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Enhanced Biogas Production through Anaerobic Co-Digestion of Agricultural Wastes and Wastewater: A Case Study in South Africa

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ABSTRACT

The sustainable management of agricultural wastes (AWs) and their valorization for biogas production offer promising alternatives to fossil fuels and contribute to environmentally responsible waste management strategies. This study examines the anaerobic co-digestion (Co-AD) of various AWs, including apples, bananas, carrots, butternuts, and potatoes, combined with wastewater (WW) from a local fruit and vegetable market, using activated sludge (AS) as the inoculum. The biomethane potential test (BMP) was performed in 1L capacity digesters with an 80% working volume and maintained at 40℃ over a 21-day period. A mixing ratio of 1:1 (% w/w) between WW and AWs and 1:2 between the co-substrates and inoculum was utilized. Biogas production was monitored daily to evaluate the effectiveness of the Co-AD process. The control group yielded a total production of 450 mL/day, while the apple and banana substrates demonstrated the highest biogas output at 595 mL/day and 585 mL/day, respectively. The potato substrate generated 525 mL/day, mixed AWs produced 485 mL/day, and butternut and carrot substrates resulted in 485 mL/day and 475 mL/day, respectively. These findings suggest that the Co-AD of AWs and WW, in combination with AS, presents a viable and eco-friendly approach to enhanced biogas production.

1. INTRODUCTION

The increasing adoption of energy-saving and renewable energy technologies is aimed at replacing fossil fuels worldwide [1, 2]. South Africa is no exception, as the country's coal-based energy sources have proven insufficient to meet its energy demands, leading to periodic power outages and load-shedding that hinder economic activity [3, 4]. Furthermore, operational and financial challenges faced by the major South African power producer have resulted in increased tariffs per kilowatt of electricity [5]. The rapid depletion of non-renewable energy sources, such as coal and fossil fuels, has caused environmental degradation, human health issues, and global climate change [3].

Globally, agricultural wastes (AWs) in the form of fruits and vegetables are produced in vast quantities, with an estimated 998 million tons generated annually [6]. However, disposing of AWs in landfills poses challenges related to cost, land security, and carbon dioxide (CO_2) emissions that contribute to global warming [7]. Landfilling also generates contaminated runoff (i.e., leachate), toxic substances, and odors with negative impacts on the environment and human health [7, 8]. Alternatively, due to their composition, AWs can serve as raw materials for animal feed, compost, and energy in the form of biogas [9]. Consequently, the conversion of AWs into environmentally friendly energy and valuable products has led to the development of technologies such as incineration, anaerobic digestion (AD), gasification, pyrolysis, and hydrothermal processes [10]. However, the current study focuses on the application of AD for biogas production.

AWs typically consist of sugars, cellulose, hemicellulose, and lignin [11], and their high carbon content makes them suitable feedstocks for biogas production [12]. To achieve optimal biogas production, a carbon to nitrogen (C/N) ratio of at least 20-30 must be maintained in the feedstock [13-16]. Potatoes and banana peels have shown promise as AW feedstocks for biogas production, with C/N ratios ranging between 35-60 and 20-30, respectively [17, 18]. Previous research has revealed some of the characteristics, such as the calorific value of apples, which ranges from 17.15 MJ/kg to 19.85 MJ/kg [19-21], and moisture content (≥80%) [22], which promotes the biodegradation process for biogas production.

The application of AD in the biodegradation of organic waste into biogas has been reported as a promising eco-friendly technology, providing opportunities for biogas production without compromising the environment [23, 24]. Biogas primarily consists of methane (35-55%), CO_2 (40-60%), and trace amounts of other gases (1-10%), depending on the feedstock and environmental factors such as operating temperature, which can be psychrophilic (10-30℃), mesophilic (32-42℃), or thermophilic (50-65℃) [16, 18]. Mesophilic conditions require less energy and are therefore predominantly used at the industrial level [25]. Another crucial factor to monitor during biogas production is pH, as microorganisms responsible for degrading organic matter are most active at pH levels between 7 and 8 [16]. To ensure the survival of microorganisms, the rate of alkaline addition to adjust the pH must coincide with the rate of volatile fatty acids (VFAs) production to prevent excessive accumulation of VFAs, which could lead to a more acidic environment and inhibit methane production [11, 16, 21, 26, 27]. The hydraulic retention time (HRT) reported in the literature ranges between 21 and 60 days [20]. The organic loading rate (OLR) indicates the amount of volatile solids (VS) that should be fed into the digester, as well as the proportion of organic-material solids that can be digested, while other solids are fixed or non-biodegradable [28]. For biomass with high VS content, such as AWs, a low OLR between 1-4 kg VS/m^3.d is recommended [12, 17].

