

# Biomethane Potential from Co-digestion of Cassava and Winery Waste in South Africa

Unathi L. Mkruqulwa, Vincent I. Okudoh, and Oluwaseun O. Oyekola

**Abstract**—Co-digestion of cassava and winery waste was investigated for the production of biogas. Cassava biomass is a good substrate due to its high carbohydrate yield per hectare (4.742kg/carb) than most plants. Winery waste is a growing problem in South Africa due to high amounts currently being dumped at landfills. Due to the chemical properties of the two substrates it is envisaged that their co-digestion will produce more biogas than use of a single substrate. Biomethane potential (BMP) tests were carried out in a batch, mesophilic (37°C±0.5) reactor using cassava and winery waste singly and in combination at a ratio of 1:1 and run for 30 days. The results showed that cumulative methane yield for cassava, winery waste and in combination were 42, 21 and 38 mLCH<sub>4</sub> respectively. It was concluded that biogas production from anaerobic digestion was dependent on many factors such as pH, substrate properties and the ratio of different feedstocks used during co-digestion.

**Keywords**—Biogas, BMP, Cassava, Co-digestion, Winery Waste

## I. INTRODUCTION

Biomethane potential (BMP) is a test done with the intention to investigate the ultimate biomethane production prospect of a substrate by anaerobic digestion [1]. This test assesses the biodegradability of substrates where microbiological, biochemical and physico-chemical aspects of the substrates are determined. During anaerobic digestion, complex high molecular weight carbohydrates, fats and/or proteins are hydrolyzed into soluble polymers by means of the enzymatic action of hydrolytic bacteria and converted into organic acids, alcohols, hydrogen (H<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) [1]. Volatile fatty acids (VFAs) and alcohols are then converted to acetic acid by acetogenic bacteria and finally methanogenic bacteria convert acetic acid formed during acetogenesis into carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) [1]. VFAs are important intermediate products during anaerobic digestion therefore VFA monitoring is of vital importance.

Cassava is a root crop mostly grown in the tropics and is used as a staple food source in Africa with Nigeria being the largest

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producer contributing 20% of the global production. More than 260 million tonnes of cassava were produced all over the world in 2012 and 60% used as a food source, while 33% was used for animal feed [2]. In South Africa cassava is used for starch production and 20 000 tons of its starch are produced commercially [3]. It is cultivated in Limpopo, Mpumalanga, Eastern Cape and northern KwaZulu-Natal.

Winery waste is generated from the winemaking process. It is characterized by high biodegradable content and produced in large quantities in South Africa especially in the Western Cape Province. According to Dillon [4], a company in Wolseley and Worcester, South Africa, processed 20 000 tonnes of grape pomace in 2008, and 25 000 tonnes in 2009 thereby making its biomass highly attractive for sustainable biogas production in the area. The aim of this study was to investigate the biomethane potential of cassava in co-digestion with winery waste for the production of biogas in South Africa.

## II. MATERIALS & METHODS

### A. Inoculum

Fresh zebra (*Equus quagga burchelli*) droppings (ZD) collected from a Stellenbosch farm game reserve were used as an inoculum to start-up the experiment (Fig. 1). The samples were collected in sterile plastic bags and stored in a refrigerator set at 4°C prior to analysis. Before utilization, zebra dung was soaked in warm water and incubated at 37°C for 24 hours. It was then sieved and used as an inoculum for all the experiments.

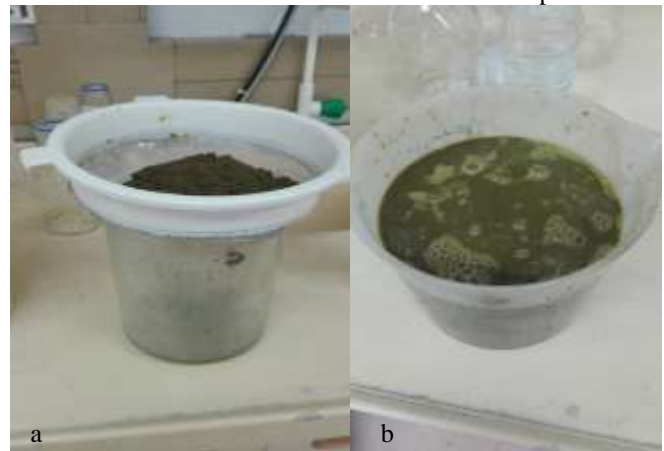


Fig 1: Preparation of inoculum: (a) zebra dung being sieved; (b) sieved inoculum

### B. Substrate

Fresh cassava (*Manihot esculenta* crantz) biomass was collected from plantation areas in Bizana, Eastern Cape in South Africa and stored in the refrigerator at 4°C until utilization (Fig. 2). Cassava was chopped into small pieces and oven dried for 48 hours. It was then milled to powder. Dried winery waste was collected from a winery in Stellenbosch, Agricultural Research Council.



