

ASSESSMENT OF ANAEROBIC CO-DIGESTION EFFECTS OF MAIZE COB AND POULTRY MANURE ON BIOGAS YIELDS AND THEIR DIGESTATE CHARACTERISTICS

ABSTRACT

Aim: The aim of the study is to assess the effects of anaerobic co-digestion of maize cob and poultry manure on biogas yields and their digestates characteristic.

Place and Duration of Study: Department of Forestry Technology, Federal College of Forestry, Jos between March and April, 2018.

Methodology: Slurries of five co-substrate treatment ratios viz 0:1(T₁), 1:3(T₂), 1:1(T₃), 3:1(T₄) and 1:0(T₅) of these wastes (in three replicates) were separately fed to 13.6L locally made batch-digesters. The anaerobic reactors were monitored for a 56 day retention period. Weekly biogas yields and some digestate characteristics were measured by standard methods.

Results: The cumulative biogas yields was in the order of T₃(2481.3ml/kg) > T₁(2197.9 ml/kg) > T₄(2163.0 ml/kg) > T₂(2116.3 ml/kg) > T₅(1713.2 ml/kg), in favour of the mixed substrates. While the percentage carbon : nitrogen reductions ranged from (12.94% - 81.80%), with T₅ and T₁ recording the highest and lowest values respectively. The chemical oxygen demand removal was in the order of T₃(80.70%) > T₄(58.00%) > T₅(46.81%) > T₁(34.15%) > T₂(13.16%). The anaerobic digestion (AD) effected reductions in Mg, C, Ca, P, Mn, Zn, Fe, Pb and increase in Cu contents of the digestates across treatments. While the K contents increased in T₂(36.72%), T₃(229.79%) and T₄(220.51%); %N in T₃(9.94%), T₄(113.19%) and T₅(291.84%) and Na increased only in T₄(4.55%). The Cu contents indicated % increase in the order of T₅(487.5%) > T₃(270.97%) > T₂(268.10%) > T₄(43.66%) > T₁(35.82%).

Conclusion: The anaerobic co-digestion of these organic wastes had unlocked the alternative energy potentials, enhanced the bioremediation tendency, while promoting sustainable public health and environmental management.

Keywords: Biogas, Co-Digestion, Digestates, Maize Cob, Poultry manure

INTRODUCTION

Fossil fuels are non-environmentally friendly and unsustainable energy. The development of renewable bioenergy is an alternative solution to meet the electricity and heat requirements of the country with a cost-efficient and beneficial for the environment technology [1].

In the light of the European Landfill Directive (1999/31/EC) states that by 2016, the disposal of biodegradable municipal waste should be reduced by 75%, the biological treatment of agricultural and bio-industrial wastes via the deployment of anaerobic digestion (AD) technology to treat and degrade organic wastes becomes imperative [1]. The technology not only produces clean renewable energy (biogas) suitable for heat and electricity production, it also generates a nutrient-rich digestate, used as bio-fertilizer [2]. The process of AD is valued for improved efficiency of the agricultural wastes management systems which is helpful in decreasing the emission rate of the greenhouse gases

Other Benefits of the AD of animal manure include pathogen reduction under mesophilic or thermophilic conditions, odor and pests reduction [3]. The process also provide a bioremediating effects of heavy metals content of the digestates. Despite the efforts over the years, full exploitation of the huge organic wastes for bio-methanogenesis in Nigeria is still at its infancy [4]. This work focuses on the assessment of effects of anaerobic co-digestion of maize cobs and poultry manure on biogas yields and its implication on their digestates characteristics.

MATERIALS AND METHODS

Substrate preparation

The bio-wastes sourced from the agricultural farms and research units of Federal College Forestry, Jos, Nigeria, were pretreated by drying, screened and pulverized, before purposely mixing in five selected ratios (w/w), parked in sterile black polythene bags and stored below 20°C until use [5]. The co-substrate mixtures of the wastes were described as follow:-

T₁ = 0:1 :- 0.0g maize cob + 1000.0g poultry manure

T₂ = 1:3 :- 250.0g maize cob + 7500.0g poultry manure

T₃ = 1:1 :- 500.0g maize cob + 500.0g poultry manure

T₄ = 3:1 :- 750.0g maize cob + 250.0g poultry manure

T₅ = 1:0 :- 1000.0g maize cob + 0.0g poultry manure

(where T₁, T₂, T₃, T₄ and T₅ represented treatments 1,2,3,4 and 5 respectively)

Anaerobic Digestion Trial

Each of these co-substrates was mixed with 3000ml of distilled water in a 1:3 ratio (w/v). Three replicates of the resulting slurries were separately fed to the 13.6L digesters, with fittings of thermometer and gas delivery pipe, and firmly sealed to achieve anaerobic condition. The 15 reactor units were arranged in an experimental chamber, using a completely randomized design (CRD), maintained under uniform temperature. The digesters were manually jolted for one minute daily at a scheduled time, to achieve homogeneity. Weekly biogas yield (dm³/kg) was measured by downward displacement of water the gas [6], throughout the 8 weeks of digestion [7].

