Impact of Substrate Composition in Biomethane Production under Thermophilic Conditions

E.J. Bambokela, A. N. Matheri, M. Belaid, E. E. Agbenyeku and E. Muzenda, *Member, IAE*

***Abstract*—In this investigation, the anaerobic digestion (AD) results from different substrates were evaluated. By means of two co-digestion processes with cow dung, it was observed a good performance in the first process and a poor production trend in the second one. NaOH was used as chemical absorbent in two different bottles in the CO_2 fixing unit to compare the volume of biomethane produced. A batch anaerobic co-digestion was carried out in 500 mL digesters which were placed into a water bath at 45°C and maintained at the initial pH of 7. Important AD factors such as pH, Carbon and Nitrogen ratio (C/N), concentration, substrate composition were observed. Through an ultimate analysis, amount of Carbon (C), Hydrogen (H), Nitrogen (N), and Sulphur (S) were also discussed. The toxicity of other elements found in substrates appeared higher in the second process than in the first. It was concluded that inhibitory elements identification and control before furthering any biogas production process were primordial.**

Index Terms— Anaerobic Digestion, Co-digestion, Substrate, Toxicity.

I. Introduction

Most substrates are usually waste derivatives. They can be part of food waste (FW), industrial waste or animal waste (Manure) [1]. Investigations have shown a preference for organic material which are obtained from FW and animal waste. FW is considered as food obtained or discarded during the food supply chain at households, restaurants or retailers [2]. This FW can further be used as substrate in the production of biogas under AD.

AD is defined as the process that involves various metabolic interactions among different groups of bacteria [3]. Co-digestion under anaerobic conditions is an oxygen-free digestion occurring with various types of wastes mixed together according to their corresponding characteristics. In other words, co-digestion refers to an AD which is performed with different categories of feedstocks with a suitable system or technology [4]. Co-digestion is usually performed by combining industrial wastes, food wastes, with liquid animal manures other organic slurries [5].

The main purpose of co-digestion is to maximize the potential biogas yield by adding co-substrate such as energy crops or silages with different level of gas generation [6]. The addition of co-substrate in digesters results to greater efficiency, however some unpleasant factors are always likely to occur if they are not well-monitored. For instance, adding animal manures with other organic material could cause an increase of ammonia or pH value which will be inhibitory to the entire digestion process [2].

Previous researches have shown that co-digestion offered better biogas production rate making it a well-preferred technology. Nevertheless, other studies are still being run to overcome challenges that prevent the full adoption of this scientific approach.

In china, for instance, the AD of FW played a key role in bringing solution for the management of organic waste by demonstrating a better approach to waste to produce energy (biogas, biomethane) [6]. According to a case study performed by Djavan De Clercq *et al.*, [6], an approximate Beijing's production of 956 300 tonnes of food waste, could generate 300 million Nm3 of CH_4 for general utilization including transportation and power consumption.

This bio-degradation takes place in the presence of microorganisms (bacteria) that develop an affiliation with hydrogen (H_2), carbon dioxide (CO_2) and acetic acid (CH_3COOH) by decomposing materials while simultaneously producing methane (CH_4). Biogas is essentially a mixture of CH_4 and CO_2 [7], [8]. It is also reported to contain other traces elements such as hydrogen sulphide (H_2S), moisture and ammonia (NH_3) on a very small scale [1], [8]. Biogas is presently seen as a potential source of energy or fuel for heating or transport purposes with no carbon emissions unlike current fossils fuels [9]. It contains a great potential of energy (power and heat) that can be used in places where energy is highly needed but the presence of contaminants such as CO_2 and H_2S constitute a hindering factor in its utilization [10]. Various factors such as the nature of substrate, the type of digester and many others that will be further developed in this study are considered in the biogas production.

Njuguna *et al.*, [1] conducted an investigation on substrate

Manuscript received October 10, 2016. This work was supported in part by University of Johannesburg, Doornfontein Campus, South Africa.

E.J. Bambokela; is with the Department of Chemical Engineering; University of Johannesburg; Doornfortein, Johannesburg, 2028 South Africa; Cell: +27789844227 (jonathanbambokela@gmail.com).