From available literature, it is evident that AW management requires attention, and anaerobic co-digestion (Co-AD) of AWs has the potential to enhance biogas production with high methane yield; however, process stability remains a limitation [14, 15]. The current study aims to investigate the biogas production potential from the co-digestion of apples, bananas, carrots, butternuts, and potatoes as representative AWs (i.e., substrate) with wastewater (WW) from a local fruit and vegetable market, using activated sludge (AS) as the inoculum. Different AWs were utilized to investigate their biogas potential measured in terms of daily biogas production.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

AWs samples were collected from a local fruit and vegetable bulk market located in Clairwood, Kwa-Zulu Natal, South Africa. The AWs consisted of different fruit and vegetables; hence the collected samples were subjected into manual separation to separate targeted waste i.e., potatoes, apples, butternuts, carrots, and bananas from the mixed AW. The selection of the AWs was based on the availability of the waste stock from the market during the experimental study, these AWs were in abundance at the dumping site and also the majority have been demonstrated by different studies to have been viable for biogas production [13, 22, 29-33]. The amount of each AWs that were taken from the site were about ± 3 kg in weight. The samples were washed with deionized water to remove dirt or any impurities. Washed samples were then kept in a refrigerator at 4°C to preserve them for a duration of less than 24 hours.

WW samples were collected from the same local fruit and vegetables bulk market, it should be noted that the wastewater is a combination of water generated during cleaning of the fruits and vegetables and sanitization of the market, water from the cooling and waste from sewerage sumps around the market system. The selection of the wastewater from the market as the second substrate was driven by the availability and the location of both substrates, which will reduce transportation costs. Characterisation was also done as can be seen from Table 1. The wastewater has a high content of microorganisms that can be digested to produce biogas [34, 35]. It is worth noting that WW sampling was conducted in accordance with the Standard Methods for the Examination of Water and Wastewater [34]. AS, which was used as an inoculum for the biodigester, was harvested from a local urban wastewater treatment plant in South Africa. The choice of the inoculum was based of the easily availability, location and that the plant produces an excellent quality AS with high organic content as seen in Table 1. Many studies have proven to show that AS has a good performance due to the high acetogenic and methanogenic microorganism's when compared to other types of inoculums like cow dung, etc. [35-37].

2.2 Characterization of substrates and inoculum

Prior to the commencement of Co-AD experimental runs, the biodigester substrates (i.e., apple; banana; potato; butternut; carrot; and mixed substrate) were subjected into a characterization process aimed at finding the substrate composition. The substrates composition was measured in terms of volatile solids (VS), total solids (TS), and moisture content. WW samples composition was measured in terms of VS, TS, pH, and chemical oxygen demand (COD). It should be noted that characterization analysis was conducted in accordance with the Standard Methods for the Examination of Water and Wastewater [34]. The pH was measured using a pH/conductivity meter (Thermo Scientific Eutech Elite PTCS, Singapore), COD was measured using spectrophotometer (HACH DR3900, Germany) using test vials, and VS, TS, moisture content, and ash were measured using the gravimetric analysis technique using an oven (Scientific oven, Trilab, South Africa) and furnace (Kiln Contracts, South Africa) and a balance (Labtech, South Africa). Characterization results are depicted in Table 1 for all substrates investigated as well as WW samples.

