

# Effects of inoculum to feedstock ratio on anaerobic digestion for biogas production

## Abstract

Biogas is considered as a clean and a renewable form of energy that could replace the increasing non-renewable energy sources such as fossil fuel for use in heat production and for electricity generation. The inchoate gain in the shift for a renewable source of energy is that the feedstock is often a by-product, a residue or waste product of other processes without the competition for arable land. In this study, five (5) Laboratory scale biodigesters were used for the anaerobic co-digestion of locally available *Miscanthus Fuscus* and cow dung, controlled at a pH range of 6.2-7.8 and at a mesophilic temperature of  $35 \pm 2^\circ\text{C}$ . Study was also carried out in batch mode at a hydraulic retention time of 33 days. The anaerobic co-digestion process was developed and optimized at varying inoculum to feedstock ratio of 1:0, 0:1, 1:3, 3:1, and 1:1 to determine the potential biogas yield from each proportion. The highest biogas potential was recorded at an inoculum to feedstock ratio of 3:1 with the least biogas potential recorded by the biodigester at a ratio of 0:1.

**Keywords:** renewable energy, co-digestion, cow dung, *miscanthus fuscus*, anaerobic digestion

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## Introduction

Generation of renewable energy sources remain a vital demand to cater for the ever-increasing energy consumption and the depletion of fossil resources from non-renewable energy.<sup>1</sup> Studies have been carried out in the past decades to find alternatives for fossil fuel replacement.<sup>2</sup> Also, urbanization has led to an increase in landfills, and it is estimated that by 2025, two-thirds of people will be living in the cities globally.<sup>3</sup> The synthesis of a renewable energy source has been evaluated, where energy is produced from biogas through anaerobic digestion (AD) and technology observed to be promising.<sup>4</sup> The AD process to produce bioenergy has gained increasing recognition for the past decades. Biogas is a renewable energy fuel that consists chiefly of 60-70% methane and 20-30% carbon dioxide with the presence of other trace compounds such as hydrogen sulfide and ammonia. The gas produced could serve as fuel for electricity generation and also its usage in the production of combined heat and power generation using appropriate technologies.<sup>5</sup> The AD process from which biogas is produced involves four major stages as a result of the biodegradation of organic matter by a consortium of microorganisms.<sup>6</sup> These include hydrolysis, acidogenesis, acetogenesis, and the methanogenesis. In brief, the methanogenic stage which is the final stage is where carbon dioxide produced from the previous processes reacts with the hydrogen present to produce methane and also, at the same time, the acetate break down to form methane and carbon dioxide.<sup>7,8</sup> Comparatively, anaerobes have been found to be most active at mesophilic conditions than thermophilic conditions as the latter tend to require higher heat input.<sup>9</sup> However, this present study focused on the former. Limitations such as process instability, process failure, poor methane yield, and longer retention time have limited the full exploitation of the AD process.<sup>10,11</sup> Processes such as anaerobic co-digestion, low organic loading to avoid overloading in biodigesters, pretreatment techniques to enhance cellulose and disrupt lignin, and the use of energy crops as feedstocks,