A. N. Matheri; is with the Department of Chemical Engineering; University of Johannesburg; Doornfortein, Johannesburg 2028 South Africa; Cell: +27616986335 (tonynjuguna22@gmail.com).

M. Belaid; Senior lecturer and HOD at Department of Chemical Engineering; University of Johannesburg; Doornfortein, Johannesburg 2028 South Africa; (mbelaid@uj.ac.za).

E. E. Agbenyeku is with Department of Chemical Engineering Technology, Faculty of Engineering and the Built Environment. He is also with Process Energy and Environmental Technology Station, University of Johannesburg (PEETS-UJ), Doornfontein, Johannesburg, South Africa. (emmaa@uj.ac.za).

E. Muzenda; is with the Department of Chemical Engineering Technology, Faculty of Engineering and the Built Environment, University of Johannesburg, Doornfontein, Johannesburg, South Africa. He is also with the Chemical, Materials and Metallurgical Engineering Department; Botswana International University of Science and technology; Botswana (muzendae@biust.ac.bw).

composition by revealing the impact that various concentrations of trace elements such as copper, silver, nickel, cadmium, and zinc could have on the efficiency of the biogas produced. Due to toxicity, these elements were reported to have inhibitory effects on biochemical reactions. Furthermore, it was added that trace elements identification and control before furthering any biogas production process were primordial.

Usually substrates used as feedstock in AD process contain mineral ions [11], [12]. Mineral ions such as sodium, potassium, calcium, and sulphur enhance the microbial growth; however, their higher concentration can be toxic [1], [11]. Mineral like copper, silver, cadmium, nickel, cadmium, zinc also contribute to microbial growth, but when their concentration is not controlled, they reduce the biogas production because of the accumulation of organic acid that causes a methanogenic microbial inhibition [1]. Toxicity depends on the level of concentration of mineral and trace elements in substrate [1].

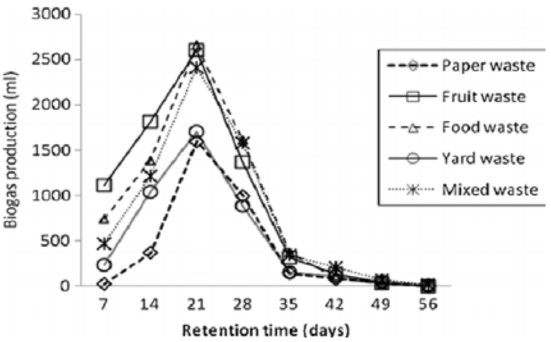

Fig. 1: Relationship between biogas production rates of different feedstocks with retention time in anaerobic digestion [13].

The figure above illustrates a difference in volume of biogas produced from different substrates (paper waste, fruit waste, food waste, lean grass, mixed waste) with respect to the retention time. It is observed that the maximum volume of biogas produced occurred between the 20th and the 22nd day. From the 7th day, the volume tends to maximize to the peak and from the 22nd day, the volume of biogas decreases considerably. This study shows that fruit waste produced biogas more abundantly than others within the same retention time. It is justified by the composition of fruit which causes bacteria to operate at a higher rate within the digester.

Temperature is a very important parameter because of the significant influence it exerts on AD process. The choice of temperature range and the exact temperature to operate with determine the efficiency and the outcome of the process [14], [15]. It was reported a direct proportionality of temperature with bio-methane production because of the rate at which microbes operate [15].The higher the temperature, the higher the bio-methane produced. Angelidaki and Sanders, [14] reported that an increase in temperature caused a subsequent increase in free ammonia which is inhibitory to AD efficiency. Abassi *et al.*, [15] suggested that mesophilic and thermophilic operating conditions were the most suitable for AD with ideal temperatures of 35°C and 45°C respectively.

II. MATERIAL AND METHODS

A. *Waste characterization*

Pig waste and cow dung samples used as main substrate were collected from a farm in Gauteng Province while the lawn grass used as co-substrate in the first co-digestion was collected from the University of Johannesburg in South Africa. The FW used in the second co-digestion consisted of fruits and vegetables that were collected at the Student Center of the University of Johannesburg, DFC Campus. Waste characterization was performed by considering physical and chemical composition of substrates. Accordingly, C/N ratio, total solids and volatile solids were determined during the proximate and ultimate analysis.