Analytical Methods

Separate fractions of the co-substrates before and after anaerobic digestion were subjected to Standard methods to determine the substrates biochemical characteristics. The chemical oxygen demand (COD) was determined, using Spectrophotometer DR 2800 [8]. Total nitrogen was determined by Kjeldahl method. The nitrogen content in the sample was calculated using the formula given below.

$$\% \text{ nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times c}{d \times e}$$

Where:- a = titre value for digested sample; b = Titre value for the blank; c = Volume to which the digest was made up with distilled water; d= Aliquot distilled; e = Weight of dried sample.

The total organic carbon (TOC) was determined according to the standard procedure of [9]. The method of [8] was adopted to determine % Phosphorous content, using the vanadate-molybdate reagent, at a wavelength of 470 nm, using the Atomic Absorption Spectrophotometer (AAS)(CTA-2000 AAS Chemtech Analytical). The P content was calculated using the following formula:-

$$P \frac{(mg)}{Kg \text{ sample}} = \frac{(GR \times TCV \times EV)}{AV \times W}$$

Where: - GR = Graph reading; Tcv = Total coloured volume; Ev = Extract volume.

A_v = Aliquot volume taken.; W = Sample weight in gram.

The Potassium, Calcium, Sodium and Magnesium Contents were determined by standard method [8], using the flame photometer, while Mg was determined using the atomic absorption spectrophotometer (AAS), based on the formula below:-

$$(K, Ca, Na, Mg) \frac{(K, Ca, Na, Mg)}{Kg \text{ sample}} = \frac{GR \times EV \times MCF}{39.1 \times 10 \times W}$$

Where: - GR= graph reading (mg/l); mcf = moisture correction factor; Ev= Extract volume (ml); Av = Aliquot volume taken (ml); W = Sample weight (g); 39.1 = Equivalent weight of potassium; 10 = Conversion factor from ppm to cmol (+)/Kg sample

The Iron, Copper, Zinc, Manganese and Lead Contents were determined by the [10] method, adopted by [11], using atomic absorption spectrophotometer (AAS).

RESULTS

Effects of Anaerobic Digestion of Samples on Biogas Yields, Chemical Oxygen Demand (COD) and Carbon-Nitrogen Ratio

There was a general increase in average biogas yield within the first six weeks of digestion (WOD), followed by a sharp decrease at the 7th and 8th week. T₅ (1:0:- maize cob + poultry manure) had the lowest biogas yields of 43.3±7.6,

78.3±6.5, 134.3±12.1, 348.7±20.8 and 303.3±6.1 at 1,2,3,6, and 7 WOD respectively. The cumulative biogas yields was in the order of T₃(2481.3ml/kg) >T₁(2197.9 ml/kg) > T₄(2163.0 ml/kg) > T₂(2116.3 ml/kg) >T₅(1713.2 ml/kg) (Table 1). Analysis of variance (ANOVA) on weekly data indicated significant difference ($P < 0.05$) in average volume of biogas produced throughout the period of digestion due to substrate type and mixing ratio.

The anaerobic digestion has effected considerable reductions in chemical oxygen demand (COD(mg/l)) contents of substrates, with the co-substrate having higher percentage reduction, in the order of T₃(80.70%) > T₄(58.00%) >T₅(46.81%) >T₁(34.15%) >T₂(13.16%). Similarly, there were percentage reductions in C:N ratios across the treatments. However, the %reduction was in the order of T₅(81.80%) > T₄(68.02%) >T₃(54.42%) >T₂(54.23%) >T₁(12.94%). (Table 2).

The biogas yields was affected by the ratios of mixing of the co-substrates. The yields followed the order: 1:1 >0:1 >3:1 >1:3 >1:0. This revealed mixed substrates with higher maize cob (C-content) and or low poultry manure (N-rich) gave higher % reduction of C/N ratio.