have been found to increase the efficacy of biogas production through anaerobic digestion.<sup>12-14</sup> Recirculation of digested slurry (washed out microbes) back into the reactor and design modification of existing biogas plants are some of the ways that have been used in literature to also improve the gas production in biogas plants.<sup>15</sup> Process parameters such as temperature, agitation, carbon-nitrogen ratio, organic loading rate, and the hydraulic retention time can be measured by studying and monitoring the variation during the AD process.<sup>16</sup> According to Simo et al.<sup>17</sup> a sharp change in these parameters could adversely affect the biogas production process. For higher efficiencies, these parameters should be varied within a desirable range to operate the biogas plant. Also, one important parameter to consider in the application of anaerobic digestion is the type of feedstock used as almost any organic material can be processed.<sup>10</sup> Feedstocks utilized in the past decades for biogas production includes waste paper, grass clippings, leftover food, sewage, sugarcane bagasse, and animal waste. However, in this study, *Miscanthus Fuscus* was used for the AD process to produce biogas. *Miscanthus Fuscus* is a bamboo-like plant that overgrows up to 3 meters high, generating a high yield of biomass with low ash content and suitable for use in electricity generation.<sup>18</sup> It is a promising non-food crop yielding a high-quality lignocellulosic material with good fiber content suitable for thatching and also for industrial use.<sup>19</sup> Literature reports that *Miscanthus Fuscus* has been found to be suitable for biogas production and has a higher methane potential per unit area.<sup>20</sup> According to Kiesel & Lewandowski,<sup>21</sup> *Miscanthus Fuscus*, when harvested before winter could increase the yield and digestibility for the AD process than after winter. The demand to seek ways to improve and increase the yield of biogas has wakened many researchers to quest for alternative ways to cater for this setback because of low yield of methane reported in various researches. This study, however, focuses on the effects of inoculum to feedstock ratio in determining the biogas potential with varying ratios of these substrates.

## Materials and methods

The biochemical methane potential (BMP) test was carried out to determine the potential of biogas from *Miscanthus Fuscus* (Figure 1) and cow dung.



**Figure 1** Photo of *Miscanthus Fuscus* used for this study.

### Material sampling

*Miscanthus Fuscus* was harvested from a local farmland at Adako Jackie in the Ashanti region of Ghana, serving as the feedstock. The choice of feedstock was due to its bioavailability for use in energy production. The inoculum, cow dung was obtained from a cattle farm within the same municipality to provide the necessary bacteria for the anaerobic digestion process which was further kept in sealed Schott bottles and stored at 4°C prior for analysis.

### Characterization of feedstock and inoculum

*Miscanthus Fuscus* was sun dried and washed to remove the unwanted particles. It was then shredded, slightly milled with a hammer miller (Fritsch Pulverisette 558, Germany) and sieved to an appreciable size of 5mm (on dry weight basis) for further analysis. This was carried out to increase the surface area for better adsorption between substrates during the AD process. The proximate analysis of the feedstock was performed with parameters such as total solids (TS), moisture content (MC), volatile solids (VS), fixed solids (FS) and ash contents (AC) as in Table 2 by the standard methods.<sup>22-24</sup> This study however did not include the ultimate analysis. Also, the values for the inoculum characterization are not included in this paper. Standard procedures were carried out in the Laboratory using a precision balance (Kern PCB 3500-2, United Kingdom), a convection oven (VWR DRY-line oven, Pennsylvania), a muffle furnace (Nabertherm, China), a 5mm sieve, and a dessicator containing dessicant for cooling.

### Experimental design for the laboratory setup

Table 1 depicts the experimental design of the inoculum to feedstock selection for this study corresponding to five (5) biodigesters. However, after feeding the biodigesters at an optimal loading rate, the pH in each biodigesters selected for this study was monitored within a desired range of 6.0-8.5 according to Kougiaris & Angelidaki,<sup>25</sup> This optimal range was selected as it was found to fall within the same range by most researchers undergoing the AD process such as that reported by Hallenbeck.<sup>26</sup> Sodium hydroxide (NaOH) was used to adjust the pH of the AD process to cater for volatile fatty acids (VFAs) such as acetates and propionates that would be generated within the biodigesters. Maile et al.<sup>27</sup> have also reported the use of calcium carbonate (CaCO<sub>3</sub>) and NaOH as preferred reagents to control

the alkalinity of the slurry during the AD process.