B. *Substrate and Inoculum*

To obtain a considerable biogas yield, the first co-digestion was preferably based on an Inoculum/Substrate ratio of 0.5 [16]. 60g of substrate (20 g of cow dung, pig waste and grass) and 120g of inoculum (Cow dung Digestate previously used) were prepared and poured into the digester. In the second co-digestion, 40g of FW (20 g of fruits + 20g of vegetables) with 20g of cow dung were prepared in the same ratio and amount of inoculum as in the first anaerobic process.

C. *Experimental Set-up*

NaOH was used as chemical absorbent in the CO2 fixing unit to compare the volume of biomethane produced after raw biogas purification. A batch anaerobic co-digestion was carried out in 500 mL digesters which were placed into a water bath at 45°C and maintained at the initial pH of 7. NaOH & H2SO4 were used to regulate the pH. The gas production was measured for 8 days using a Gas Chromatography (GC) device. This investigation was performed in accordance with the method described by APHA (2005) [17], and the Automatic Methane Potential Test System (AMPTS) II which is widely adopted as a standardized method for Biomethane Potential (BMP) testing [18].

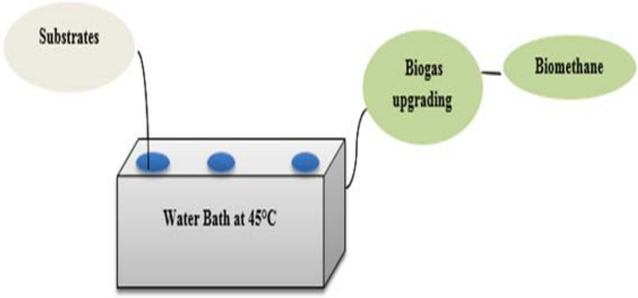

Fig. 2: Steps considered during anaerobic co-digestions.

III. RESULTS AND DISCUSSIONS

This study aimed at evaluating the impact of substrates in the production of biomethane (upgraded raw biogas) by comparing the pattern of different substrates.

A. *Substrates composition*

As per table I, an absence of sulphur in grass and food was noticeable comparatively to pig manure and cow dung where sulphur was present. A large C/N ratio was also observed in grass, cow dung and FW than in pig waste. The amount of N was highly observed in pig waste and lowly in grass. The largest amount of total solid and volatile solid was present in grass although cow and pig waste had more nutrients. These values were in the acceptable range [1].

TABLE I: ELEMENTAL ANALYSIS OF SUBSTRATES

Item	Unit	Pig waste	Cow dung	Food waste	Lawn Grass
C	%	23.15	41.61	43.85	42.49
H	%	3.41	5.44	5.79	5.62
N	%	2.52	1.97	1.58	0.66
S	%	1.40	1.81	0.00	0.00
TS	%	76.49	56.79	14,64	85.52
VS/TS	%	43.02	81.01	94,4	98.02
C/N		9.18	21.12	27.71	64.87

It is important to add that this type of analysis enables the regulation of key factors of biogas production [1]. When they are uncontrolled, they can become hindering to the AD [16]. For instance, the optimum C/N ratio can be obtained by adjustment from a mixture of highly biodegradable substrate with high nitrogen content with low biodegradability and nitrogen component [16]. As per this statement, mixtures of substrate for co-digestions were done in this study.

B. Calorific Value of substrates

In this study, the calorific value (CV) of substrates was also analyzed. CV is defined as the potential amount of energy per unit mass obtained after complete combustion [19]. It can also be seen as an indicator of the chemical energy stored in substrate, and a factor of good quality of components [19]. To determine and predict the performance of each component during the AD, the amount of energy per unit mass of cow dung, grass clippings, FW, and pig waste were compared in table II.

