# Effect of Substrate to Inoculum Ratio on Biogas Yield

Emmanuel Pax Makhura, Edison Muzenda, and Tumeletso Lekgoba

***Abstract*—This paper aims at finding the effect of substrate to inoculum ratio on biogas yield. The batch anaerobic digestion experiment was conducted using the Automatic Biogas Potential Test System (AMPTS II). Substrate to inoculum ratios of 1:1, 2:1 and 3:1 were used for the study on anaerobic digestion of both food waste and cow dung. Tests were carried out starting with the preparation of substrates, substrate characterization to determine the moisture content (MS), ash content (AC), total solids (TS), volatile solids (VS) and ultimately batch anaerobic digestion experiments under thermophilic conditions (37$^0$C). The TS and VS contents of food waste ranged between 19.6–22.0% and 90.7 – 92.6% respectively while moisture content, total solids and volatile solids for cow dung were 67.2, 32.8 and 96.0% respectively. The cumulative biogas yields of reactors with cow dung were 12847 Nml, 3598.3 Nml and 4199.4 Nml for the ratios of substrate to inoculum of 2:1, 3:1 and 1:1 respectively. A 67% increase in accumulated volume of biogas was obtained by increased Substrate/Inoculum ratio from 1:1 to 2:1. On the other hand the cumulative biogas yields of reactors with food waste and inoculum were 110.2 Nml, 70.1 Nml and 46.7 Nml for the ratios of substrate to inoculum of 2:1, 3:1 and 1:1 respectively. When compared with those from cow dung, a 58% increase in cumulative biogas yield was obtained through an increased Substrate/Inoculum ratio from 1:1 to 2:1 from food waste.**

***Index Terms*—Anaerobic digestion, inoculum, substrate, total solids, volatile solids.**

## I. Introduction

Energy is a very pivotal aspect in the growth and development of any country and the whole world at large. Combustible fuels such as coal, gas and oil are the main sources of energy to the world [1]. These energy sources are non-renewable and therefore are depleting with time hence limited in quantity. Fossil fuels also have adverse effects on the environment more so that the high energy demand leads to the large-scale use of these combustible fuels. Global warming and climate change are some of the problems associated with the use of fossil fuels [2]. Many rural communities in Botswana rely on traditional energy sources such as firewood and cow dung. These can be time consuming and not reliable during certain conditions such as rainy seasons when the firewood moisture content would rise thus having a negative effect on its calorific value. Firewood being the main source of energy, not only in households but also in schools and communal centres leads to deforestation which contribute to the problem of climate change. In order to alleviate the problem of deforestation and reduce reliance on fossil fuel energy sources, biogas becomes a good substitute [3]. The biogas is often used for cooking, heating, lighting and electricity generation. The sludge resulting from anaerobic digestion of biomass is often used as fertiliser. Engineers and scientists have taken the initiative to ensure that the application of rural biogas production is widely spread.

## II. Experimental Method

### A. Materials

The cow dung used for the tests was collected from one of the local farmhouses in Palapye, Botswana whereas the food waste was sourced from Botswana International University of Science and Technology campus (BIUST) cafeteria [4]. In order to prevent early digestion of the substrates, they were stored in the refrigerator at a temperature of 4 ℃. In addition to the two substrates was sodium hydroxide solution which was mainly used to maintain a constant pH of the solution throughout the experiment, distilled water (tap water was not used so as to eliminate the possibility of the ions influencing digestion process) which was used to prepare the slurry while Thymolphthalein pH indicator was used to show any change in pH during the experiments.

Fig. 1. Substrates (a) food waste and (b) cow dung [4].

### B. Equipment Used

A calibrated analytical balance was used for weighing the samples while a knife was used to reduce the size of substrates as they come from the refrigerator especially cow dung. Crucibles were used for holding the substrates during the determination of the moisture content, total and volatile solids. Samples in the crucibles were put in the electric hot air oven and heated to a temperature of 105$^0$C to determine the moisture content and total solids (TS) while the furnace was used for heating the samples to a temperature (500$^0$C) to determine the ash content and volatile solids (VS) of the substrates [5]. pH of the different samples was monitored through the use of a Jenco pH 6810 – Handheld pH meter. Anaerobic batch digestion tests were carried out using the AMPTS ll.