The experimental values obtained from Table 2 are illustrated by the calculations from Equations 1-5 below according to APHA<sup>22</sup> as;

$$\%Total\ solids = \frac{(A - B) \times 100\%}{(C - B)} \quad (1)$$

$$\%Volatile\ solids = \frac{(A - D) \times 100\%}{(A - B)} \quad (2)$$

$$\%Fixed\ solids = \frac{(D - B) \times 100\%}{(A - B)} \quad (3)$$

$$\%Moisture\ solids = 100\% - \frac{(A - B) \times 100\%}{(C - B)} \quad (4)$$

$$\%Ash\ solids = \frac{(M_d - M_a) \times 100\%}{(M_b - M_a)} \quad (5)$$

Where A=weight of dried residue + dish, in g; B=weight of dish only, in g; C=weight of wet sample + dish, in g; D=weight of residue + weight of dish after ignition, in g; M<sub>d</sub>=mass of crucible + mass of sample after 600°C ashing; M<sub>a</sub>=mass of crucible only, in g; M<sub>b</sub>=mass of crucible + mass of sample before ashing, in g.

**Table 1** Feedstock and the Inoculum experimental design

Biodigester ID	Inoculum (I)	Feedstock (S)	I/F
A	100	0	1:0
B	0	100	0:1
C	25	75	1:3
D	75	25	3:1
E	50	50	1:1

**Table 2** Results for the proximate analysis of *Miscanthus Fuscus*

Parameters	Experimental values (%)
Total solids	91.0
Moisture content	9.0
Volatile solids	76.1
Fixed solids	23.8
Ash contents	3.5

### The biochemical methane potential (BMP) test

The characterization values were used to calculate the organic loading rate for each biodigester prior for the BMP test. Each biodigester was a 1000mL Duran Schott bottle with a working volume of 800mL leaving a headspace of 200mL. This headspace was purged with nitrogen gas (N<sub>2</sub>) to create the anaerobic environment for about 45 seconds each. Biodigesters were closed air-tight with rubber caps and incubated in a circulating water bath regulated at a mesophilic

temperature of  $35 \pm 2$  °C. Since it is a batch system, it was made to run until the AD process was complete at a hydraulic retention time (HRT) of 33 days. Stirring was done after both substrates were kept in each biodigester prior for the BMP test to ensure uniformity. Biogas yield was verified to determine both the methane and the carbon dioxide contents since these constitute the largest components of gases in the biogas. Qualitatively, biogas was determined using a Gas chromatograph (SRI 8610 GC) equipped with thermal conductivity detector, packed with 6' Hayesep-D/6' Molecular Sieve-13 X. The volume of biogas produced was determined using the downward displacement method on daily basis as depicted in Figure 2.

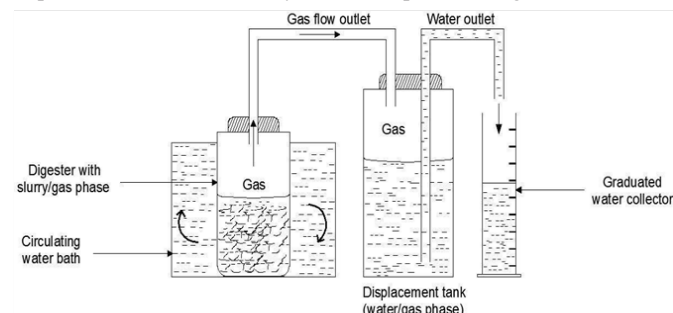


Figure 2 The experimental setup of a single biodigester for the BMP test.

## Results and discussion

A known quantity of inoculum (cow dung reported as raw material) and the feedstock (*Miscanthus Fuscus*, reported as dry matter) were characterized according to standard methods. The results showed a great methane potential as a significant biodegradable fraction existing in the feedstock.