TABLE II: CALORIFIC VALUE OF SUBSTRATES

Substrate	Energy (MJ/Kg)
Cow dung	17.309
Lawn grass	18.242
FW	18.358
Pig waste	9.906

FW had the highest CV and pig waste had the lowest CV. It was also seen that lawn grass and cow dung had a good CV comparatively to pig waste. Thus, it can be reported that FW releases more energy upon complete combustion than cow dung, lawn grass and pig waste. As per the investigation conducted by Matin and Chelgani [19], it can be concluded that FW, grass and cow dung possess a highly energy potential than pig waste.

C. Comparison of co-digestions

In fig. 2 and 3 where two different co-digestions were conducted, the first co-digestion increasingly produced methane up to 415 ml of volume. Under thermophilic condition at exactly 45°C, methanogens continuously multiplied thus increasing the rate of production. In the course of the AD, the rate of pH normally changed. However, after the 5th day, the curve showed a static tendency.

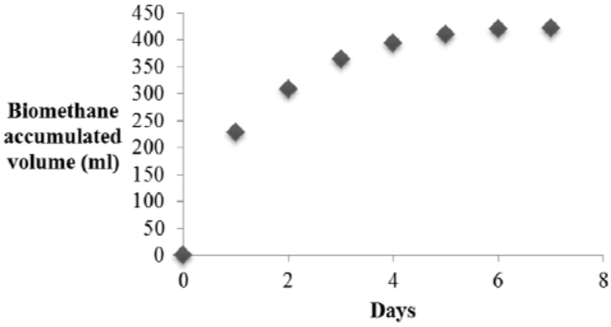

Fig. 3: Biomethane acccumulated volume from Cow, pig and grass co-digestion (1st co-digestion).

The rate of production remained constant and the production stopped after couple of days. From a close observation, it can be said that the trend of production and the behavior of the curve in fig. 2, reflected a normal biomethane production process. The biomethane yield was obtained under good anaerobic co-digestion conditions. All important factors were observed but more essentially, substrates used, were not composed of inhibitory elements.

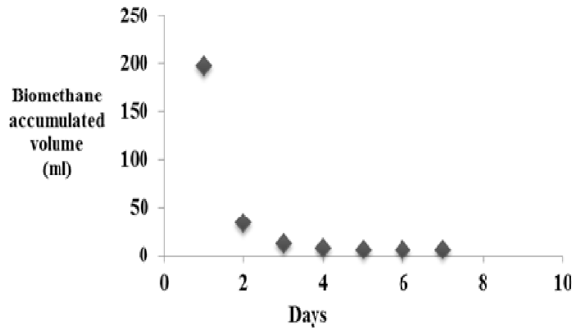

Fig.4: Biomethane Production from Food waste and cow dung co-digestion (2nd Co-digestion).

Unlike the first co-digestion, the second co-digestion was decreasingly producing biomethane from 200 ml of accumulated biomethane generated the first day. Under similar conditions, the curve showed poor performance during the entire process although all important factors such as pH, concentration, TS and VS were observed. It was understood that the toxicity of elements found in these substrates was higher than those of the first co-digestion. This means that the concentration of toxicity found in trace elements contained in the FW was not well regulated [1], [20]. In other words, the regulation of trace element's concentration was not performed in order to avoid inhibitory factors such as this. FW did not have trace elements concentration in acceptable range [1].

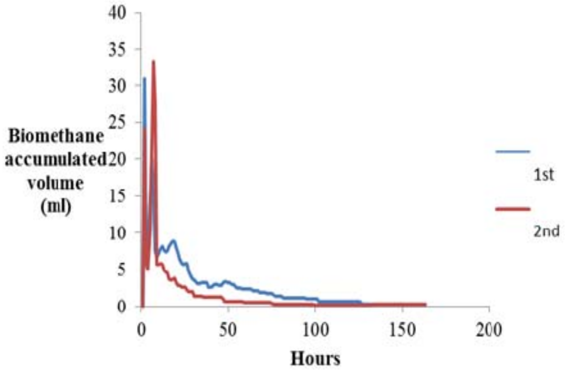

Fig. 5: Comparison of rate of production (ml. h^{-1}) between the first and the second co-digestion.