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### C. Determining the Biogas Methane Potential (BMP)

As shown in Fig. 2, the bioprocess control AMPTS II is made up of three sections being the digesters, carbon dioxide fixing unit and the gas collection system. The experimental setup was made up of 2000 mL glass bottles which were used as reactors with a working volume of 1800 mL and headspace of 200 mL. The glass bottles were sealed with a rubber stopper with two metal tubing for purging and gas exit and a plastic cap fitted with a stirrer and motor. The mixture was then transferred to the assay bottles and put in a water bath and covered with a lid to maintain the mesophilic temperature of 37 ˚C. 500 mL glass bottles with 350 mL working volume were used as $CO_2$ scrubbers which were fitted with plastic screw caps and rubber stoppers with two metal tubing for sealing the bottles. A scrubbing solution was prepared (NaOH) following standard procedures to a desired concentration of 3M. A pH indicator solution was added to determine the saturation point for the scrubbing solution to be replaced. Gas collecting unit was made up of a water bath which included a water tank, flow cell holder, 15 injection mould flow cells containing magnetic metal pieces, base and protection plate and plastic glass lid for the water tank. The water tank was filled with deionized water to the maximum level. The motors were switched on and the flow cell calibrated. The AMPTS software was now used from the computer to start the process by first filing the experimental data and start the run. The experiments were run for 21 days HRT.

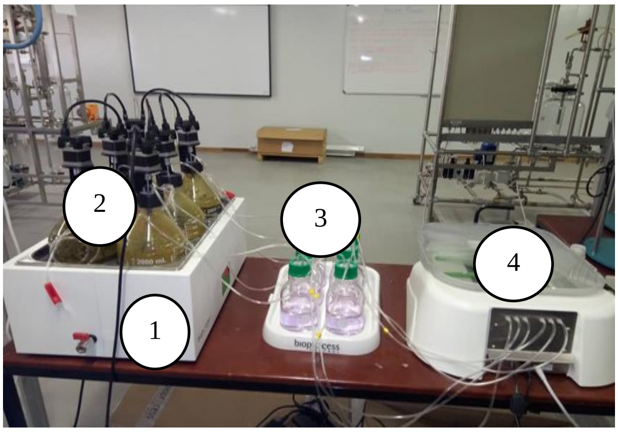

Fig. 2. Biochemical biogas potential test in AMPTS(II), (1) thermostatic water bath, (2) glass bottle reactor, (3) $CO_2$-fixing unit and (4) gas volume measuring device [4], [6].

TABLE I: CHARACTERISTICS OF FOOD WASTE AND INOCULUM AT DIFFERENT S/I RATIO

| Property | 1:1 S/I Ratio | 2:1 S/I Ratio | 3:1 S/I Ratio |
|---|---|---|---|
| Moisture content (%) | 78 | 80.4 | 79.7 |
| Total solids (%) | 22 | 19.6 | 20.3 |
| Volatile solids (%) | 90.7 | 92.6 | 91.1 |

## III. RESULTS AND DISCUSSION

### A. Characterisation of the Samples

The characterization tests results of feedstocks were presented and discussed in [4], [6]. The measured pH and moisture values of the food waste reported by the authors agree with those of [7]. The high moisture content of the food waste is attributed to the specific contents of the waste, more especially fruit peels and vegetables.

### B. Effect of Cow Dung to Inoculum Ratio on Biogas Yield

From the results, a ratio of 2:1 produced the highest volume of biogas as shown in Fig. 4. An increase in the ratio of substrate to inoculum from 2:1 to 3:1 leads to a decrease in biogas yield. Similar trends where observed for food waste as a ratio of 2:1 favours higher biogas yields. An important trend that was observed during digestion of food waste is that, a drastic drop in pH deactivated the bacteria and ultimately stopping the digestion process. The cumulative biogas yields of reactors with cow dung were 12847 Nml, 3598.3 Nml and 4199.4 Nml for the ratios of substrate to inoculum of 2:1, 3:1 and 1:1 respectively. The maximum gas volume recorded was 12847.4 Nml from the 2:1 ratio. A 67% increase in accumulated volume of biogas was obtained by increased Substrate/Inoculum ratio from 1:1 to 2:1 as shown in Fig. 3. However, it should be noted that a further increase in the ratio decreased the yield significantly by 72% (12847.4 to 3598.3 Nml). This result was probably due to a larger population of the methanogens hence favouring the production of biogas [8].

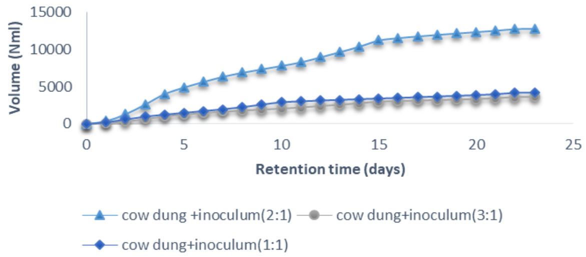

Fig. 3. Accumulated Gas volume with time.