Table 1. Substrate and inoculum composition

	Parameters				
	VS[1] (%)	TS[2] (%)	pH	COD[3] (mg/L)	Moisture content (%)
Substrate					
Bananas	91	19	-	-	78
Apples	88	22	-	-	73
Carrots	85	8	-	-	84
Potatoes	82	9	-	-	85
Butternuts	87	15	-	-	75
Mix[4]	93	20	-	-	86
WW[5]	47	12.5	5.5	1858	-
AS[6]	61	14	6.8	8456	-

Notes: 1. VS (volatile solids), 2. TS (Total solids), 3. COD (Chemical oxygen demand), 4. Mix (mixed AWs before co-digestion), 5. WW (wastewater from the market), 6. AS (activated sludge)

2.3 Anaerobic co-digestion experimental setup

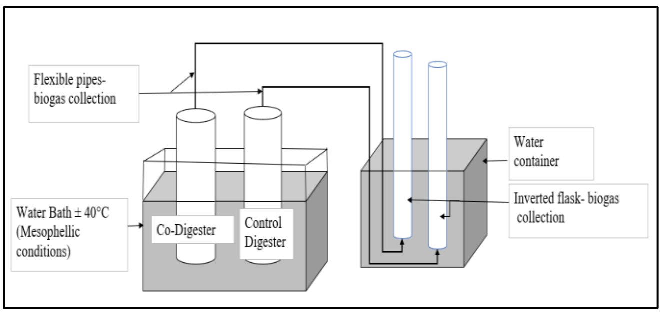

Figure 1. Co-AD experimental setup

A laboratory scale batch Co-AD system (Figure 1) was used for biogas generation. The system consisted of seven 1 L blue-cap Schott bottles; it is worth noting that a working volume of 800 mL was used. The blue-capped bottles were used as bio-digesters and were immersed in a water-bath which was incorporated with a temperature-control device to maintain mesophilic conditions of ± 40°C. Each bio-digester consisted of a flexible pipe which was attached to a biogas collecting system. The biogas collecting system consisted of seven graduated flasks which were placed up-side down in another 10 L container of water to create a downward displacement connection between the outlet gas nozzle and the biogas collecting system as depicted in Figure 1.

In preparation of the substrate for experimental runs, samples were sliced into small cubes and blended and grinded for duration necessary to produce a consistent paste (± 15 minutes). The blending was done using an 800 W power kitchen blender (LOGIK, China) to form a consistent paste. The blending and grinding of the samples is a pre-treatment method used increase the surface area for adsorbing the substrate, which would improve biodegradation process subsequently improving the biogas production [38]. The substrate was preserved in a refrigerator, to avoid any possible microbial activities which might affect its composition.

Moreover, experiments for each substrate were conducted at a weight percentage (% w/w) ratio of 1:1:2 (i.e., AWs: WW: inoculum) for a hydraulic retention time of 21 days, at a fixed organic load of 2.5 kgVS/m^3.day. The design of this experiment and operating conditions was supported from previous studies that conducted co-digestion of the AWs with different co-substrates [39, 40]. It is imperative to note that prior to the commencement of each run, each biodigester was purged with nitrogen gas into the headspace for 2 min, to induce anaerobic conditions by eliminating the oxygen [41]. The experiment had a total of seven digesters for each experimental run with the substrates and inoculums mixtures as shown in Table 2. The biodigester temperature and pH were monitored on a regular basis by means of inserting a thermometer and pH probe, respectively. In cases of temperature variations, the water bath temperature was adjusted to the desired temperature conditions. It should be noted that the biogas production was measured on daily basis using the water displacement method [42, 43]. The biogas was sampled from the sample point and the methane composition was characterised using a Gas chromatography (Shimadzu GC-2014, Japan).

Table 2. Biodigesters fed into the Co-AD system

Digester	Label	Description
1	Apple-WW	Apples and WW in AS (1:1:2) ratio (% w/w)[1]
2	Banana-WW	Bananas and WW in AS (1:1:2) ratio (% w/w)[1]
3	Carrot-WW	Carrots and WW in AS (1:1:2) ratio (w/w)[1]
4	Butternut-WW	Butternuts and WW in AS (1:1:2) ratio (% w/w)[1]
5	Potato-WW	Potatoes and WW in AS (1:1:2) ratio (% w/w)[1]
6	Mix-WW	Mixed AWs and WW in AS (1:1:2) ratio (% w/w)[1]
7	Control	WW and AS (1:2) ratio (% w/w)[1]