### Biogas production

The relative daily biogas production rates from the biodigesters containing the substrates were observed under different mixing ratios (I/F) as depicted in Figure 3. The lag phase occurred during the 1<sup>st</sup> day as biogas production commenced afterwards. It was evident that there was pressure build-up in the headspace of each biodigester as biogas production rate remained constant until the 15<sup>th</sup> day. It is however observed that the biogas rate increased in all the biodigesters from the 15<sup>th</sup> day, a sharp drop on the 18<sup>th</sup> day and rose again on the 22<sup>nd</sup> day as shown in Figures 3–5. However, biogas production started to decrease from the 30<sup>th</sup> day until on the 33<sup>rd</sup> day, when it ceased. This is because the microorganisms responsible for the degradation during the AD process might have been consumed up leading to the seizure of the entire process on the 33<sup>rd</sup> day. The results also show that on the 21<sup>st</sup> day, a higher biogas rate of 1 ml/kg was produced from biodigester D (I/F ratio of 3:1), followed by E (ratio 1:1) of 0.9 ml/kg and then C (ratio 1:3) of 0.8 ml/kg as in Figure 3. Similarly, biodigesters A and B produced the least amount of biogas, both at a rate of 0.7 ml/kg. Therefore, exposing cow dung and *Miscanthus Fuscus* to same operating conditions can resolve in producing almost the same amount of biogas.<sup>16</sup>

### Biogas composition of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>)

Comparatively, there have been several reports from literature on the percentage yield of methane and carbon dioxide. Biogas consists of about 60% methane (CH<sub>4</sub>) and 36-37% carbon dioxide (CO<sub>2</sub>) with the presence of other trace gases such as H<sub>2</sub>S and NH<sub>3</sub>.<sup>28</sup> However, in

this study, the biogas produced shows a higher methane production on daily basis as compared to carbon dioxide as depicted in Figure 4 & Figure 5 respectively, an indication of trend as presented in literature. It was also shown that biodigester D with I/F ratio of 3:1 contributed to the highest methane yield as compared to biodigesters A, B, C and E. This is attributed to the balance between the amount of inoculum and the feedstock presence in the biodigester D while the rest of the biodigesters received setbacks of unbalanced inoculum to feedstock ratio. This eventually affected the microorganism and, in some cases, could lead to shortage rendering the process unstable and could lead to possible reactor failure. For 33 days, the average methane recorded was 0.31, 0.3, 0.36, 0.46 and 0.4 ml/kg for biodigesters A, B, C, D, and E respectively. Likewise, the CO<sub>2</sub> recorded were 0.14, 0.14, 0.17, 0.21 and 0.18 ml/kg for biodigesters A, B, C, D, and E respectively. Besides biodigester D generating the highest amount of biogas, a combination of the inoculum and feedstock in the ratio of 1:1 also showed a high probability of generating biogas as depicted in Figure 6.

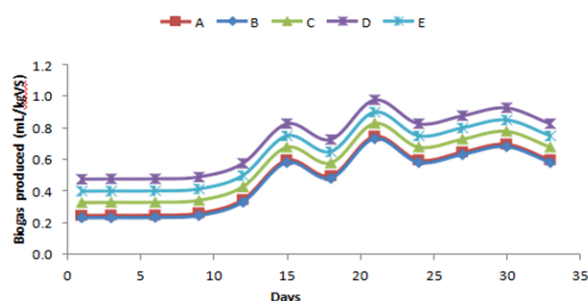


Figure 3 Effect of I/F ratio on daily biogas production (ml/kgVS).

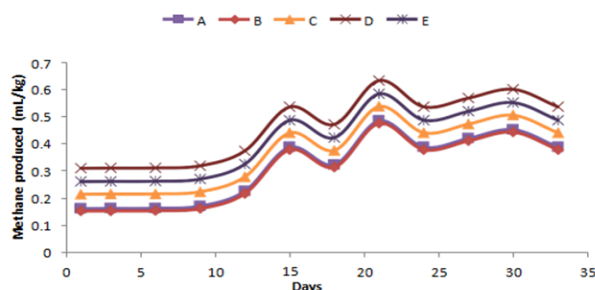


Figure 4 Effect of I/F ratio on daily methane production.

