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Effect of Microbial Species and Inoculum Dosage on Anaerobic Digestion

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Abstract

This work aims at studying the effect of microbial species and inoculum dosage on anaerobic digestion of food waste using cattle rumen content as inoculum. Four different batch runs were carried out with different percentage inoculations which were (control sample), 25% dosage, 50% dosage and 75% dosage. To have both logical and scientific explanation why the inoculated samples had better performance than the control sample, microbial identification was carried out using standard method during the reaction period. The result of the microbial identification showed that inoculation was essential for better biogas quality and yield. The better biogas quality from the inoculated samples was traceable to the presence of Thermoplasma volcanium and Archaeoglobus fulgidus which are sulfur reducing bacteria as well as Methanosarcina barkeri which forms methane using different metabolic pathways. However, while the CRC furnishes the reacting media with the essential microbes, the results of the effects of inoculum dosage showed that inoculation using CRC should be done within 25% and 50% with the former slightly better. This was because; beyond 50% dosage, there was sharp decline in the performance of the system which is an indication of self-inhibition. Therefore, it was concluded that to improve the performance of anaerobic digestion without the risk of inhibition at high inoculum dosage, biostimulation of the system with isolated samples of necessary species of microbes was recommended and where isolation is not used, inoculation should not exceed 25% when using CRC.

Keywords: Anaerobic digestion, cattle rumen content, food waste, inoculum dosage, microbial species

INTRODUCTION

Biogas is produced by the decomposition of organic matter by microorganisms in the absence of oxygen in a process called anaerobic digestion, also referred to as methane fermentation, biodigestion, or biogas fermentation. Biodigestion is a complex process involving various bacterial and microbial groups. The main groups of microorganisms being acid- and methane-forming bacteria differ with respect to nutritional needs, growth kinetics, physiology, and sensitivity to environmental conditions. As a result, it has been shown that the yields of volatile fatty acids (VFA) and methane are the most sensitive to the archaea microorganisms responsible for methanogenesis and are more irritable to inhibitory compounds that may be present in the feed (Delgadillo et al., 2018) [11]. Identifying these species of microbes with subsequent isolation and cultivation can improve the performance of the digestion process.

The inoculum-to-substrate ratio (I/S) is the ratio of the biostimulant to the substrate used during digestion and measured on the same basis (unit).

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Alternatively, the percentage (dosage) of the inoculum was used to biostimulate the digestion process. Research has shown that different inoculum dosages result in varying performances depending on the substrate used as well as the biostimulant. Even the same dosage used with different substrate does not guarantee the same outcome [1–4].

The effects of inoculum to substrate ratio on anaerobic digestion were studied by Asante-Sackey et al. (2018) [5]. In this study, they used cow dung as the biostimulant and Miscanthus fuscus (a plant similar to elephant grass that grows as tall as 3m) as the feedstock. The I/S for the different batch runs were and 3 for 33 d HRT at a controlled pH range of 6.2–7.8 and a temperature range of. The results of this experiment showed that I/S gave the highest biogas production, while I/S had the least biogas production. In all samples, the biogas, methane, and carbon dioxide daily production increased overall [6–16].

Mohamed et al. (2016) [17] compared the production of biogas from MSW using cow dung and sewage sludge as inocula. The 60 days reaction was carried out at mesophilic temperature (not specified) and a controlled pH range of 6.8–7.3 using a sodium hydroxide (NaOH) solution. The I/S ratios were 0%, 10%, 20%, and 30% for cow dung and sewage sludge, respectively. At the end of the digestion period, it was observed that an increase in I/S led to a corresponding increase in biogas production for both samples seeded with cow dung and sewage sludge. The maximum cumulative biogas production for the 30% biostimulants using cow dung and sewage sludge was 567 ml and 383.52 ml respectively. This indicated that cow dung was more effective as an inoculum than sewage sludge. It is uncertain how these dosages affect the methane content of gas [18–21].

Ihoeghian et al. (2022) [12] studied the effects of cattle rumen content (CRC) on anaerobic digestion of food waste. Seven different samples were prepared with inoculum-to-substrate dosages of 0:1, 1:4, 2:3, 1:1, 3:2, 4:1, and 1:0. They found that the sample with an equal blend of CRC and food waste produced a maximum biogas yield of. From the results of this study, we can assert that although cattle rumen content is a good biostimulator, it becomes counterproductive beyond a certain dosage. Hence, it must be used in the right proportion [22].

Bello et al. (2015) [8] studied the effects of rumen fluid dosage during co-digestion with food waste. Their results indicated that there was a general antagonistic effect during co-digestion as the quantity of rumen fluid increased.

Rumen fluid has also been used as an inoculum in the co-digestion of cattle manure with kitchen waste, as studied by Argaw et al. (2013) [4]. According to them, increasing the dosage of cow manure resulted in poorer performance, while increasing the dosage of kitchen waste improved the result. Similar to CRC, cow manure exhibits an inhibitory effect beyond a certain dosage [23].