Effects of Anaerobic Digestion on Mineral Element and Heavy Metal Compositions of Digestates

There were variations in the mineral and heavy metal composition of the digestates due to anaerobic digestion (AD). Before AD, the contents of Mg ranged from 793.00 to 2002.20mg/kg, OC(37.03-52.99%), Na(0.08-0.26%), Ca(450.50-16234.00mg/kg), P(1608.75-15843.75mg/kg), but reduced to 0.39-1.17mg/kg; 17.52-37.78%; 0.06-0.14%; 0.07-3.96 mg/kg and 0.096-0.982mg/kg respectively. The contents of K increased in T₂(36.72%), T₃(229.79%) and T₄(220.51%); %N in T₃(9.94%), T₄(113.19%) and T₅(291.84%). Na increased only in T₄(4.55%)(Figure 3). After AD, all treatments had % reductions in heavy metals (Mn, Zn, Fe and Pb), except Cu, which indicated % increase in the order of T₅(487.5%) > T₃(270.97%) > T₂(268.10%) > T₄(43.66%) > T₁(35.82%). (Figure 4).

Table 1: Mean biogas Production (ml/wk) During Eight Weeks of Anaerobic Digestion

Treatments/Weeks	1	2	3	4	5	6	7	8	Total
T₁	93.3±4.2 ^d	150.7±19.0 ^c	262.7±16.6 ^d	316.3±15.0 ^d	382.3±12.5 ^c	423.3±14.0 ^b	385.0±7.0 ^c	184.3±12.1 ^b	2197.9
T₂	60.0±9.2 ^b	108.0±15.1 ^b	193.3±14.0 ^b	262.3±11.2 ^a	310.0±5.0 ^a	464.0±20.3 ^d	382.7±5.0 ^c	336.0±8.5 ^d	2116.3
T₃	63.0±4.2 ^c	113.0±8.2 ^b	240.0±12.0 ^c	309.7±4.0 ^d	462.3±12.5 ^d	512.0±5.3 ^e	418.0±5.3 ^d	363.3±13.3 ^e	2481.3
T₄	62.0±15.1 ^c	102.3±12.5 ^b	190.0±13.1 ^b	295.0±11.8 ^c	398.0±5.3 ^c	442.7±5.0 ^c	366.7±6.1 ^b	306.3±8.5 ^c	2163.0
T₅	43.3±7.6 ^a	78.3±6.5 ^a	134.3±12.1 ^a	287.3±15.7 ^b	321.3±8.1 ^b	348.7±20.8 ^a	303.3±6.1 ^a	196.7±16.7 ^a	1713.2
Σ	321.60	552.30	1020.30	1470.60	1873.90	2190.70	1855.70	1386.60	10671.70

Means along each column bearing different superscripts are significantly different ($P < 0.05$) at 5% level by Duncan's New Multiple Range Test; T₁ = 0:1 :- 0.0g maize cob + 1000.0g poultry manure; T₂ = 1:3 :- 250.0g maize cob + 7500.0g poultry manure; T₃ = 1:1 :- 500.0g maize cob + 500.0g poultry manure; T₄ = 3:1 :- 750.0g maize cob + 250.0g poultry manure; T₅ = 1:0 :- 1000.0g maize cob + 0.0g poultry manure (where T₁, T₂, T₃, T₄ and T₅ represented treatments 1,2,3,4 and 5 respectively)

Table 2: Chemical Oxygen Demand (COD) (x10³ mg/l) and Carbon-Nitrogen (C/N) ratio of Substrates Before and After Anaerobic Digestion

Tmt	COD _{BAD}	COD _{AAD}	CODR(%)	C/N _{BAD}	C/N _{AAD}	%C/N _{Red}
T₁	41	27	34.15	14.30	12.45	12.94
T₂	38	33	13.16	19.73	9.03	54.42
T₃	57	11	80.70	23.52	10.72	54.42
T₄	50	21	58.00	43.49	13.91	68.02
T₅	47	25	46.81	108.14	19.68	81.80

T₁ (0:1:- 0.0g maize cob + 1000.0g poultry manure); T₂ (1:3:- 250.0g maize cob + 7500.0g poultry manure); T₃ (1:1:- 500.0g maize cob + 500.0g poultry manure); T₄ (3:1:- 750.0g maize cob + 250.0g poultry manure); T₅ (1:0 :- 1000.0g maize cob + 0.0g poultry manure) (where T₁, T₂, T₃, T₄ and T₅ represented treatments 1,2,3,4 and 5 respectively)