### C. Effect of pH on Food Waste to Inoculum Ratio on Biogas Yield

According to Fig. 3 and Fig. 4, the AD yielded significant differences in biogas production depending on the substrate to inoculum ratio. The type of substrate also played a significant influence on the results. The cumulative biogas yields of reactors with food waste and inoculum were 110.2 Nml, 70.1 Nml and 46.7 Nml for the ratios of substrate to inoculum of 2:1, 3:1 and 1:1 respectively (Fig. 5) where the maximum volume recorded was 110.2 Nml from the 2:1 ratio. There was a 58% increase in cumulative biogas volume from FW compared to 67% that was obtained from cow dung for an increased Substrate/Inoculum ratio from 1:1 to 2:1 (Fig. 4). However, it should be noted that a further increase in the ratio decreased the yield significantly by 36.4% (110.2 to 70.1 Nml).

Moreover, the results revealed possible acidification in the digesters loaded with S/I of 3:1 and 1:1. As has been reported in the previous works, the S/I ratio does not affect the microbial properties only but it affects the non-microbial properties, such as alkalinity, TS, and micronutrients [10]. In this study, the increased S/I ratio also increased the TS content, the trend conformed with what was reported by [10] for batch AD. Food waste had a higher total solids percentage as compared to cow dung. Total biogas yield reduces at higher TS content because of compromised mass transfer of solutes in the slurry [11]. Higher S/I ratios usually reduced populations of methanogens and alkalinity and lead to inadequate reactor capacity to consume VFAs produced in the early stage of digestion, resulting in a pH drop and digester failure [10], [12]. It is notable that the average cumulative volume of biogas yield in the digesters with S/I ratio of 2:1 was higher than the ratio of 1:1 for the digestion time, the daily gas flow was high as well possibly because reactors with S/I ratio of 2:1 might have had a higher concentration of feedstock compared with that in reactors with S/I ratio of 1:1.

TABLE II: CHANGE IN PH DURING DIGESTION

| | cow dung + Inoculum (1:1) | cow dung + Inoculum (2:1) | cow dung + Inoculum (3:1) | Food waste + Inoculum (1:1) | Food waste + Inoculum (2:1) | Food waste + Inoculum (3:1) |
|---|---|---|---|---|---|---|
| **Initial pH** | 6.97 | 6.95 | 6.97 | 6.95 | 6.90 | 6.89 |
| **Final pH** | 6.89 | 6.89 | 6.68 | 6.89 | 3.53 | 2.97 |
| **Change** | -0.08 | -0.06 | -0.29 | -0.06 | -3.37 | -3.92 |


Fig. 4. Accumulated gas volume with time.

### D. Degradation of Volatile Solids (VS)

The reduction in VS gave a strongly positive correlation when compared with biogas yield, and a higher VS reduction was observed in reactors with higher biogas yields; [13]. The digesters which recorded the highest gas yield (2:1 ratio) for both cow dung and food waste was selected for the study of VS reduction, the highest VS reduction was achieved in reactors with cow dung (58.7%) compared with a reduction of 7.3% in food waste digester as shown in Table III.

TABLE III: CHANGE IN VOLATILE SOLIDS

| | cow dung | Food waste |
|---|---|---|
| **Initial VS (%)** | 80 | 91.5 |
| **Final VS (%)** | 21.3 | 84.2 |
| **Digested solids** | 58.7 | 7.3 |

## IV. CONCLUSION

From the study, cow dung has been found to quickly initiate anaerobic digestion processes compared to food waste. The faster start-up may indicate that cow dung slurry had higher concentration of methanogens that reduced the risk of severe VFA accumulation at the initial stage of digestion. It is to be noted that digesters containing cow dung had higher pH values before and after the reaction when compared to those containing food waste as the substrate, which indicated that cow dung could provide higher buffering capacity to digesters, plus a more robust community of methanogens, which are beneficial to the anaerobic digestion process. The former could be the main reason why food waste stopped producing any gas after a few days. Initiation of anaerobic digestion process from food waste has been found to be slower, this is shown by the decrease in peak values as the S/I ratio increases in all the digesters. It can further be concluded that substrate to inoculum ratio plays an important part in anaerobic digestion. Increasing the substrate/inoculum ratio from 1:1 to 2:1 increased the biogas yield but a further increase in the ratio resulted in a decrease in the yield. An optimum of 2:1 was observed for both cow dung and food waste but the only comparable difference was the fact that more biogas was produced from digesters loaded with cow dung (12847.4 Nml) compared to 110.7 Nml of food waste.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Emmanuel Pax Makhura carried out the research and literature of the work; Tumeletso Lekgoba assisted Emmanuel with analyzing the data and composing the paper while Edison Muzenda was responsible for reviewing and proof reading the paper ensuring it meets the journal requirements and all authors had approved the final version.

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