Notes: 1. (% w/w) -Weight percentage of the feed

3. RESULTS AND DISCUSSIONS

3.1 Synergistic effect on biogas production

The current study is focusing on the co-digestion of fruits (i.e., apples and bananas) and vegetables (i.e., potatoes, butternut, and carrots) with wastewater using AS as an inoculum on biogas production. The study was conducted under mesophilic conditions i.e., ± 40°C, at an HRT of 21 days and the findings of the study on biogas production are presented in Figures 2-4. The findings of the current study are presented in terms of the daily, cumulative, and total biogas production. As such, the results depicted in Figures 3-4 explicitly indicate a synergistic effect in the Co-AD of the model AWs with WW, which is produced by combination of desirable characteristic of both substrates which makes them easily biodegradable [44]. The highest biogas production as shown in Figure 4, was recorded in the order of apple-WW (595 mL/day) > banana peels-WW (585 mL/day) > potato-WW (525 mL/day) > mix-WW (490 mL/day) > butternut-WW (485mL/day) > carrot-WW (475 mL/day) and control (450 mL/day).

From the biogas production results obtained, it is apparent that the co-digestion of apples-WW and banana peels-WW produced a higher yield of biogas of more than 32% and 30%, respectively when compared with the control.

The marketplace has a low C/N ratio of 12 and VS percentage of 47% as shown in Table 1. The WW requires Co-AD with other organic waste with a high C/N ratio and VS, to improve the nutritional balance and increase the quantity of degradable carbon subsequently resulting to an increase in the generation of biogas [45]. This was demonstrated in this study by the addition of AWs.

The increase in biogas production from the Co-AD of AWs is attributed to the readily biodegradable organic content such as carbohydrates, lipids, and proteins, which plays a vital role in their conversion into biogas by microbial species such as AS [46-49]. The moisture content (Table 1**)** of apples (73%) and banana-peels (77%) was also conducive for the AD process as it promotes in the dissolving of easily/readily degradable organic content within the framework of the substrate composition [50]. It is noted that despite the moisture content of apples and banana-peels substrate being lower than the recommended value of 80% [22], the AD system for the aforementioned substrates gave a relatively high yield of biogas production in terms of biogas production as depicted in Figures 3-4. The current study had bananas with VS of 91% of is comparable to the results obtained by Tumutegyereize [51] of 87%. Furthermore, the high biogas yield demonstrated by the co-digestion of apples and banana-peels, is attributed to their high C/N ratio of 35 and 25, respectively which is well within the recommended range of 20-30 [13-16].

On the other hand, the Co-AD of carrots recorded the lowest percentage biogas increase of 8%, when compared to the co-digestion of potato, banana-peels, apples, and butternut, this is attributed to the low carbohydrates content in carrots [52]. The Potato-WW digester produced an increase of 16%. Potatoes had a good performance which can be linked to the high moisture content of 85% (Table 1) and this moisture is consistent with results obtained by Butnariu and Butu [52] of a moisture content of about 80% and approximately 18% carbohydrates. A Co-AD study by Parawira et al. [29] produced an increase in methane yield between 31 to 62%.

Whereas, the low biogas production (490 mL/day) for the mixed substrate suggest that, the model mixed substrate is composed of complex compounds which are not readily biodegradable thus compromising the biogas production [53].

3.2 Biogas production through the Co-AD of different AWs with WW

It is worth noting that, at the initial stages (i.e., first three days) the was a gradual increase in the daily production of biogas as can be seen in Figure 2, all the digesters were exhibiting the same trend and were in a lag phase [54]. This phase is defined as an acclimatization of microorganism's substrates and ambient conditions during digestion and signals methanogenic activity [54-56]. However, the acclimatisation of the microbial population is not explicitly accounted for in the current study. The length of the lag phase for all the digesters was less than 2 days, this can be attributed to the high amount of microorganisms the inoculum AS in use and also the Inoculum-Substates (I/S) ratio of 2.0, which is greater than 1:1 and was determined to be within the desired range [35, 39, 54].

The average daily biogas production (Figure 2) of the digesters was Apple-WW (28.33 mL/day) > Banana-WW (27.86 mL/day) > Potato-WW (25 mL/day) > Mix-WW (23.33 mL/day) > Butternut-WW (23.10 mL/day) > Carrot-WW (22.62 mL/day) > Control (21.43 mL/day).

It can be observed from Figures 2 and 3 that there was a significant decrease in daily biogas production post day three (3). Moreover, the reduction in biogas can also be attributed to the high VS content of the model substrates under investigation as indicated in Table 1 [28]. It should be noted that VS can undergo the hydrolysis process during the biodegradation process, thus resulting to a decrease in pH which does not favour microbial activities of methanogenic bacteria [57].