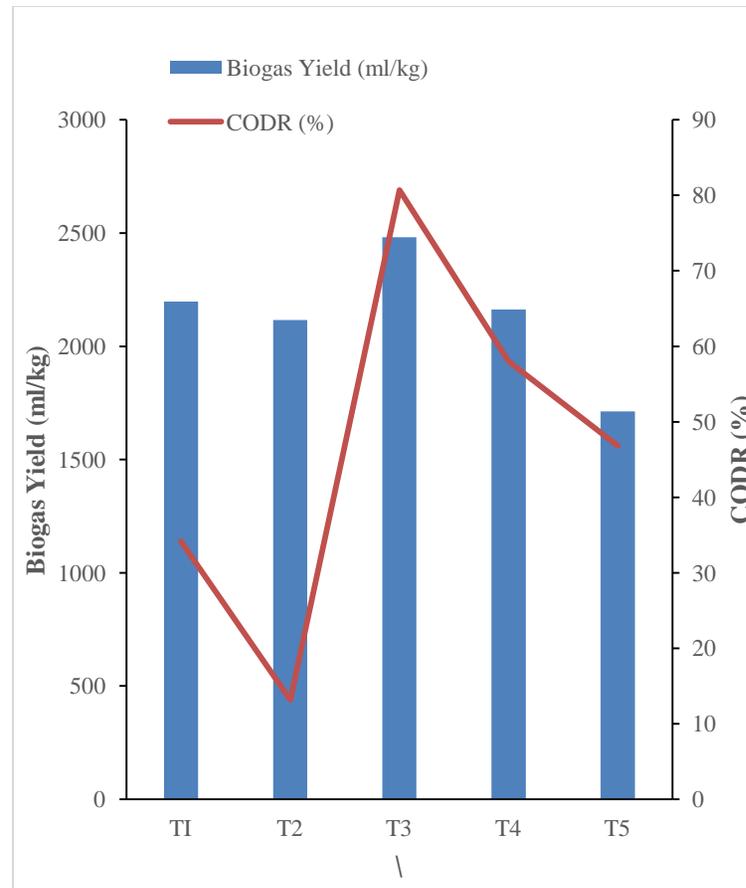


Figure 1: Effects of Anaerobic Digestion on Biogas yields and COD_{reduction}

T₁ (0:1:- 0.0g maize cob + 1000.0g poultry manure); T₂ (1:3:- 250.0g maize cob + 7500.0g poultry manure); T₃(1:1:- 500.0g maize cob + 500.0g poultry manure); T₄(3:1:- 750.0g maize cob + 250.0g poultry manure); T₅ (1:0 :- 1000.0g maize cob + 0.0g poultry manure) (where T₁,T₂, T₃, T₄ and T₅ represented treatments 1,2,3,4 and 5 respectively)

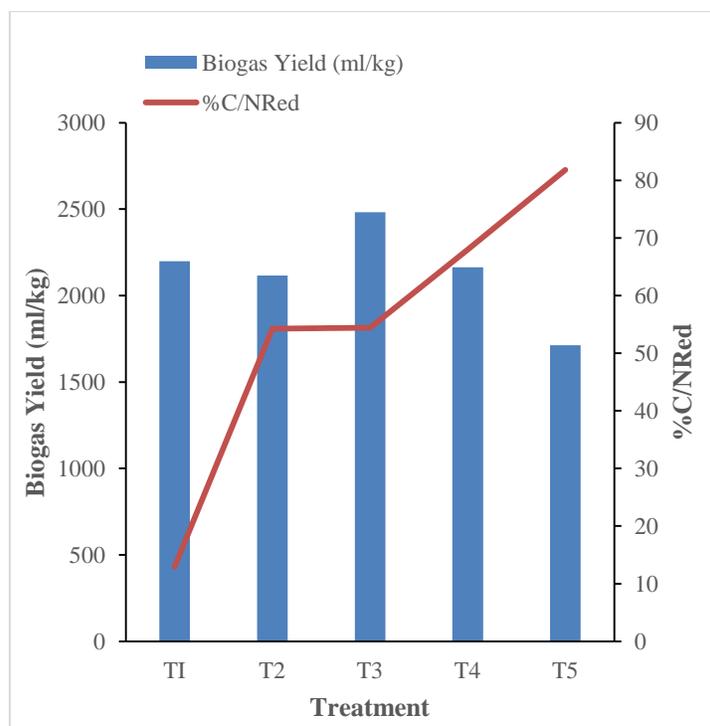


Figure 2: Effects of Anaerobic digestion on Biogas and Carbon-Nitrogen_{Reduction}

T₁ (0:1:- 0.0g maize cob + 1000.0g poultry manure); T₂ (1:3:- 250.0g maize cob + 7500.0g poultry manure); T₃(1:1:- 500.0g maize cob + 500.0g poultry manure); T₄(3:1:- 750.0g maize cob + 250.0g poultry manure); T₅ (1:0 :- 1000.0g maize cob + 0.0g poultry manure) (where T₁,T₂, T₃, T₄ and T₅ represented treatments 1,2,3,4 and 5 respectively)