Pathak and Srivastava (2020) [26] studied the effects of inoculum dose on methane production from food industry effluent. Five different percentages of inocula were used: 0%, 10%, 20%, 30%, and 40%. The inoculums were obtained from the digested slurry of a cow dung biogas plant, while the substrate or feedstock was wastewater from the food industry. The outcome of the studies revealed that the biogas yields were respectively: ml/ml, 0.97 ml/ml, 1.05 ml/ml, 1.35 ml/ml and 1.23 ml/ml. The 30% concentration of biostimulant exhibited the best performance in terms of both biogas production and methane content. This result attests to the inhibitory effect of cow dung above a certain concentration [24].

The agreement between the results of different researchers using different substrates cannot be a mere coincidence. It can be concluded that for the biodigestion of any organic feedstock, using an appropriate inoculum dosage will ensure maximum productivity as well as gas quality [25].

MATERIALS AND METHOD

Materials

The following materials were used in this research: nylon bags, blender (shredder/crusher), storage container for cattle rumen content, digital weighing balance M411L by M-METLAR, refrigerator, and food waste (substrate), which is a blend of several kitchen wastes such as rice, beans, bread, carrot, cucumber, banana, plantain, baked cassava flakes, meat, fish fragments, and spaghetti.

Methods

A known amount (kg VS) of inoculum and crushed substrate were measured using a weighing balance and mixed with water using a water-to-substrate ratio proportional to the percentage MC of the feedstock. Four different samples with different inoculum-to-substrate ratios of, and were used.

Each mixed sample was charged into the reactor and covered tightly to prevent air and light from gaining access. All kinetic data, such as temperature, pressure, pH, concentrations, volume of biogas, and volume of its constituent gases, were measured daily for each digester for a period of 20 days.

Readings of temperature, pressure, and pH were performed using instruments embedded in the reactor. The bacterial concentration was determined using the cell count technique and substrate concentration was determined by measuring the VS of the reactor's content, while the gas composition was determined using gas chromatography fitted with a mass spectrophotometer (GC/MS). Gas volume was measured using the water displacement method described by Ojikutu and Osokoya (2014).

RESULTS AND DISCUSSION

Bacterial Species and Identification.

Table 1 shows the outcome of bacterial species identification for the digestion of FW inoculated with CRC, while Table 2 shows the digestion of FW without inoculation (control).

Gram staining, motility, methyl red, indole, citrate utilization, sugar fermentation, and Voges-Proskauer were the seven indicators used to determine the presence of different microbial species. The results indicated that the control sample lacked the presence of some microorganisms, such as Thermoplasma volcanium, which is responsible for the degradation of sulfur. On the other hand, the results on the effects of the inoculum-to-substrate ratio revealed that the control sample had a higher percentage of hydrogen sulfide and indicated that there was Thermoplasma volcanium feeding on the elemental sulfur, thereby reducing the amount of hydrogen sulfide formed. Therefore, the sulfur content in the control experiment was much higher than that in other experimental runs. In addition, Halobacteri salinarum, a salt-degrading aerobic microbe capable of survival in a very low oxygen environment, was present in the inoculated food wastes. It might have been able to survive by using traces of oxygen produced during anaerobic degradation. Other microbes found in the inoculated samples that were not in the control experiment included Archaeoglobus fulgidus, which is usually found in high-temperature oil fields and grows anaerobically at a high temperature range of with optimal at. They are sulfate-reducing archaea, coupled with the reduction of sulfate to sulfide with the oxidation of many different organic carbons, as well as complex polymers. Methanosarcina barkeri can anaerobically produce methane through different metabolic pathways. This might have contributed to the quality of biogas produced from the inoculated samples compared to the control experiment. Methanobrevibacter smithii was not detected in control experiments. This microbe plays an important role in the efficient digestion of polysaccharides by consuming the products of bacterial fermentation. It is both a methanogen and hydrogenotroph that recycles hydrogen in a reaction by combining it with carbon dioxide to form methane. This could be another reason for the higher methane content yield at the 25%, 50%, and 75% inoculum dosages. Generally, from the effects of inoculum dosage, while inoculation with cattle rumen content favors the percentage of methane content, there is a limit beyond which the inoculum becomes inhibitory.

Table 1. Microorganism result identification for inoculated food waste.

Micro organism	Gram staining	Motility	Methyl red	Indol	Citerate utilization	Sugar fermentation	Voguesproskauer
Methanosphaera stadtmaniae	+	+	+	-	+	+	+
Methanopyrus Kandleri	+	+	-	-	-	+	+
Methanococcus Maripaludis	-	+	-	+	-	+	-
Methanobacterium Thermoautotrophicum	-	-	+	+	+	-	+
Methanosracina Mazei	-	+	-	+	+	+	+
Methanospirilum Hungatei	-	-	+	+	-	+	+
Methanocella Paludicola	+	+	-	-	+	+	-
Halobacterium Salinarum	+	-	+	-	+	+	+
Thermoplasma Volcanium	+	+	-	+	-	+	-
Archaeoglobus Fulgidus	+	+	-	-	+	-	+
Methanosarcina Barkeri	+	+	+	-	+	+	-
Methanobrevibacter Smithii	+	+	-	+	+	+	+

Table 2. Micro-organism identification for non-inoculated food waste.