The maximum biogas production of 80 mL/day which was for the control system (i.e., WW and inoculum), despite the significant decrease on biogas production from 80 mL/day in day 3 to 45 mL/day in day four, a significant biogas increase of 60 mL/day was observed in day five. This can be attributed to the acclimation of microbial population in day 5 resulting to the increase in biogas production [56]. Between day 10 and 12 as well as day 13 and 15 (Figures 2 and 3**)**, there was an insignificant difference in biogas production However, a decline in biogas production was observed from day six which can be attributed to the decrease in soluble biodegradable organic substrates essential for biogas production. Between day 10 and 12 as well as day 13 and 15 (Figures 2 and 3**)**, there was an insignificant difference in biogas production. This is attributed to the fact that, WW composition consist of slowly biodegradable substrates as reported by Khumalo et al. [58] consequently hindering microbial growth rate leading to low biogas production.

It can be noted from (Figure 2**)** that the digesters with AWs stopped production between day 10 and day 14 and this is consistent with the results obtained by Seswoya [39] and Park et al. [59]. This suggests that microbial population was well acclimated at day six and day ten for the potato and carrot substrates, respectively. For butternut, and potato substrates, biogas production did not take place post day thirteen after a rapid substrate biodegradation from day eleven as indicated by the sharp negative slope. Biogas production ceased from day 14 which can be attributed to the high VS content when compared to vegetables substrates [60].

From day four, the mixed substrate system recorded higher biogas production rate as compared to the control system. The results suggest that the mixed substrate of apple, banana, butternut, potatoes, and carrots have high fraction of soluble readily biodegradable organic substrate that is essential for biogas production. Moreover, the results suggest that despite the high VS content of 93% for the mixed substrate, the microbial activity of methanogens was not inhibited [53].