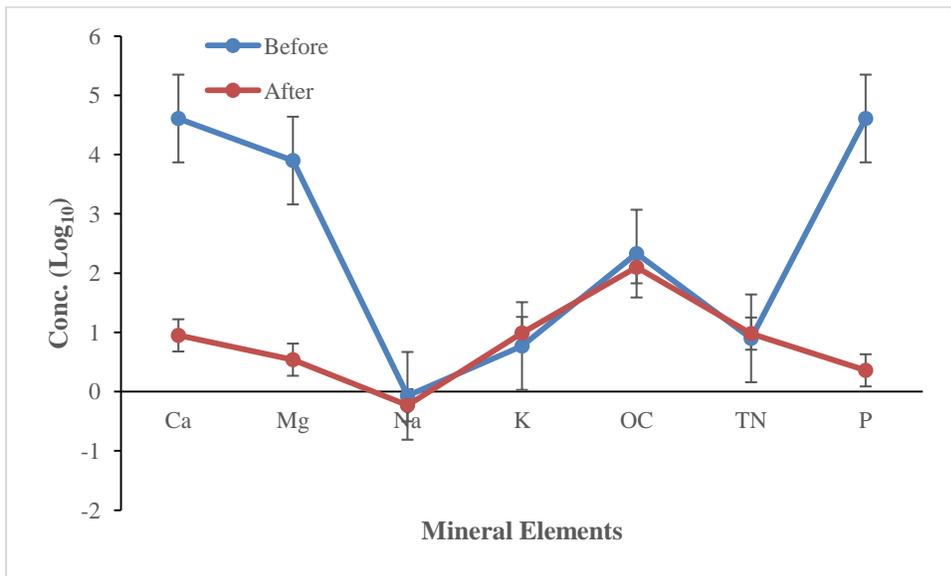


Figure 3 : Mineral Element Composition off Substrates Before and after Anaerobic Digestion

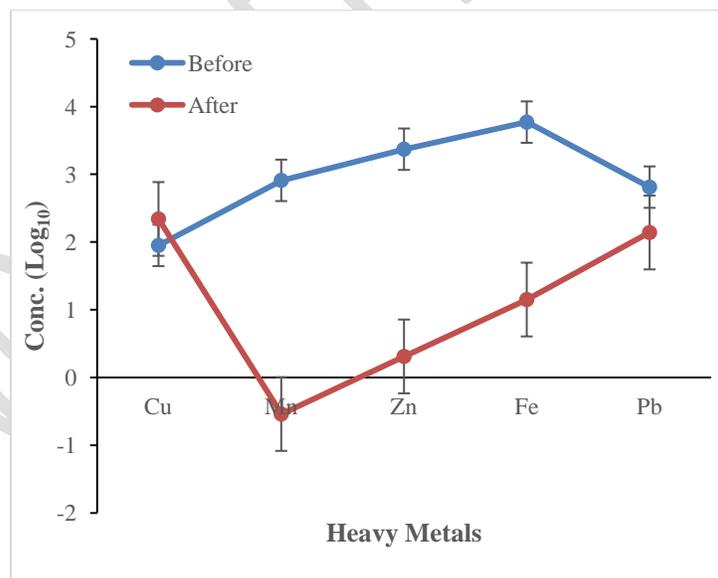


Figure 4 : Heavy Metal Composition of Substrates Before and after Anaerobic Digestion

DISCUSSION

The pattern of biogas yields corroborated findings of [12], who inclined the increase to the presence of biodegradable organic matter and high load of microbial communities of the slurries. The reduction in gas volume after an initial sharp increase, strengthen the opinions of [13], to lack or reduction of soluble biodegradable fraction of the substrates, accumulation of volatile fatty acids (VFAs) and a low pH. Before digestion, all substrates had higher values of % chemical oxygen demand %COD, which became reduced after the process. [14], reported close relationships between biogas yield and COD removal, while [15], maintained that biogas yields proportionately increased with COD removal as observed in this study (Figure 1), suggesting that the methanogenic consortium acclimated very well and consequently leads to the digestion of organic matter (COD) and volatile solid (VS).