Micro organism	Gram staining	Motility	Methyl red	Indol	Citerate utilization	Sugar fermentation	Voguesproskauer
Methanosphaera stadtmaniae	-	-	+	+	+	-	+
Methanopyrus Kandleri	+	+	-	-	-	+	+
Methanococcus Maripaludis	+	+	-	+	+	-	+
Methanobacterium Thermoautotrophicum	-	+	+	-	-	+	+
Methanosracina Mazei	+	+	+	+	+	-	-
Methanospirilum Hungatei	+	+	-	+	-	+	+
Methanocella Paludicola	+	+	+	-	+	+	-
Halobacterium Salinarum	-	+	-	+	-	+	-
Thermoplasma Volcanium	-	-	-	+	-	+	+

Effects of Inoculum Dosage on Biogas Volume and Quality

Figure 1 shows the results of the cumulative biogas volume and gas quality for the control sample (0% biostimulation). A total of $29.7l$ of biogas was produced from 574.6gVS of food waste. This translates to a biogas yield of $0.0517l/gVS$.

The quality of the biogas was quantified in terms of gas composition. Spectrophotometric analysis revealed that the biogas had an average methane content of 43.4%. When expressed in terms of yield, the biogas had a methane yield of $0.0224l/gVS$. Because of the high cost associated with gas analysis via GC/MS and other contingencies, gas analysis was carried out at certain intervals beginning on the day each reactor started producing a significant amount of gas.

Figure 2 shows the variation in the percentage of the selected biogas composition with time. The results showed that the biogas quality increased with time, while the impurity decreased over time. This is because microbes always try to detoxify media and attain stability during anaerobic digestion. More importantly, very high biogas purity was observed during this continuous operation because of the use of one-time biostimulation instead of continuous codigestion of food waste and cattle rumen content. Hence, the inhibitory substances or toxins accompanying the inoculum were eventually washed off by gradual digestate removal.

Figure 3 shows that a total of $28.75l$ was produced from 2 kg (511.4gVS) of codigestion of food waste and rumen content in a ratio $3:1$. This translates to a biogas yield of $0.0562l/gVS$. The average methane content for the entire duration was 64.08%.

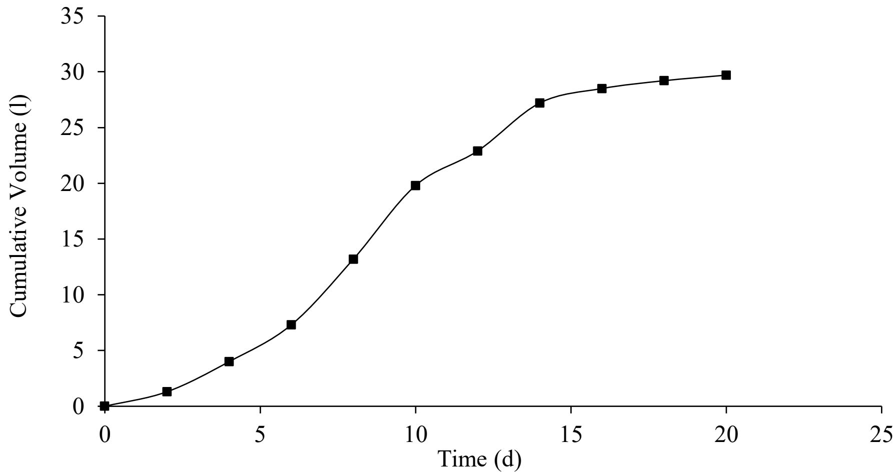

Figure 1. Effect of 0% biostimulant on biogas volume.

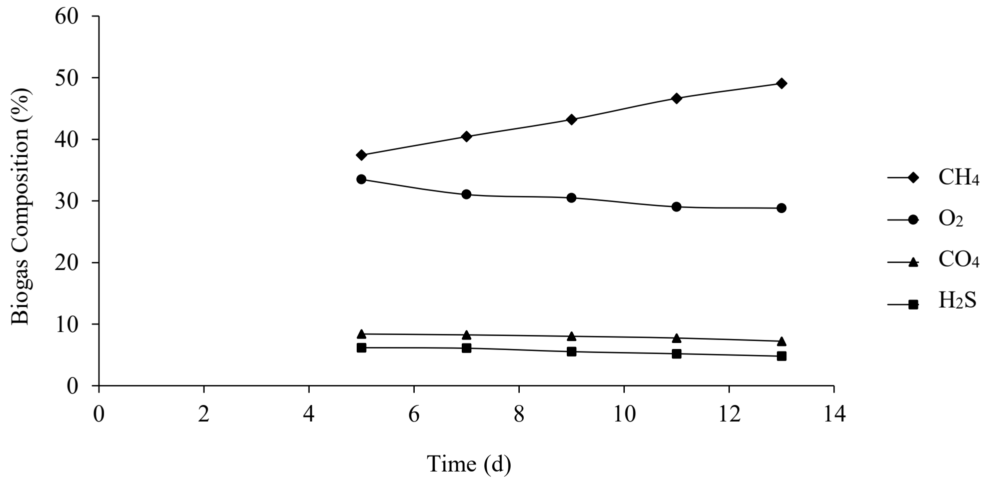

Figure 2. Effects of 0% biostimulant on select biogas composition.

