microbial colonies indicated phosphate solubilisation ability after cultivation for 3 – 5 days. For the screening of diazotrophic nitrogen-fixing potential, isolates were grown on Burk’s medium and the plates were incubated anaerobically. Organisms that appeared after incubation at 37°C indicated the potential to fix atmospheric nitrogen [34].

2.5 Isolation of Methanogens

Methanogens associated with anaerobic digestion process were isolated by selective enrichment on low phosphate basal medium [35]. The plates were incubated at 40°C for 2 days under strict anaerobic condition using gaspak anaerobic system. Representative colonies were characterized and identified based on their ability to utilize catabolic substrates such as acetate, formate, methanol, methanethiol and dimethylsulphide.

2.6 Statistical Analyses

All experiments were performed in triplicates and reported as mean values. The obtained data were subjected to statistical analyses (two-way ANOVA) using Microsoft excel version 2013 with *P* = 0.05 confidence interval.

3. RESULTS AND DISCUSSION

3.1 Effect of Codigestion on Daily and Cumulative Biogas Production

The rates of biogas production for codigestion and monodigestion assay are presented in Figs. 1a, 1b, 2a and 2b respectively. During the same retention time of 35 days, a two-fold increase in biogas production was generally observed across all codigestion experiments when compared to monodigestion. The highest daily biogas yield was ranked in the sequence RS/GM>CS/GM>RS/CS/GM (Fig 1a). Biogas production progressed rapidly with peak values obtained at day 18 in all codigestion experiments whereas it was initially low and rose very slowly in single (mono) substrate digestion with maximum production on day 21 across all digesters. These observation was similar to earlier reports by Zhang et al. [16] and Zhang et al. [36], who indicated that the mixture of harvest residues with livestock manure was beneficial by supplying missing nutrients and boosting microbial synergism necessary for efficient
biogas production. Codigestion of rice straw and goat manure recorded the highest cumulative biogas yield (573 ml.gVS⁻¹) compared to other co-substrates (Fig. 2a). The cumulative biogas yield of codigested rice straw and goat manure (RS/GM) demonstrated 51.48% and 27.3% improvement than monosubstrate digestion of RS or GM respectively (Fig. 2a and 2b). A similar trend was observed for codigested corn stover and goat manure (CS/GM) as well as mixture of RS/CS/GM. Whether daily or cumulative biogas yield, the mixture of rice straw and goat manure (RS/GM) was more efficient in terms of biogas generation. This increase was significantly higher (P = 0.05) in all codigestion assays than in monodigestion assays. The result of this study validated other studies [15,16,37], and revealed that co-fermentation of goat manure with crop residues can significantly improve biogas production.

**Fig. 1a. Daily biogas production from codigestion assay**

**Fig. 1b. Daily biogas production from monodigestion assay**
3.2 Effect of Codigestion on Process Performance Indicators (TS, VS and pH) During Anaerobic Digestion Process

The efficiency of codigestion was evaluated in terms of TS and VS removal rates as well as pH stability and the results are shown in Fig. 3a, b and c. Notably, codigestion caused a remarkable increase in initial TS and VS concentrations of the ingestate (raw substrate) compared to single substrate (Fig. 3a and b). However at the termination of experiment, the total solid contents in codigestion assay decreased from 62.3 to 36.6%, 65.4 to 38.3% and 67.5 to 40.5% in RS/GM, CS/GM and RS/CS/GM respectively. While reductions in monodigestion assay was from 43.2 to 23.5%, 42.3 to 21.3% and 40 to 20.1% for RS/GM, CS/GM and RS/CS/GM respectively. Average TS and VS removal
achieved from codigestion of corn stover and goat manure (CS/GM) were 41.4% and 38.6% respectively which was comparatively higher than all single substrate digestion indicating that codigestion influenced the TS and VS removal efficiencies. Harvest residue (HR) are highly complex lignocellulosic material which is difficult to degrade and may account for the low organic matter (TS and VS) removal in single substrate digestion [11]. Codigestion provided nutrients, diluted inhibitory substances and encouraged microbial interaction in the co-substrates needed to promote anaerobic degradation and methane formation. Overall results from this study showed a strong linear and positive relationship between biogas production and TS and VS removal \( r = 0.95 \).