The highest cumulative biogas yield recorded by treatment T₃ (1:1:- maize cob:poultry manure) after 8 week of digestion was in line with the position of [16], indicating that co-digestate of ratio 1:1 of cattle manure blended with some plant residues gave an optimal yields. The yield was significantly ($P < 0.05$) influenced by co-digestion as well as substrate ratios. This result is similar to those of [17], accounting that substrate ratio 1:1 of livestock wastes blended with cassava peels gave significant increase average biogas yield. They maintained that substrates with very high C/N ratio, produced very low biogas (Figure 2). However, when co-digested with materials of lower C/N ratio, enhanced methanogenesis was observed, due to stabilized ratio at optimal range between 22 and 30 [18]. Co-digestion has been thought to enhance buffering capacity, microbial diversity, positive synergisms, more balanced and complementary nutrients supply [12]. Blending phyto-biomass with livestock wastes was found to lowers the C/N ratio of the blend, enhance digestibility, due to high microbial community. Mixing ratio affects yields as higher mixing ratios meant higher C/N as well as lignin content which could hinder microbial activities and methanogenesis [17].

Extremely high C:N ratio implied increased acidification and retarded methanogenesis [19], consequently, the yield pattern of T₅ with 81.80% C/N_{Reduction}. The C/N ratio obtained for the substrates before digestion were in line with [19], stressing that an excessively high C:N ratio would increase acidity of the medium which retards methanogenesis. When the C:N ratio is too low, nitrogen is converted to ammonium-N at a faster rate than it can be assimilated by the methanogens, leading to NH₃ poisoning. Co-digestion provides supplementary and complementary nutrient requirements which trigger increase in digestion performance and methane yield, [20]. This is because animal manure fraction of co-substrate provides high buffer capacity which mainly contains wide variety of nutrients necessary for optimal bacterial growth [21]. It also promotes synergistic effects, which overcomes the imbalance in nutrients resulting in higher mass conversion and lower weight and volume of digested waste thereby improving biodegradability.

Based on their findings [22], reported a higher values of C, N, K, P, Zn, Cu, Mn, Na, and Pb in undigested poultry manure, which corroborated the current findings except for higher values for Cu.[23], opined that besides C, H, O needs, N, S, P, Ca, Mg and a number of trace elements required for methanogenesis are predominantly found in most organic wastes. The reduction in Ca, Mg, Fe, Zn, Mn and Pb contents after anaerobic digestion(AD) was viewed as their utilization by degradating microbes to power the process[19], the extent of which determined their residual in the digestates. [24], claimed that Mg²⁺ enhances bio-remediating tendency of certain methanogenic strains by reducing K⁺ toxicity during anaerobic digestion. It shows synergistic effects, when combined with Ca and Na at certain levels, helping the anaerobic process to recover from K inhibition [25]. Trace level of heavy metals during anaerobic biodegradation of organic matter is essential for the proper enzyme functioning, which could be inhibitory at high concentrations [26]. Heavy metals are only toxic to anaerobic bacteria in their soluble form. Microorganisms exposed to heavy metals consequently activate a wide variety of intracellular detoxification defense strategies. The reduction of heavy metals such as Fe, Zn, Pb, Mn but Cu assayed, in the present study revealed the bio-remediating tendency of the process. [27], attributed the reduction in concentration of Ca, Mg, Fe, Zn, Mn and Pb after digestion to the bio-remediating tendencies of microbial consortium present in the substrates. This involves mechanisms of metal binding to microbial biomass in the form of intracellular accumulation, sorption or complex formation on cell surface and extracellular accumulation or precipitation [28]. Manganese is required by microbes for the formation of manganese peroxidase, an enzyme which aids in the Lignin and lingo-cellulosic degradation [29]. The variation in contents of Na, K, Ca, Mg, and increase in N corroborated the findings of [30], pointing out that the buffering properties of the co-substrates favour the degrading microbes.

Microbial community under co-digestion could experience selective inhibition by heavy metal due to different tolerant levels leading to microbial community structure and functional stratification [31]. Thus, disrupt some microbial

pathways, making them more sensitive to some metals than others, resulting in selective inhibition and decline in numbers and diversity of microbes relying on those pathways [32]. [33], related heavy metal removal to reductions in the COD removal with increasing metal concentrations. Also, [34] reported Cu toxicity on COD removal which recorded much higher levels in the absence of Cu ions for all hydraulic residence time levels (HRTs) tested. [35], reported factors such as pH, metal concentrations before treatment, quantity biomass, temperature, retention time, presence of other ions could affect the reduction of heavy metal in digestive medium.