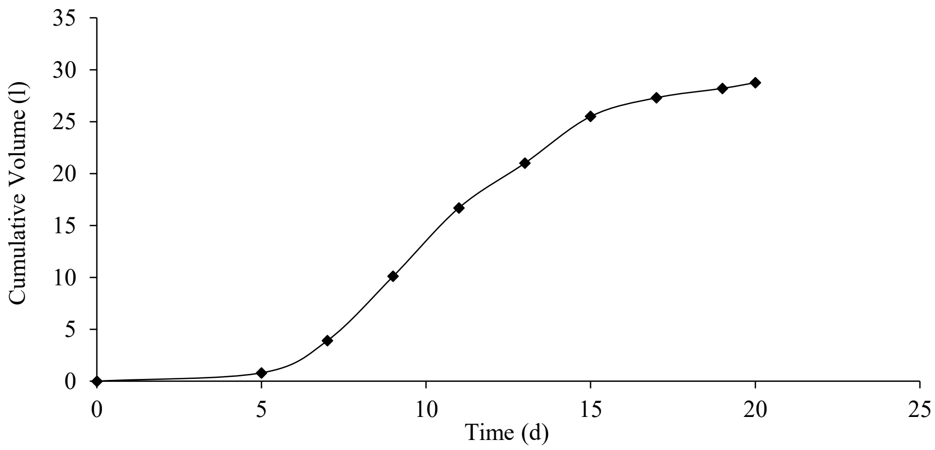

Figure 3. Effects of 25% biostimulant on cumulative volume.

At this percentage, the methane yield of methane gas was $0.036l/gVS$. Because of the biogas yield, one might think that the control sample had close competition with the 25% dosage. However, based on the fuel (methane), the 25% inoculum dosage was more than 1.5 times better.

Figure 4 shows the variation in the percentages of selected biogas compositions over time. For this batch run, the quality of the gas increased with an increase in HRT, whereas the presence of impurities decreased with time. This indicated that the microbes adapted better with time and, as a result, were able to properly digest the substrates.

The outcomes for the digestion of food waste with cattle rumen content in equal ratios are shown in Figure 5. A total of $23.8l$ of biogas was produced from 2 kg (448.2gVS) of waste food, translating to a biogas yield of 0.0531l/gVS.

The average methane content calculated from Figure 6 was 58.950%. The methane yield was 0.0313l/gVS. Given this value of biogas and methane yields, the 50% dosage was close to the 25% biostimulant. In addition, biogas quality improved over time during the course of digestion, as shown in Figure 6.

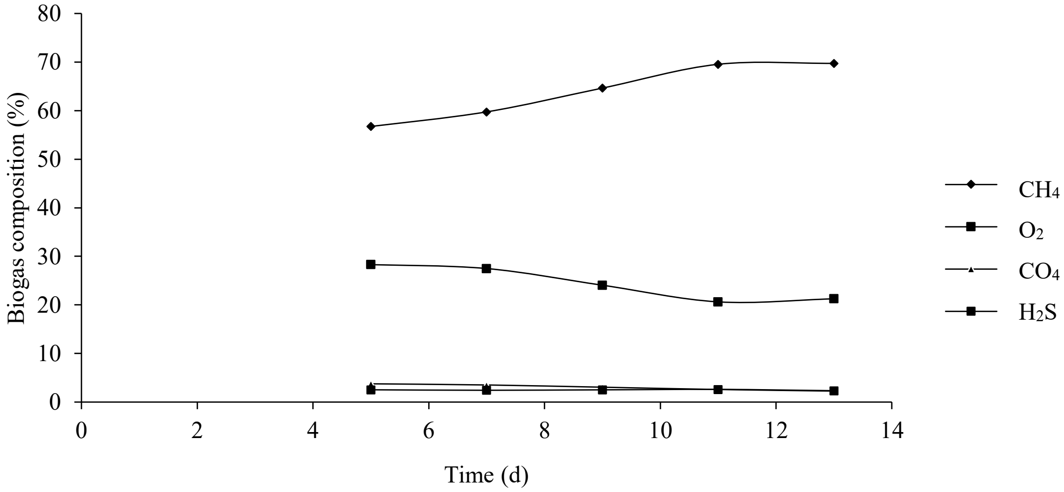

Figure 4. Effects of 25% biostimulant on select biogas composition.