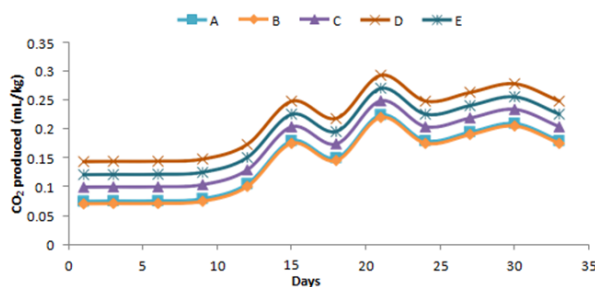
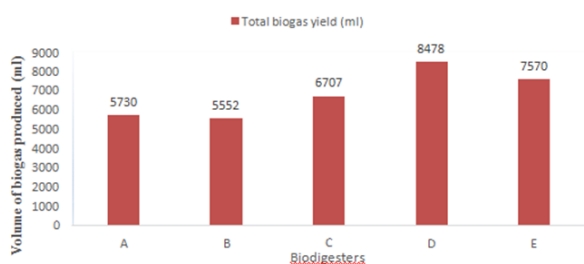


Figure 5 Effect of I/F ratio on daily CO<sub>2</sub> production.

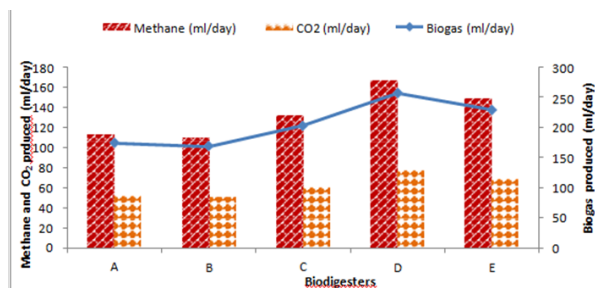
### Inoculum effects on biogas production

Figure 6 shows the effect of the inoculum in each biodigester for cow dung and *Miscanthus Fuscus* for the total biogas production. The results show the biodigesters in decreasing order of biogas production as D<E<C<A<B respectively in the ratio (I/F) of 3:1, 1:1, 1:3, 1:0 and

0:1. The total volumes recorded were 8478 for biodigester D, 7570 for biodigester E, 6707 for biodigester C, 5730 for biodigester A and 5552 ml for biodigester B. From Figure 7, the study also shows that the total biogas production for most of the biodigesters was observed to be higher than for those biodigesters with the absence of an inoculum. This result indicates that a well-balanced mixture of cow dung and *Miscanthus Fuscus* is an effective and an alternative option to improve the biogas and methane yield within a specified hydraulic retention time. Thus, the presence of an inoculum in a biodigester during an AD process contributes greatly to the produced biogas.<sup>29</sup> Also, in brief, it could be stated that the greater the ratio of the inoculum to a feedstock in a biodigester, the better the biogas output as compared to biodigesters with less or without inoculum fractions. The lowest biogas and methane yields observed in biodigester B (containing only *Miscanthus Fuscus*) gives an indication for the absence of bacteria responsible for the biodegradation during the AD process.<sup>16</sup> In other studies, such loading in the absence of an inoculum for biodegradation could easily lead to reactor failure and a much shorter retention time lasting for less than 10 days.<sup>11</sup>



**Figure 6** Biogas produced per each biodigester (I/F ratio).



**Figure 7** Overview of biogas composition produced per each biodigester (I/F ratio).

## Conclusion

Biogas production via anaerobic co-digestion of *Miscanthus Fuscus* with cow dung was found to be a potential resource for energy production. Biodigester D containing these substrates at an I/F ratio of 3:1 reported the highest potential for biogas production with a total volume of biogas of 8478 mL in this study for the BMP test. Average biogas production for the same biodigester was reported to be 0.4 ml/kg during the 33 days of anaerobic digestion. The results indicated that biogas and methane production in the presence of an inoculum increases the yield output than biodigester's without the presence of an inoculum. Therefore, a well-balanced inoculum to feedstock ratio is an effective way of improving the yield of biogas via anaerobic digestion. It is therefore warranted to researchers to focus on the utilization of *Miscanthus Fuscus* with an inoculum in a definite proportion as both substrates have the potential to produce higher

biogas yield. This could in turn be used to ease the dependency on fossil fuels and other non-renewable sources of energy.

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## Conflict of interest

The authors of this manuscript declare that there is no conflict of interest.

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