As one of the process performance indicator, pH variations reflected changes in the anaerobic digestion process (Fig. 3c). The pH data showed a similar trend in all experimental mixtures. Studies have indicated that the growth of methane forming bacteria can be significantly influenced by pH level \([6,38]\). The initial pH of the digesters were within the optimal range (6.5 – 7.5) required for efficient AD, but decreased gradually between 4.2 and 4.5 at the end of the experiment. This drop in pH may be linked to the accumulation of VFAs which have deleterious effect in the anaerobic digestion process \([39]\).

![Fig. 3. Percentage total solid (TS) and volatile solids (VS) concentration of (a) influent substrate (b) digestate effluent in co- and monodigestion experiments. RS= Rice Straw, CS= Corn Stover and GM= Goat Manure](image-url)
Fig. 3c. Variations in pH during anaerobic digestion of harvest residues and goat manure

3.3 Bacterial Load Profile of Influent Substrate and Digestate Effluent

Prior to anaerobic codigestion, the initial bacterial characteristics of influent substrate revealed higher densities and rich assemblage of faecal coliform, total coliform, *Staphylococcus aureus*, heterotrophic bacteria, anaerobic bacteria, *Vibrio*, *Salmonella* and *Shigella* species in goat manure (co-substrate) compared to other substrates 4a). The occurrence and preponderance of these organisms is needed for biomass fermentation to produce metabolic intermediates before methanogenesis can occur hence the need for codigestion. Except for total anaerobic bacteria which ranged from 2.5 to 4.6 log CFU/g, a general decline in microbial load was observed for all bacterial groups at the end of the experiment (Fig. 4b). Remarkably, potentially pathogenic and indicator bacterial species such as faecal coliform were completely decimated highlighting the sanitary quality of the digestate sample for potential use as biofertilizer. This trend corroborates the findings of previous studies [22,40].

The reduction of bacterial load in the present study validates the use of AD process for hygienic waste treatment and management.

3.4 Plant Growth Promoting Bacterial Populations in Anaerobic Digestate

Anaerobic digestion residue (digestate) were evaluated for plant growth promoting bacteria and their concentrations are illustrated in Fig. 5. Results showed that the digestate harboured high densities of beneficial plant growth promoting bacteria (PGPB) thus increasing its potential for use as biofertilizer. The PGPB group were *Bacillus* species which ranged from 1.5 to 5.3 log CFU/ml and was significantly higher than the *Pseudomonas* species (1.0 – 3.7 log CFU/ml) across all biodigesters. It was also observed that digestates from co-digested substrates contained higher populations of plant growth promoting bacteria (PGPB) than digestates from single substrate digestion. Hence codigestion improved and enriched the biofertilizer properties of the resultant digestion product. This increase in populations of PGPB could be attributed to the use of goat manure as co-substrate and studies have shown that anaerobic digestate can serve as sources of biofertilizing bacteria through the addition of readily available nutrients to soil [21,34,41].

Altogether, twenty two (22) isolates were encountered in the feedstock and identified based on their morphological and biochemical characteristics. However, only seven (7) isolates remained (in the digestate) at the end of the digestion and were predominantly *Clostridium, Bacillus, Pseudomonas* and *Lactobacillus* species including methanogens (*Methanothrix, Methanobacterium* and *Methanosarcina* species). These organisms are similar to those reported by Luo et al. [42] and de Diego-Díaz et al. [43] and have been implicated with
Fig. 4. Initial bacterial load of (a) influent substrate before codigestion and (b) digestate effluent after codigestion