Fig 2: Picture of cassava tuber from Bizana, Eastern Cape, South Africa

### C. BMP Test Set-up

The BMP test system for this study was conducted under reproducible and controlled conditions (Fig. 3). Four experiments were conducted in triplicates i.e. twelve glass bottles with a volume of 500 ml each were submerged in a water bath. The water bath was kept constant at 37°C±0.5 throughout the duration of the experiments. After the bottles were filled up with the inoculum and substrates, pH was measured and when necessary was adjusted to pH 7 using 1M sodium hydroxide (NaOH) or 32% hydrochloric acid (HCl) solution prior to fermentation.



Fig 3: Picture showing the set-up of BMP test

### D. Procedure

The contents of the bottle were bubbled with nitrogen gas for 3 minutes so as to remove all dissolved oxygen and then sealed immediately to maintain an anaerobic environment. The bottles were shaken manually twice a day. This was done to achieve homogeneity inside the reactor, free trapped gases and prevent scum accumulation. The 12 batch reactors were inoculated as shown in (Table 1). 25g of zebra dung was measured and diluted with water to 250ml for each experiment. Bottles 1-3 were each

inoculated with 250ml of zebra dung. These served as control experiments and were run as a baseline for comparison. For bottles 4–6, each bottle was inoculated with 250ml zebra dung and 25ml cassava. Bottles 7–9 were each inoculated with 250ml zebra dung and 25ml winery waste. Lastly, bottles 10–12 were inoculated with 250ml zebra dung, 12.5ml cassava and 12.5ml winery waste. These served as co-digestion experiments. The experiments were terminated after 30 days

TABLE I: BMP INOCULATION

	Bottles 1-3	Bottles 4-6	Bottles 7-9	Bottles 10-12
Inoculum (ml)	250	250	250	250
Cassava (g)	0	25	0	12.5
Winery waste (g)	0	0	25	12.5

### E. Basic Substrate Parameters

Constant temperature was maintained by a water bath at 37°C±0.5 and thermometers were also dipped in the bath to measure the temperature. The pH measurement was carried out at room temperature before and after the experiments. Total solids, volatile solids, COD and ash content were also measured on the samples. All analyses were determined by standard methods [5]. Volatile fatty acid content was measured and quantified using HPLC before and after digestion.

### F. Biogas Yield

Biogas formed was measured by downward displacement of water. The digesters were kept air tight, thereby preventing biogas from escaping. The net biogas formed in each bottle with both substrate and inoculum was subtracted from the gas formed from the bottle that has the inoculum only. This was done to account for the biogas formed from just the inoculum as in Equation (1).

$$\text{Biogas produced (ml)} = \text{biogas from substrate (ml)} - \text{biogas from control (ml)} \quad (1)$$

The biogas was normalized using Equation (2)

$$\text{Cumulative methane yield (mLCH}_4\text{/gVS)} = \frac{\text{Net cumulative methane (mLCH}_4\text{)}}{\text{Mass of VS added (g)}} \quad (2)$$

## III. RESULTS & DISCUSSION

### A. Biomass Characterization

Cassava and winery wastes were characterized and the results showed major differences on some of the properties that were tested for. The physical and chemical characteristics of fresh cassava and winery waste are shown in Table 2. There are major differences between the protein contents (2.25 %) for fresh cassava and (11%) for winery waste. The iron content was also found to be lower in cassava (1.15%) than in winery waste (28.05). Sodium was found to be lower in cassava (359.75 mg/kg) compared to winery waste (1191.9 mg/kg). Both substrates (cassava and winery waste) have a high total solids content of 94.45% and 95.92% respectively and also a high volatile solids content of 98.20% for cassava and 83.86% for winery waste. A substrate with a high volatile solids amount is of vital importance for biogas production as this depicts the