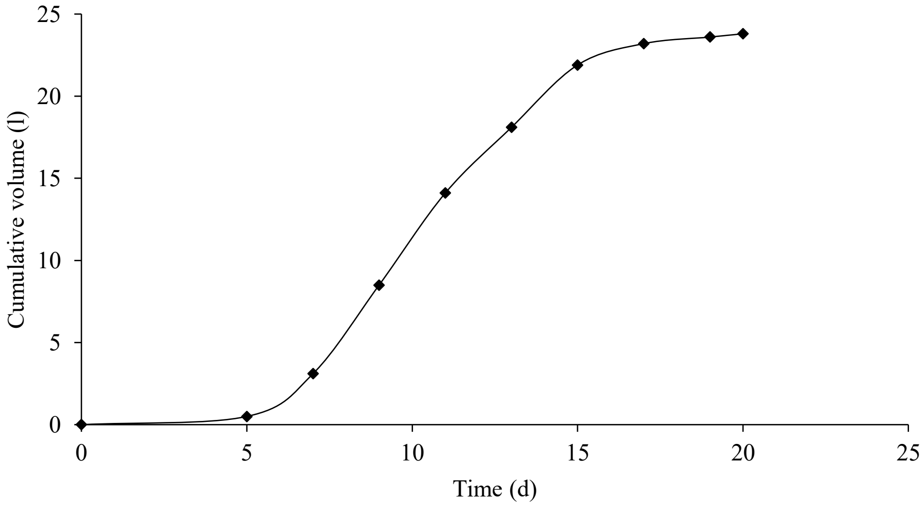

Figure 5. Effects of 50% biostimulant on cumulative volume.

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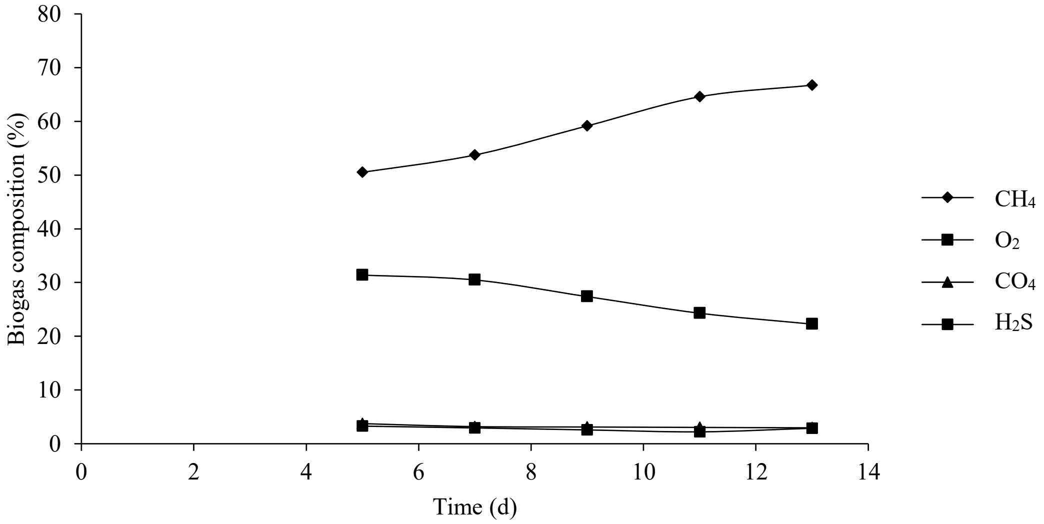

Figure 6. Effects of 50% biostimulant on select biogas composition.

As shown in Figure 7, a cumulative of $15.4l$ of biogas was produced from the codigestion of 2 kg (385 gVS) of food waste and CRC in a ratio $1:3$. Based on this, the gas yield was calculated to be $0.04l/gVS$. This quantity of gas is less than the $28.48l$ produced by Bagudo *et al.,* (2011) from an equivalent of 2 kg of cow dung. This may be attributed to CRC, which might have contained some toxic compounds alongside the requisite microbes required for anaerobic digestion.

Figure 8 reveals that the percentage of methane increased with time, whereas the other components of the biogas that constituted impurities decreased with time. The average percentage of methane was 53.96%, which was used to calculate the methane yield of the sample as $0.0216l/gVS$.

For all samples, the cumulative biogas curve initially steeply flattened out from the 18th day of digestion, indicating that the reaction ended.

Generally, the volume of biogas improves with a decrease in the inoculum (cattle rumen content) dosage. That is, while CRC supplies the necessary species of microbes to the substrate, it has an inhibitory effect such that beyond a certain dosage, a decline in performance occurs. This was confirmed by the pH of the samples. The pH of the control fluctuated between 6.8 and 7.2, which is within the acceptable optimal pH range for anaerobic digestion, while the pH of the other samples decreased with increasing percentage dosage.

From this perspective, comparative analyses of different samples are presented in Figure 9–11. It was observed that gas production commenced much earlier with the control sample on day 2, while the other dosages commenced at approximately on the 5th day, with the 25% dosage starting around the 4th day.

This increase in the time required for the commencement of gas production with a higher inoculum could be attributed to the adaptation period required by these microbes in their new environment. For this reason, the control sample commenced earlier because the microbes came naturally from the food waste; as such, there was rapid acclimatization.

The amount of biogas that can be extracted from organic waste depends on the waste itself and process conditions. The results of all the different dosages samples fall within the range of $0.02m^3$ to $0.8m^3$ per kilogram of waste reported by Biogas World (2022).