CONCLUSION AND RECOMMENDATIONS

The biodegradative capacity of maize cob and poultry manure mixtures to produce biogas at five ratios was assessed. Co-substrates especially ratio 1:1(T₃) yielded 2481.3ml/kg as the highest biogas, while 1:0(T₅) had the least (1713.2 ml/kg). The C/N ratio and COD removals proportionately affected gas yield. Higher volumes of biogas are produced at relatively higher C/N ratio higher COD removal. The anaerobic digestion of these organic wastes has enhanced the heavy metal reduction, thus elucidating the bioremediating tendency. However, further studies involving other agricultural and industrial organic wastes should be undertaken under varying controlled conditions for process optimization.

REFERENCES

- [1] Franke-Whittle, I. H., Confalonieri, A., Insam, H., Schlegelmilch, M. and Körner, I. (2014). Changes in the microbial communities during co-composting of digestates. *Waste Management* 34, 632–641.
- [2] Körner, I., Amon, B., Bade, O., Balsari, P., Bioteau, T., Dabert, P., Dach, J., Deipser, A., Kupper, T., Ferreira, L.J.M., Moller, H., Mantovi, P., Schnüre, A., Soldano, M., Ward, A., Fabbri, C., (2010). Anaerobic digestion and digestate utilization in Europe. In: Marques dos Santos Cordovil, C.S.C., Ferreira, L., (Eds.), RAMIRAN2010 – Treatment and use of organic residues in agriculture-challenges and opportunities towards sustainable management, Proceedings of the 14th International Conference, ISA-Press, Lissabon, Portugal, Art. 0177.
- [3] Babae, A., Shayegan, J. & Roshani, A. (2013). Anaerobic slurry co-digestion of poultry manure and straw: effect of organic loading and temperature. *Journal of Environmental Health Sciences & Engineering*. 11:15.
- [4] Gupta, P.R.S. Singh, A. Sachan, A.S. Vidyarthi & A. Gupta (2012): A re'appriassal on Intensification of Biogas Production. *Renewable and Sustainable Energy Review*. 16: 4908-4916.
- [5] Chomini, M.S. (2017). Comparative Studies on Biogas Production From Some Selected Indigenous Substrates and The Effects of Their End-Products on Growth and Performance of *Zea mays L.*(Maize). (Ph.D Thesis), University of Jos. p. 292.
- [6] Salam, B., Biswas, S. and Rabbi, M S. (2015). Biogas from Mesophilic Anaerobic Digestion of Cow Dung Using Silica Gel as Catalyst. 6th BSME International Conference on Thermal Engineering (ICTE 2014). *Procedia Engineering*, 105, 652 – 657.
- [7] Chomini, M. S. Ogbonna, C.I.C., Falemara, B.C. & Micah, P. (2015). Effect of Co-Digestion of Cow Dung and Poultry Manure on Biogas Yield, Proximate and Amino Acid Contents of Their Effluents e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 11 Ver. I (Nov. 2015), PP 48-56 www.iosrjournals.org
- [8] APHA, (2005). Standard Method for Examination of Water and Wastewater. American Public Health Association, Washington, D. C. 1368p.
- [9] AOAC (2005). Official Methods of Analysis, 18th ed., Association of Official Analytical Chemist, Washington, DC, 1168p.
- [10] AOAC. (1990). Official Methods of Analysis. Association of Official Analytical Chemists, 15th ed Washington DC. USA. pp. 123-126.
- [11] Hamed, T. B., Soyngbe, A. A. & Adewole, D. O. (2011). An Abattoir waste water management through composting: A case study of alesinloye waste recycling complex. *The International Journal of Interdisciplinary Social Sciences*, 6(2) : 67-78.