biodegradable amount in total solids. The nitrogen content (cassava - 0.36% and winery waste - 0.4%), carbon content (cassava - 45.6% and winery waste - 50.40%), calcium (cassava - 0.01% and winery waste 0.06%), potassium (cassava - 0.26% and winery waste - 1.77%), phosphorus (cassava- 0.05% and winery waste - 0.16%) and cyanide (cassava - 0.88 mg/kg and winery waste 0.92 mg/kg) showed very little difference. The moisture content of the substrates was found to be low (5.5% for cassava and 1.15% for winery waste). Moisture content is important for anaerobic digestion. It also depends on the type of cassava and place of cultivation. High moisture content results in more biogas yield whereas low moisture content yields low biogas [6]. Several authors [7- 9] also reported the moisture content of cassava to be around 15 – 19% dry weight. For winery waste, the ash content, protein, calcium and phosphorus contents are comparable with values reported by [10]. Seenappa [10] reported that winery waste contains 5% ash, 11% protein, 0.35% calcium and 0.4% phosphorus.

The carbon/nitrogen (C/N) ratio of the two substrates was found to be high (45.6:0.36 for cassava and 50.40:1.76 for winery waste). The optimum carbon/nitrogen ratio is 20-30:1 for appreciable biogas production during anaerobic digestion. High C/N ratio indicates that the substrate is not good for anaerobic digestion and thus will not appreciably yield biogas [11]. According to Ward[11], when one substrate has a high C/N ratio it can be co-digested with a substrate that has low C/N ratio in order to balance the ratio and drop it to a value between 20-30:1. One of the reasons for co-digestion is to balance the C/N ratio of substrates. However, in the case of cassava and winery waste both substrates have a high C/N ratio thereby causing low biogas yield when digested anaerobically. The biogas volume from cassava digestion was greater than the biogas volume from the co-digestion of cassava and winery waste (Table 3). Addition of urea as a nitrogen source could be of vital importance in order to increase the nitrogen content of the digester thereby balancing the C/N ratio to 20-30:1.

The protein content of cassava was found to be 2.25% and 11% for winery waste (Table 2). During anaerobic digestion, carbohydrates and proteins are hydrolyzed to soluble polymers by means of hydrolytic bacteria. High carbohydrate and protein contents result in high biogas yield with carbohydrates degrading more efficiently than protein [12]. However, according to Kovacs et al [13], protein content of the substrates should be kept minimal to avoid inhibition by ammonia. At high concentrations, free ammonia can inhibit biogas production during anaerobic digestion whereas at normal concentrations, it is an important nutrient for bacterial growth. Ammonia in the form of nitrogen which is generated by the deamination of amino acids can be used to monitor the degradation rate of the amino acids [14].

TABLE II: PHYSICAL & CHEMICAL CHARACTERISTICS OF DRIED CASSAVA AND WINERY WASTE

Characteristics	Unit	Dried cassava	Dried winery waste
Moisture content	%	5.5	1.15
Total solids	%	94.45	95.92
Volatile solids	%	98.20	83.86
Protein	%	2.25	11
Total nitrogen	%	0.36	1.76
Total carbon	%	45.6	50.40
Ash	%	1.7	15.95
Calcium	%	0.01	0.06
Phosphorus	%	0.05	0.16
Potassium	%	0.26	1.77
Iron	mg/kg	1.15	28.05
Sodium	mg/kg	359.75	1191.9
Cyanide	mg/kg	0.88	0.92

### B. Biomethane Potential

The biomethane potential was determined for all samples in triplicates and the average results are expressed in Table 3. Biogas production for cassava digestion started on day 4 of the digestion and dropped on day 22 whereas it started on day 4 of digestion and dropped on day 17 for winery waste. For co-digestion of both cassava and winery waste, it started from day 5 and dropped on day 20 (Fig. 4) After which the biogas production became normalized using Equation (2). The amount of methane in the biogas was found to be 62% of the total biogas produced which was comparable to values obtained by Abdeshahian [15].