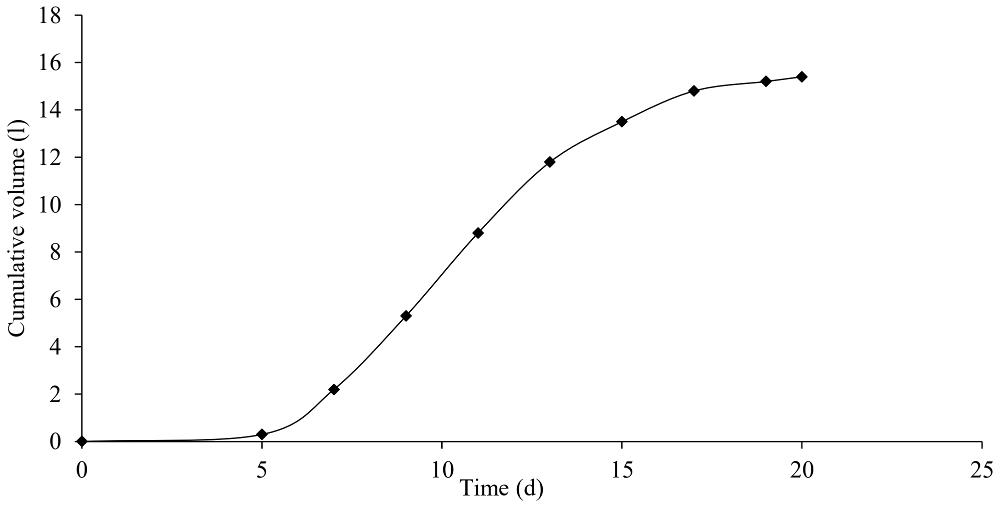

Figure 7. Effects of 75% biostimulant on cumulative volume.

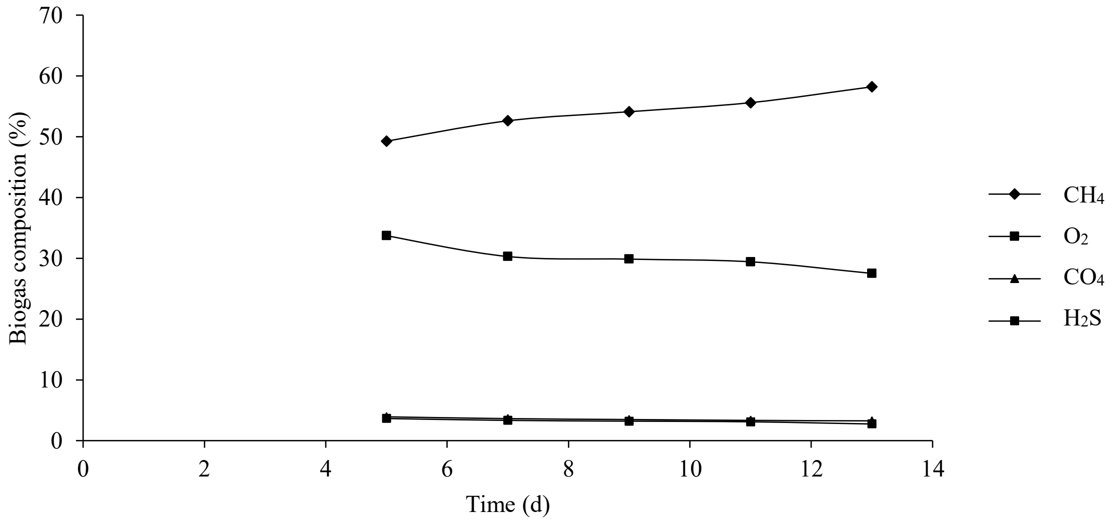

Figure 8. Effects of 75% biostimulant on select biogas composition.

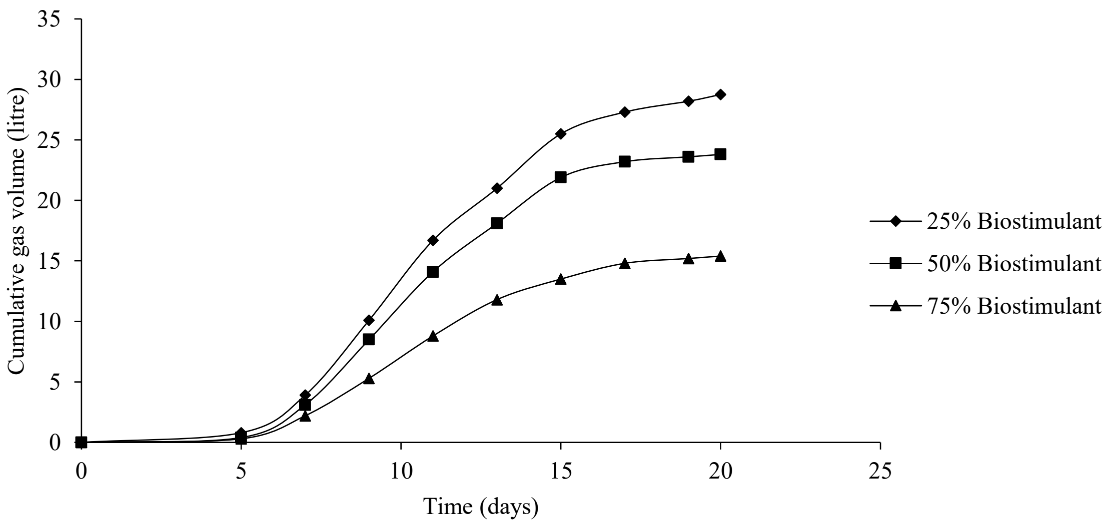

Figure 9. Effects of i/s ratio on biogas volume for 20 days HRT.