From several experiments performed, it can be reported that first hours of biomethane generation required higher rate of production due to higher rate of methanogenesis caused by the activation energy demand from bacteria. In other words, to start the production, there had to be enough energy to generate methanogens responsible of producing biomethane. In fig. 5, the second co-digestion highly produced biomethane during the 10 first hours but poorly performed during the remaining time.

D. *Further investigations*

A study performed by Stuart., [21] explains how the AD process is mostly subjected to pH sensitivity due to the presence of methanogen bacteria. It was explained that the sudden increase of acidogens in a digester caused by a pH decrease would cause a slow growth of methogenes which will consequently affect the performance of acidogens and acetogens. It was additionally stipulated that heavy metals (trace elements) contained in the initial feedstocks could not be removed by digestion [21], [22]. This makes AD a sensitive and limited process as well in terms of trace elements control.

As per the investigation conducted by Njuguna *et al*., [1] the impact of copper, silver, nickel, cadmium, and zinc found in substrates could always hinder the production of biogas with their inhibitory effects on biochemical reactions.

Very high methane yields were obtained in a co-digestion of cow manure with grass silage, sugar beet tops and oat straw during an investigation performed by Lehtoma ki *et al*., [23] who reported a final volumetric increase in methane produced. It was concluded that the amount of methane generated in the digester was affected by the volume of the feedstock added, which caused a portion of methane produced to remain in the digester during extraction [23].

In a co-digestion of whey with chicken manure, Gelegenis *et al*., [24] noticed the instability of the co-digestion process caused by the insufficiency of Chemical Oxygen Demand (COD) of whey in respect to manure. This instability led to a decrease of pH and a change in C/N ratio which lowered the biogas yield.

IV. CONCLUSION

This study aimed at evaluating the impact of substrates in the production of biomethane from raw biogas by comparing patterns of different substrates. Two different co-digestion processes were conducted. The first process was based on cow dung, pig manure and grass clippings while the second process was based on FW which was a mixture of fruits and vegetables. Despite the fact that FW possessed the highest energy potential, the second co-digestion with FW decreasingly produced biomethane unlike the first co-digestion. Under similar conditions, the curve of the second co-digestion showed poor performance during the entire process. From literature, it was understood that the toxicity of elements found in these substrates was higher than those of the first co-digestion. The toxicity found in trace elements contained in the FW was not regulated. This investigation revealed how important waste characterisation, proximate and ultimate analysis are. It emphasized on the determination of substrate composition before AD. The production of biogas was dependent of important factors such as C/N ratio, pH, and operating temperatures; however the substrates composition had to be primordially observed.

V. OTHER RECOMMENDATIONS

It is highly recommended that further studies be conducted on trace elements such as copper, silver, nickel, cadmium, and zinc contained in FW in order to prevent inhibitory effects they might have on AD. We also observed that a good Inoculum/Substrate ratio and a suitable C/N ratio should be used in order to avoid hindering factors such as the augmentation of Volatile Fatty Acids (VFA) during co-digestion of FW.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Process Energy, Environmental and Technology Station (PEETS) funded by the South African National Energy Development Institute (SANEDI) and the Technology Innovation Agency (TIA); Applied Chemistry and Metallurgy Departments at the University of Johannesburg for allowing us to work in their laboratories.

REFERENCES

[1] Anthony Njuguna Matheri, Mohamed Belaid, Tumisang Seodigeng and Catherine Jane Ngila, **“***Role and Impact of Trace Elements on the Anaerobic Co-digestion in Biogas Production”*. 24th World Congress on Engineering (WCE 2016) London-UK, 2016 .

[2] Wikipedia, 2016. Biogas. [Online] Available at: https://en.wikipedia.org/?title=Biogas [Accessed on the 19 May 2016].

[3] Tippayawong, N. and Thanompongchart, P., 2010. Biogas quality upgrade by simultaneous removal of CO 2 and H 2 S in a packed column reactor. *Energy*,*35*(12), pp.4531-4535.

[4] Co-digestion: A Developing Concept and Market. 2009. *Co-digestion: A Developing Concept and Market*. [ONLINE] Available at: http://www.ati-ae.com/resources/techtalk/176-codigestion.html. [Accessed on the 22 May 2016].