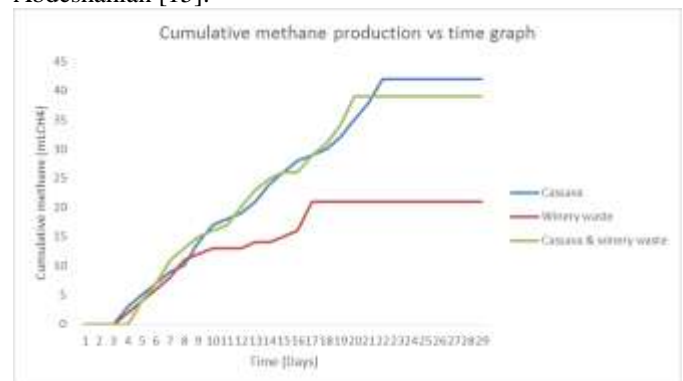


Fig 4: BMP test graph that shows the average methane yield

The results in Table 3 show that cassava digestion produced more biogas than winery waste and was also found to have produced more biogas than the co-digestion of cassava and winery waste. This result was found to be contradictory to literature which showed that co-digestion of substrates should produce more biogas than a single substrate [16]. This could be due to a number of factors which include the high C/N ratio of both substrates. This could be rectified by adding urea as a nitrogen source to adjust the C/N ratio to the optimum of 20-30:1. The other reason for less biogas production during co-digestion could be due to the cassava to winery waste ratio. Winery waste has been found to have inhibiting factors for biogas production such as the presence of phenolics which inhibit biogas production [17]. Lafka et al [18] studied winery waste phenolics using HPLC and found it to have major phenolics like gallic acid, catechin and epicatechin. Some of the



other identified phenolics from winery waste were caffeic, syringic, vanillic, p-coumaric and o-coumaric acids.

Another possible reason could be that, during co-digestion, the combination of the substrates increased the cyanide content of the digester making the environment more acidic. This acidity inhibits the anaerobic microbial activity such that they can't operate at their optimum best. This can also result in decreased biogas yield when the two substrates (e.g. cassava and winery waste) with high cyanide contents are co-digested compared to when a single substrate is used. Fresh cassava and winery waste were found to have 0.88 and 0.92mg/kg (1mg/kg~1ppm) cyanide content respectively. However, Eze [19] converted cassava waste from Gari processing industry to energy and biofertilizer and concluded that cyanide in cassava had no effect on the lack of biogas production if the amount of cyanide was less than 1mg/kg. Another study found that during anaerobic digestion of cassava the cyanide content of cassava was reduced concluding that the cyanide content does not have a negative impact on biogas production [20].

TABLE III: CUMULATIVE BIOGAS PRODUCED FROM CASSAVA, WINERY WASTE AND CO-DIGESTION OF BOTH SUBSTATES

Sample	Cumulative methane (mLCH <sub>4</sub> )	Cumulative methane (mLCH <sub>4</sub> /gVSadded)
Zebra dung + cassava	42	1.62
Zebra dung + winery waste	21	0.9
Zebra dung + cassava + winery waste	38	1.58

### C. Volatile Fatty Acids

The results of the VFA analyses obtained using an HPLC are shown in (Table 4). The concentrations of acetate, propionate and butyrate were determined for samples containing zebra dung only, zebra dung + cassava, zebra dung + winery waste and zebra dung + cassava + winery waste.

Acetate, propionate and butyrate concentrations obtained at the beginning and on the last day of digestion during the BMP tests are shown in (Table 4). The results show that during the digestion of zebra dung, there was no acetate at the beginning of the digestion process but was present at the end. This means that acetate was formed during AD. Propionate was found to be present at the beginning of digestion and was found to have increased at the end. Butyrate was not present both at the beginning and at the end of digestion. For cassava digestion, acetate was found at the beginning of digestion, increased significantly and persisted till the end. Butyrate was also present at the beginning of digestion but was not found at the end. For winery waste, acetate was present at the beginning, increased slightly during digestion and had a large increase towards the end. Propionate and butyrate were present at the beginning of the digestion but were not found at the end. During co-digestion of the two substrates, acetate was present at the beginning of the digestion and increased during digestion and was found to be more at the end. Propionate and butyrate were also present at the beginning of digestion and were not found at the end of the digestion period. According to [21] acetic and butyric acids are the most predominant VFA during anaerobic digestion. Acetic acid is necessary for anaerobic digestion as it is directly linked to methane and carbon dioxide formation.

Gorris et al [22] found propionic acid to be completely degraded when acetic acid levels in the digester were low (less than 100mg/L) and that high acetic acid levels (more than 4700mg/L) inside the digester blocked propionic acid degradation. This observation may be applicable to this experiment. For winery waste digestion and co-digestion of the two substrates, low acetate present in the digester resulted in propionate being completely degraded. According to Wijekoon et al [21], methanogenic bacteria has been found to be vulnerable to propionic acid concentration greater than 1.000~2.000 mg/L. Gourdon and Vermande [23] also observed no inhibitory effect for propionate levels above 600mg/L.