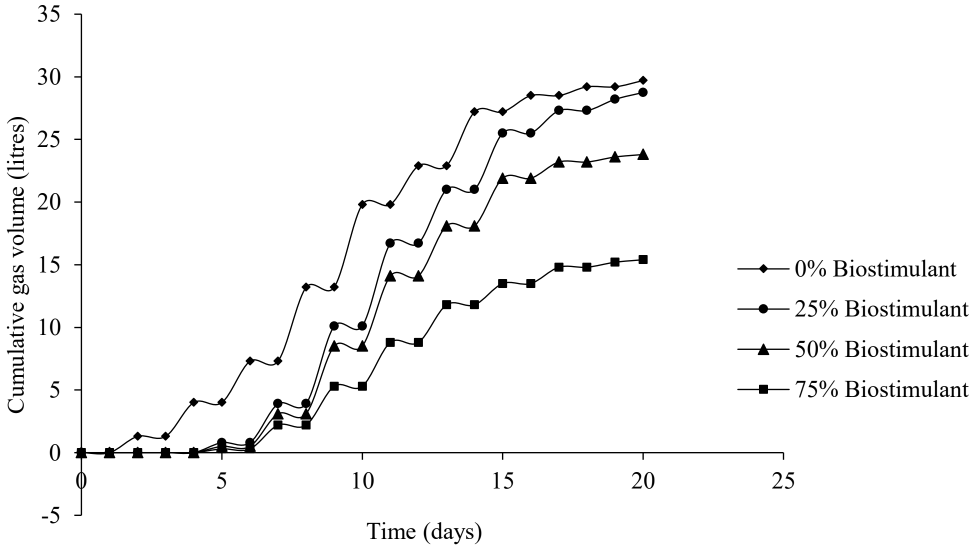

Figure 10. Effects of i/s ratio on biogas volume.

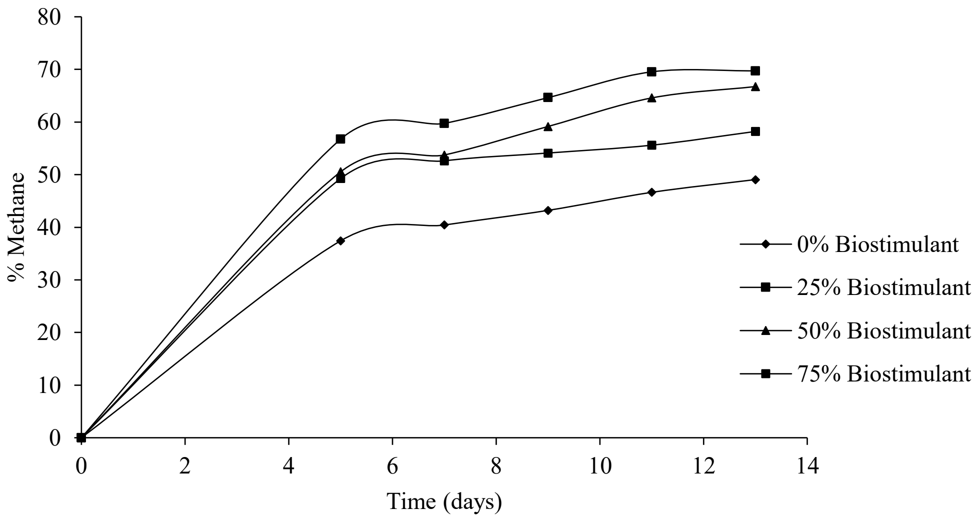

Figure 11. Effects of inoculums to substrate ratio on methane content.

Effects of Inoculum Dosage on Biogas and Methane Yield

As mentioned earlier, the inoculum to substrate ratio I/S affects both gas and methane yields. It is evident that at certain concentrations of rumen content, the rumen content becomes counterproductive to bioprocesses. These effects were more pronounced when the methane yields were plotted against the I/S ratio, as shown in Figure 12 and 13 for the biogas and methane yields, respectively. Although the 50% biostimulant had close competition with the 25% biostimulant, there was a gradual decline beyond the I/S ratio of 0.25. Severity increased as the I/S ratio increased. This observation is consistent with that reported by Ihoeghian et al. (2022) [12]. However, in their investigation, the 50% equal blend gave a maximum cumulative biogas yield of 0.32052l/gVS. Similarly, Bello et al. (2015) [8] observed a general decline in biogas production as the inoculum dosage increased.