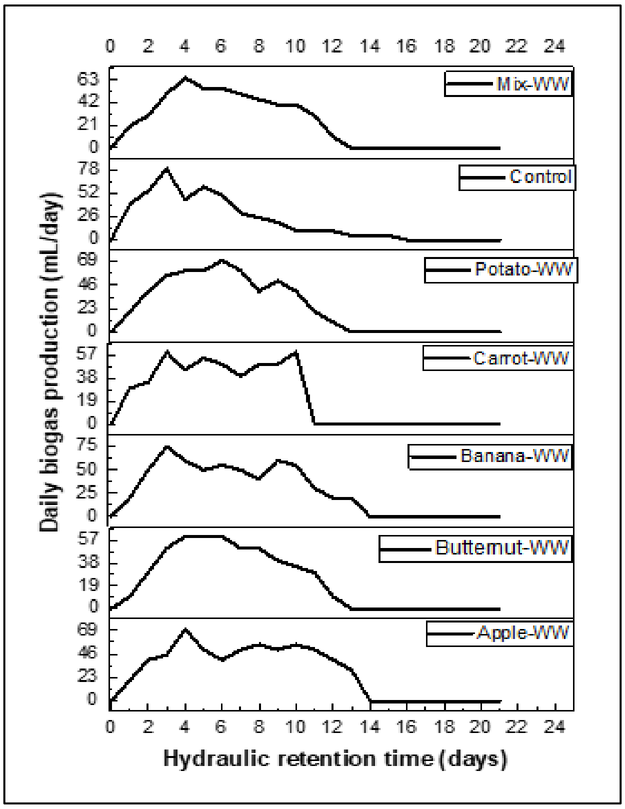

Figure 2. Daily biogas production at mesophilic conditions

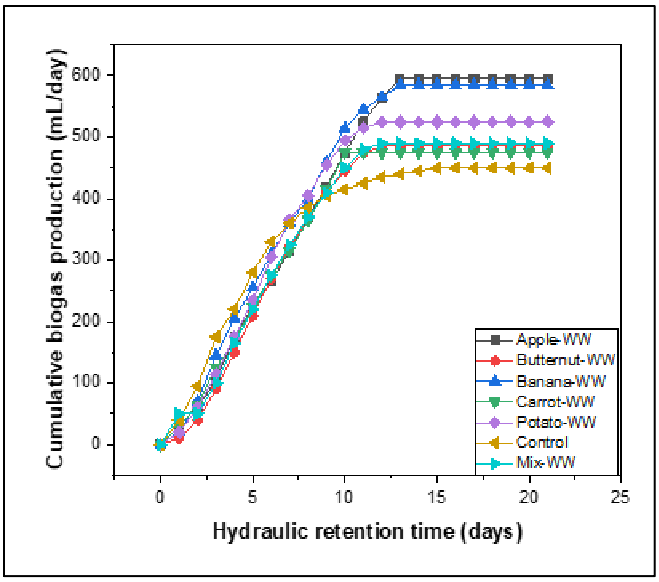

Figure 3. Cumulative biogas production mesophilic conditions

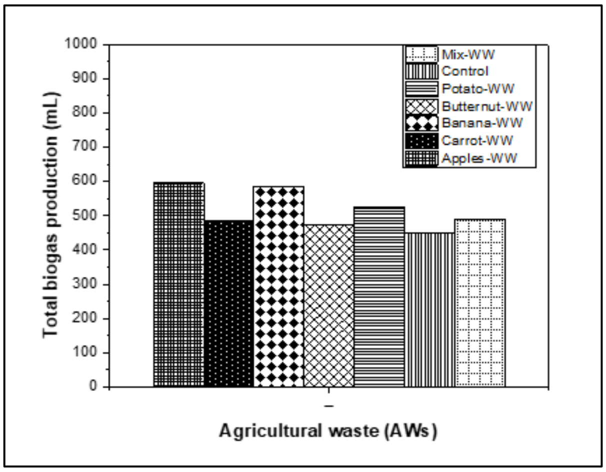

Figure 4. Total biogas production mesophilic conditions

3.3 Effect of pH on biogas production

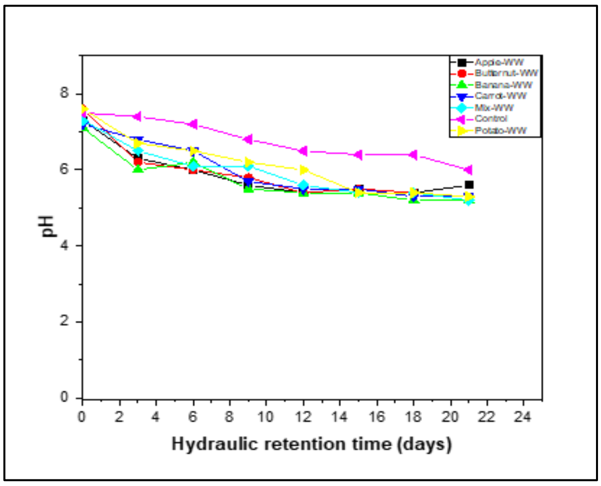

Figure 5. PH trend during co-digestion of AWs and WW

Figure 5 shows the effect on pH during the 21 days Co-AD period. The pH inside each biodigester was monitored throughout the duration of the Co-AD process to ensure that microbial activity was not inhibited by low pH [14, 57]. The desirable pH for hydrolysis and acidogenesis is (5.5-6.5) and is lesser than that for methanogenesis (6.5-8.2) [57]. The initial pH of the digesters was kept at above a pH of 7 [14]. The trend for all the biodigesters with AWs demonstrates a decrease in pH from the early stages of Co-AD, demonstrating a phenomena which is caused by the sugars in the AWs, which results in acidification which increases accumulation of VFAs, which causes the pH inside the bio-digesters to decrease [61]. The pH began to increase and was above 6 for the majority of the digesters after day 4 and 5 and operates at ranges that are good for Co-AD throughout the consecutive days, which can be caused by the microorganisms using up the VFAs after the acidogenesis and hydrolysis processes [14, 36, 62]. The pH is then stabled for the duration of the process and operates above 6 for all the digesters. The performance of pH in digestion of the control shows a trend where the pH is stable and does not drop towards the acidic regions for the control. The pH will therefore not hamper the methanogenic activity and this demonstrates that the system had sufficient buffering capacity to maintain the pH at a stable level [63].