- [12] Li, J., Jha, A. K., He, J., Ban, Q., Chang, S. & Wang, P. (2011). Assessment of the effects of dry anaerobic codigestion of cow dung with waste water sludge on biogas yield and biodegradability. *International Journal of the Physical Sciences*, 6(15): 3723-3732.
- [13] Xie, S., Lawlor, P.G., Frost, J.P., Hud, Z. & Zhan, X. (2011). Effects of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bio resource Technology*, 102: 5728–5733.
- [14] Jha, A.K., He, J., Li, J. & Zheng G. (2010): Effect of substrate concentration on methane fermentation of cattle dung. In: Proceedings of International conference on challenges in environmental Science and computer engineering. Wuhan, P. R. China. March 6-7. Part, 1: 512-515.
- [15] El-Mashad, H. M. & Zhang, R. (2010). Biogas production from co-digestion of dairy manure and food waste. *Bioresource Technology*, 101, 4021–4028.
- [16] Lehtomaki, A., Huttunen, S. & Rintala, J.A. (2007). Laboratory investigations on co- digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resource Conservation and Recycling*, 51 (3): 591-609.
- [17] Adelekan, B. A. & Bamgboye, A. I. (2009). Comparison of biogas productivity of cassava peels mixed in selected ratios with major livestock waste types. *African Journal of Agricultural Research*, 4 (7), 571-577.
- [18] Karki, B.A., Gautam, K.M. & Karki, A. (1994). Biogas Installation from Elephant Dung at Machan Wildlife Resort, Chitwan, Nepal. *Biogas Newsletter*, Issue No. 45. pp 26-27.
- [19] Ghasimi, S. M. D., Idris, A., Chuah, T. G. & Tey, B. T. (2009). The Effect of C:N:P ratio, volatile fatty acids and Na⁺ levels on the performance of an anaerobic treatment of fresh leachate from municipal solid waste transfer station. *African Journal of Biotechnology*, 8 (18), 4572-4581.
- [20] Kacprzak, A., Krzystek, L. & Ledakowicz, S. (2010). Co-digestion of agricultural and industrial wastes. *Chemical Paper*, 64, 127-131.
- [21] Macias-Corral, M., Samani, Z., Hanson, A., Smith, G., Funk, P., Yu, H. & Longworth J. (2008). Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. *Bioresource Technology*, 99, 8288–8293.
- [22] Adelekan, B. A., Oluwatoyinbo, F. I. & Bamgboye, A. I. (2010). Comparative effects of undigested and anaerobically digested poultry manure on the growth and yield of maize (*Zea mays*, L). *African Journal of Environmental Science and Technology*, 4 (2), 100-107.
- [23] Ofori, M. A. (2009). Anaerobic Digestion of Shea Waste For Energy Generation. (Ph.D Thesis) University of Cape Coast. pp 1-179.
- [24] Bashir, B. H. & Matin, A. (2004). Sodium toxicity control by the use of magnesium in an anaerobic reactor. *Journal of Applied Sciences and Environmental Management*, 8 (1), 17– 21.
- [25] Chen, Y, Cheng, J. J. & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. *Bioresources Technology*, 99, 4044–4064.
- [26] Lebiocka, M., Montusiewicz, A. and Depta, M. (2016). Co-digestion of Sewage Sludge and Organic Fraction of Municipal Solid Waste in the Aspect of Heavy Metals Content. Middle Pomeranian Scientific Society of the Environment Protection Annual Set the Environment Protection. 18. 555-566.
- [27] Bhatnagar, S. & Kumari, R. (2013). Bioremediation: A sustainable tool for environmental management – A Review. *Annual Review and Research in Biology*, 3(4), 974-993.
- [28] Bishnoi, N. & Garima, R. (2005). Fungus: An alternative for bioremediation of heavy metal containing wastewater: a review. *Journal of Science Indigenous Research*, 64, 93-100.
- [29] Isroi, M. R., Syamsiah, S., Niklasson, C., Cahyanto, M. N., Lundquist, K. & Taherzadeh, M. J. (2011). Biological Pretreatment of Lignocelluloses with White-Rot Fungi and Its Applications: A Review. *Bioresources*, 6(4), 5224-5259.
- [30] Baharuddin, A. S., Hock, L. S., Yusof, M. Z. M., Rahman, N. A. A., Shah, U. K. M., Hassan, M. A. & Shirai, Y. (2010). Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m³ of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process. *African Journal of Biotechnology*, 9(16), 2427-2436.

- [31] Sobolev, D. & Begonia, M. F. T. (2008). Effects of Heavy Metal Contamination upon Soil Microbes: Lead-induced Changes in General and Denitrifying Microbial Communities as Evidenced by Molecular Markers. *International Journal of Environmental Research and Public Health*, 5 (5), 450-456.
- [32] Holtan-Hartwig, L., Bechmann, M., Høyås, T. R., Linjordet, R. & Bakken, L. R. (2002). Heavy metals tolerance of soil denitrifying communities: N₂O dynamics. *Soil Biology and Biochemistry*, 34, 1181-1190.
- [33] Gikas, P. (2007). Kinetic responses of activated sludge to individual and joint nickel (Ni (II)) and cobalt (Co (II)): an isobolographic approach. *J Hazard Mat* 143:246–256
- [34] Pamukoglu, Y. and Kargi, F. 2007. “Biosorption of Copper (II) Ions onto Powdered Waste Sludge in a Completely Mixed Fed-Batch Reactor: Estimation of Design Parameters”. *Bioresour. Technol*, 98: 1155-1162.
- [35] Congeevaram, S, Dhanarani, S, Park, J, Dexilin, M. & Thamaraiselvi, K. (2007) Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J Hazard Mat* 146:270–277.

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