In previous studies where cow dung was used as inoculum, Pathak and Srivastava (2020) [26] observed a decline beyond a certain optimum inoculum dosage. It suffices to say from the current study that at a certain concentration of cattle rumen content, the biostimulant becomes inhibitory. Hence, biodigestion must be performed within an acceptable dosage range to obtain optimal results.

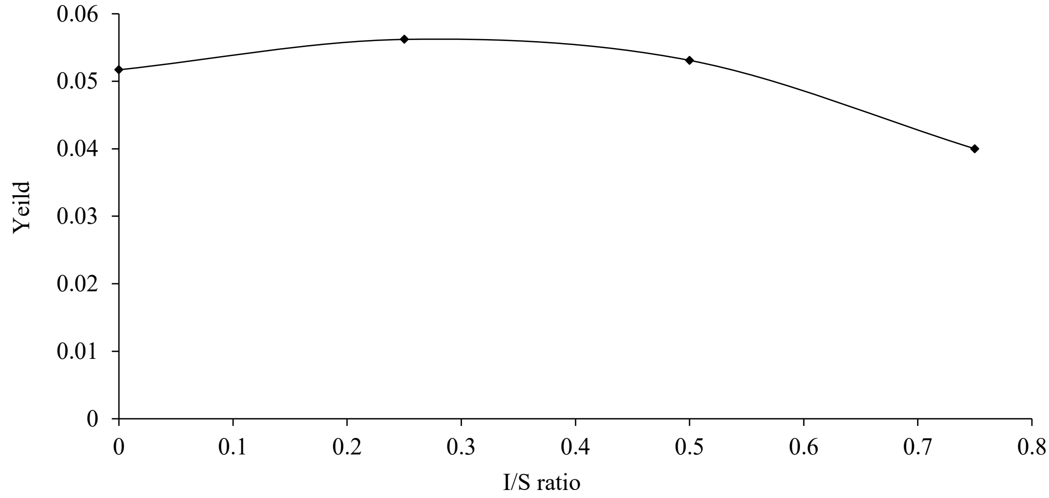

Figure 12. Effects of I/S Ratio on biogas yield.

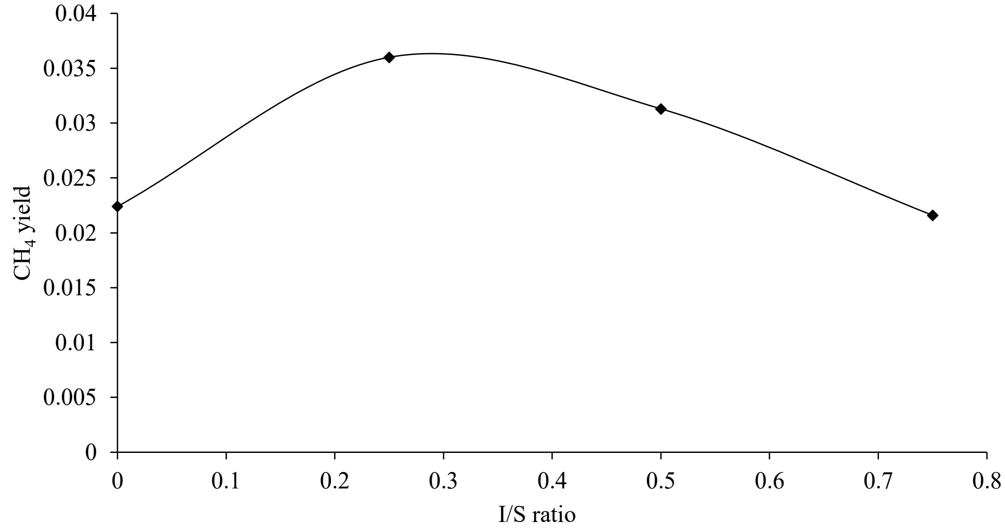

Figure 13. Effects of I/S Ratio on methane yield.

CONCLUSION

The effects of microbial species and inoculum dosage on anaerobic digestion of food waste were investigated. The microbial cell species present in the digester played a significant role in the quality of biogas produced. As expected, these microbes underwent adaptation as a result of alteration in their natural home for approximately five days, a period known as the lag phase. It was observed that the control sample started production within two days compared to 5–7 days for biostimulated samples, confirming that the latter's environment did not change as much. In addition, all the biostimulated samples had similar microbial species, which made them have better methane content than the control (no biostimulation), which lacked the presence of some essential microbes, such as Archaeoglobus Fulgidus, Archaeoglobus Fulgidus and Methanobrevibacter Smithii, which are needed for proper methane fermentation. Hence, it is sufficient to state that inoculation was essential for optimal production of biogas from food waste.

In addition to knowing the necessity of inoculation, the problem of dosage is equally important. Regarding the effect of the I/S dosage, it was found that the 25% biostimulant gave the best result in terms of both biogas and methane yields, followed by 50% and then 75%. The poorer performance at higher concentrations of the other inoculums was attributed to inhibition. We can conclude that while inoculation is necessary for digestion of food waste, it must be done in the right proportion to avoid inhibition, and better performance could be achieved by using isolated microbial species to avoid inhibitory substances present in CRC.

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