3.4 Methane composition

Table 3 below shows the methane composition of the biogas that was analysed throughout the co-digestion process for the current study and previous studies. The comparisons of the anaerobic mono-digestion/ Co-AD of the substrates is shown in Table 3. The comparisons are complex as the conditions in each study are different and thus the behaviour will be different. The focus will be on methane composition. The anaerobic digestion of fruits rather than potatoes and vegetables is predicted to be high largest methane compositions which are greater than > 60% [21].

The co-digestion of banana-peels as conducted for the current study produced biogas with methane composition of 62%. Velmurugan [13] conducted a study using banana stems as AWs substrate for biogas production via the AD process, it was reported that biogas production was achieved with a methane composition of 65% for an OLR of kgVS/m^3.day and HRT of 30 days. The difference in the performance for the current study can be attributed to the types of substrates as the current study used banana peels while the other used banana stems. Another study on banana-peels conducted by Divyabharathi et al. [22], produced biogas with a methane composition of 54.8%, for a HRT of 42 days. The other factors can be the different design of experiment in terms of, type of digester, amount of volatile substances, type of inoculum and nature and makeup of the food that needs to be digested [49, 64].

Table 3. Comparison of methane composition

	Parameter			
Substrate	C/N[1] ratio	Co-substrate	Methane composition (%)	Reference
Bananas	25	WW[2]	62	This study
	NR[3]	-	65	[13]
	29.03	-	54.8	[22]
Apples	35	WW[2]	68	This study
	NR[3]	-	77	[32]
	NR[3]	Swine manure	62	[31]
Carrots	27	WW[2]	55	This study
	30	Corn silage	51.08 - 53.59	[64]
Potatoes	25	WW[2]	66	This study
	35	Sugar beet	84	[29]
	35		62	[29]
Butternuts	20	WW[2]	57	This study
Mix[4]	21	WW[2]	60	This study
WW[2]	12		65	This study

Notes: 1. C/N (Carbon to Nitrogen), 2. Wastewater local (Market) 3. NR (Not recorded) 4. Mix (Mixed AWs substrates)

The current study of Co-AD apples at 2.5 kgVS/L.d produced a methane composition of 63%. Coalla et al. [32] conducted a study using apple waste with OLR 1.5 kg/m^3.d and HRT of 52 days that produced methane composition of 77-80%, and González-García et al. [31] conducted a study using apple pomace pulp (7.5% w/w) co-digested with swine manure with an HRT of 33 days and produced a methane composition of 61.96%. The difference in methane composition can be attributed to the longer HRT and the type of substrate (apple waste) [49, 64].

This stud produced a methane composition of 62% for the Co-AD with potatoes. Parawira et al. [29] conducted a study which was using mono digestion of potato waste and produced a methane composition of 62% and potato waste co-digested with sugar beet with HRT of 14 days which produced a methane composition of 84%. The results for this study show a consistency with the mono-digestion but are lesser than the Co-AD with sugar beet.

The current study had the mixed substrates producing a methane composition of 60%, this was a satisfactory performance for the mixed AWs, this could be enhanced by using more fruits in the mix and thus increasing the potential to have a higher composition [65].

The Co-AD of carrots for this study produced a methane composition of 55% for carrots and The results from this study are consistent with literature. Kacprzak et al. [64] co-digested carrots with corn silage and produced a methane composition between 51.08 and 53.59%.

The butternut had a methane composition of 57%. There is limited data on previous studies that were conducted on butternuts. The control had the highest methane composition of 65%.

4. CONCLUSION

The performance of the control which had only WW and AS produced the lowest volume of biogas of 450 mL/day. The performance of the Co-AD of apples and banana) had the highest 32% and 30% increase in production when compared to the control, while the Carrot-WW recorded the lowest increase at 8%. with 475 mL/day. However, the vegetables substrate recorded the highest biogas production of 525 mL/day for Potato-WW, followed by 485 mL/day for Butternut-WW. The biogas produced with the highest methane composition was from the Apple- WW digester, with a composition of 68%. The lowest was butternut-WW with a composition of 57%. The Mix-WW had a production of 490 mL/day. The results obtained show evidence that that the Co-AD of the model AWs substrates did not inhibit the microbial activity of methanogenic bacteria which was indicated by the biogas and methane production. Moreover, the findings of the current work suggest that there is a need to conduct further investigations aimed at optimizing the substrate mix ratios and substrate combinations to increase the production of biogas.

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