[5] Braun. R. 2002. Potential of Co-digestion. [ONLINE] Available at:http://www.novaenergie.ch/iea-bioenergytask37/Dokumente/final. PDF. [Accessed on the 22 May 2016].

[6] Biomethane production potential from restaurant food waste in megacities and project level-bottlenecks: A case study in Beijing by Djavan De Clercq, Zongguo Wen n , Fei Fan, Luis Caicedo State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China.

[7] Lallawmsanga et al., 2012. Ameliorating effect vermicompost and cow dung compost on growth and biochemical characteristics of Solanum melongena L. treated with piant industrial effuent. 3(5), pp.2268-74.

[8] Chen Y, Cheng JJ,Creamer KS, Inhibitory anaerobic digestion process: a review,Bioresource. Technol 2008; 99: 4044-64.

[9] Dioha, I. J., et al. "Effect of carbon to nitrogen ratio on biogas production." *International Research Journal of Natural Sciences* 1 (2013): 1-10.

[10] Persson, Margareta, Owe Jönsson, and Arthur Wellinger. "Biogas upgrading to vehicle fuel standards and grid injection." *IEA Bioenergy task*. Vol. 37. 2006.

[11] Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z. and Yu, X., 2015. Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renewable and Sustainable Energy Reviews*, *51*, pp.521-532.

[12] Mengistu, M.G., Simane, B., Eshete, G. and Workneh, T.S., 2015. A review on biogas technology and its contributions to sustainable rural livelihood in Ethiopia. *Renewable and Sustainable Energy Reviews*, *48*, pp.306-316.

[13] Biogas production rate of different feedstocks and retention time in anaerobic digestion. 2016. [ONLINE] Available at: https://www.researchgate.net/figure/261374115_fig4_Fig-4-Biogas-production-rate-of-different-feedstocks-and-retention-time-in-anaerobic. [Accessed on the 20 May 2016].

[14] Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Bio/Technology*, 3, 117-129.

[15] T Abbasi, S.M. Tauseef, S.A. Abbasi, Biogas Energy, 2012.

[16] A.P.H.A and F.W. Environmental., *Standard methods for the examination of water and wastewater*. American Public HealthAssociation (APHA): Washington, DC, USA, 2005.

[17] Haider, M.R., Yousaf, S., Malik, R.N. and Visvanathan, C., 2015. Effect of mixing ratio of food waste and rice husk co-digestion and substrate to inoculum ratio on biogas production. *Bioresource technology*, *190*, pp.451-457.

[18] Kleinheinz, G. and Hernandez, J., 2016. Comparison of two laboratory methods for the determination of biomethane potential of organic feedstocks.*Journal of Microbiological Methods*, *130*, pp.54-60.

[19] Matin, S.S. and Chelgani, S.C., 2016. Estimation of coal gross calorific value based on various analyses by random forest method. *Fuel*, *177*, pp.274-278.

[20] Dioha, I.J., Ikeme, C.H., Nafi'u, T., Soba, N.I. and Yusuf, M.B.S., 2013. Effect of carbon to nitrogen ratio on biogas production. *International Research Journal of Natural Sciences*, *1*, pp.1-10.

[21] Stuart, P., 2006. The Advantages And Disadvantages Of Anaerobic Digestion As A Renewable Energy Source. Loughborough University, Loughborough.

[22] Hosseini, S.E. and Wahid, M.A., 2014. Development of biogas combustion in combined heat and power generation. *Renewable and Sustainable Energy Reviews*, *40*, pp.868-875.

[23] Lehtom¨aki, A.; S. Huttunen, J.A. Rintala. 2007. Laboratory investigations on codigestion of energy crops and crop residues with cow manure for methane production:Effect of crop to manure ratio. *Resources, Conservation and Recycling.* **51**: 591–609.

[24] Gelegenis, J.; D. Georgakakis; I. Angelidaki; and V. Mavris. 2007. Optimization of biogas production by co-digesting whey with diluted poultry manure. Renewable Energy 32:2147- 2160.