RS = Rice Straw, CS = Corn Stover and GM = Goat Manure

degradation/metabolism of complex lignocellulosic substrates. As per their biofertilizer characteristics, the bacterial isolates demonstrated either or both abilities to solubilize phosphate and fix nitrogen. All *Clostridium* and *Bacillus* species showed phosphate solubilization and nitrogen-fixing abilities whereas *Pseudomonas* species showed mostly phosphate solubilization ability (Table 2). Nitrogen and phosphorus are indispensable nutrients required for plant growth. Nevertheless, these nutrients are unavailable for plants since they are usually present in insoluble forms. In this study, anaerobic digestion residue was shown to be laden with potential biofertilizing bacteria capable of diazotrophic nitrogen fixation and solubilizing phosphates to plant-utilizable forms. Studies have pointed that the application of digestate can affect phosphorus and nitrogen availability in soil either directly by adding inorganic phosphorus/nitrogen or indirectly by influencing soil microbial activity [44–46]. Phosphate solubilizing bacterial species and nitrogen-fixing diazotrophs were detected in the
Present study thus the direct application of the digestate on agricultural soil can introduce phosphate solubilizing and nitrogen fixing bacteria into the soil and subsequently enhance plant phosphorus and nitrogen uptake and availability.

![Fig. 5. Concentration of plant growth promoting bacteria in digestate biofertilizer](image)

**Table 2. Plant Growth Promoting Characteristics of Bacteria Isolated from Digestate**

| Isolate Identity       | Nitrogen Fixation | Phosphate Solubilization | Isolate Source |
|------------------------|-------------------|--------------------------|----------------|
| Bacillus megaterium    | +                 | +                        | RS/GM          |
| Pseudomonas fluorescens| -                 | +                        | RS/GM          |
| Bacillus subtilis      | +                 | +                        | CS/GM          |
| Lactobacillus species  | -                 | +                        | CS/GM          |
| Clostridium species    | +                 | +                        | RS/CS/GM       |
| Pseudomonas fluorescens| -                 | +                        | RS/CS/GM       |
| Bacillus subtilis      | +                 | +                        | CS             |
| Lactobacillus species  | -                 | +                        | GM             |
| Bacillus megaterium    | +                 | +                        | GM             |
| Clostridium species    | +                 | +                        | GM             |
| Pseudomonas fluorescens| -                 | -                        | GM             |
| Bacillus subtilis      | +                 | +                        | GM             |
| Clostridium species    | +                 | +                        | RS/CS/GM       |
| Bacillus megaterium    | +                 | -                        | RS/GM          |
| Pseudomonas fluorescens| -                 | +                        | CS/GM          |
| Bacillus subtilis      | +                 | +                        | RS/CS/GM       |
| Lactobacillus species  | +                 | -                        | CS/GM          |
| Bacillus megaterium    | +                 | +                        | RS/CS/GM       |
| Pseudomonas fluorescens| -                 | +                        | RS/GM          |
| Bacillus subtilis      | +                 | +                        | CS/GM          |
| Lactobacillus species  | -                 | -                        | CS/GM          |
| Bacillus megaterium    | +                 | +                        | RS/CS/GM       |
| Pseudomonas fluorescens| -                 | +                        | RS/CS/GM       |
| Bacillus subtilis      | +                 | +                        | RS/GM          |
| Lactobacillus species  | -                 | -                        | RS             |
| Bacillus megaterium    | +                 | +                        | CS             |
| Pseudomonas fluorescens| -                 | +                        | GM             |
| Bacillus subtilis      | +                 | +                        | GM             |

*Key: RS = rice straw; CS = corn stover; GM = goat manure*
4. CONCLUSION

The anaerobic codigestion of goat manure with harvest residues is a veritable technology for engaging the environmental problems caused by the burning of rice straws and corn stover. Biogas production was enhanced by overcoming the pH instability associated with monosubstrate digestion, supplying unavailable nutrients and boosting microbial synergism. Codigestion also improved the biofertilizer property of the digestate owing to the preponderance and increased concentration of phosphate solubilizing and nitrogen-fixing bacteria in the biogas digestate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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