TABLE IV: VFA COMPARISON BEFORE AND AFTER DIGESTION

Sample	VFA	Inlet concentration (mg/L)	Outlet concentration (mg/L)
Zebra dung only	Acetate	0	136.62
	Propionate	144.11	149.2
	Butyrate	0	0
Zebra + cassava	Acetate	79.87	726.34
	Propionate	17.10	20.93
	Butyrate	56.73	0
Zebra dung + winery waste	Acetate	235.94	859.90
	Propionate	25.05	0
	Butyrate	52.91	0
Zebra dung + winery waste + cassava	Acetate	110.77	791.87
	Propionate	51.70	0
	Butyrate	154.02	0

## IV. CONCLUSION

The chemical composition of winery waste and cassava showed that both substrates were favorable for biogas production due to their high volatile solids and moisture content. However, the C/N ratio is higher than normal and may have to be lowered by using urea as a nitrogen source. Higher amounts of trace metals from both cassava and winery waste were optimal for anaerobic digestion. The optimum temperature of 37°C±05 showed great results for anaerobic digestion of the two substrates (cassava and winery waste). The obtained results from co-digestion of cassava and winery waste compared to the digestion of cassava alone were surprising. It was expected that the co-digestion of the two substrates would produce more biogas than a single substrate, however, the digestion of cassava (zebra dung + cassava) produced more biogas than the co-digestion of cassava and winery waste (zebra dung + cassava + winery waste). The following aspects will be further investigated on the co-digestion of these two substrates;

- Biogas optimization (substrate ratio, temperature & pH) will be performed on these substrates
- Biogas composition and quantification to determine the methane content
- The microbial dynamics inside the anaerobic digester to identify the cellulolytic microorganisms involved.

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He started work as a Medical Representative for a multinational pharmaceutical company (WYETH/Delpitano) in 1991 for 2 years. He then moved to REGENT Pharmaceuticals where he was promoted to Sales Executive and spent another 3 years before joining the academia. After his Postdoc he joined the Cape Peninsula University of Technology (CPUT) as a Biotechnology Lecturer in 2013 for 2 years. He was recently promoted and now a Senior Lecturer in the Department of Biotechnology & Consumer Science, CPUT, Cape Town campus. He has published a book titled "Biogas Production in Africa: Benefit Potentials of Cassava Biomass" published by Saarbrücken: LAP Publishing GmbH in 2015 and also contributed to the technical report "The State of Waste to Energy Research in South Africa: A Review" published by SA DoE Renewable Energy Centre for Research and Development. He has published many articles in top-rated peer-reviewed journals and conference proceedings. He published in the following: Elsevier: *Renewable & Sustainable Energy Reviews*, August 9, 2014, vol. 39(2014)1035 - 1052 with impact factor of 6.0 for 2015; *Tropical Journal of Pharmaceutical Research*, October 2012; 11(5): 729-737 and *South African Journal of Science*, May/June 2007, vol.103, No. 5/6: 216 - 222. His ambition is to lead a

research group that will use anaerobic fermentation techniques to convert any locally available waste to bioenergy specifically biogas. His research targets also include the use of modern isolation and screening methods to discover novel antibiotics from soil actinomycetes.

Dr. Okudoh has received numerous awards and currently an NRF Thuthuka grant holder and has supervised a number of postgraduate students. He is a FUTA Regional coordinator for Africa (South Africa) representing the Alumni diaspora group and a member of the Golden Key International Honor Society for top academic achievers. Dr. Okudoh belongs to the American Society for Microbiology [ASM], Society for Industrial Microbiologists & Biotechnology (SIMB) and South African Society for Microbiologists (SASM). Dr. Okudoh serves on the Advisory Board and Service Learning committees of the Biotechnology programme since 2013 and has been involved in coordinating Work-Integrated-Learning (WIL) helping students find placements for industrial attachment.



Oluwaseun Oyekola was born in Oyo, Nigeria. He holds a PhD in Chemical Engineering from the University of Cape Town, South Africa, Master's degree in Biochemistry from Rhodes University, South Africa and Bachelors (Honours) degree in Biochemistry from the University of Ibadan, Nigeria.

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Dr Oyekola's commitments include being part of the South African team of The PEESA (Programme on Energy Efficiency in Southern Africa). Further, he was recently selected to be part of the WasteEng2018 (International conference on Engineering for waste & Biomass valorisation) Scientific Committee.