DETERMINATIONS OF SOME TYPHA MINERAL COMPOSITION AND ORGANIC LOADING RATE FOR ANAEROBIC DIGESTIONS

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ABSTRACT

Choice of good substrate and organic loading rate in anaerobic digestion of biomass for biogas generation was among the critical factors needed to achieve a stable biogas system. This study determined some nutrient compositions require by anaerobic microorganism for biogas production from typha biomass and evaluate biogas production at different organic loading rates under laboratory condition. The nutrients compositions from typha plants were determined from standard laboratory procedures while the organic loading rate a single factor experiment was design in completely randomized design (CRD) at four level of organic loading rate: 0.2, 0.4, 0.6, and 0.8 g/100 ml and hydraulic retention time of 34 days. The experiments were repeated three times for four weeks. The results were analysed using statistical analysis Software (SAS). The digesters were feed after 4 - 5 days intervals and the system operated at mesophilic temperature (38 – 39 °C). The system has a total volume of 125 ml with a working volume of 105 ml. Volume of biogas produced from each unit of the digester was measured using a glass syringe while the methane was measured with the used of sodium hydroxide solution. The study established that the plant has a nitrogen content of 0.34 - 1.58%, while organic carbon had 52.42 - 55.78%. Calcium concentration found to be 0.69 -1.36 % and phosphorous, magnesium, potassium, and sodium obtained to be: 0.03 - 0.29, 0.07 - 0.23, 0.73 - 3.13 and 0.13 - 1.21%, respectively. Similarly, iron was within the range of 196 - 707 ppm while manganese 261 - 752 ppm. The zinc range was 7 - 26 ppm while copper 2 - 9 ppm. Neutral detergent fiber (NDF) varies from 59 - 75%. Acid detergent fiber (ADF) ranged 34 - 60%, while lignin concentrations range 12 - 21%. High carbon to nitrogen ratio obtained with the range from 34 – 162/1. Ash content was 5.05 - 10.74%. For the gas generation, 0.6 g produced the highest cumulative volume of gas while 0.4 g with the highest methane yields. However, 0.2 g has the highest efficacy with the gas yield of 366 ml/ g volatile solid (VS). It is advantageous to operate with 0.2 g due to higher volatile solid.

KEYWORDS: Typha; nutrients; composition; organic loading; anaerobic digestion;

1. INTRODUCTION

Some mineral components are essential for the growth and survival of anaerobic microorganisms because insufficient of these elements and their too high in the substrate may cause inhibition and disturbance of the biogas systems (Schnurer and Javis, 2009). Different organisms have different requirements for nutrients. Some organisms can form vitamins themselves, while other organisms need to absorb some vitamins from their environment. The substrate should supply these nutrients to microorganisms. However, the quality of substrate can also be assessed based on their chemical composition and energy content. Substrate characteristic determined whether the addition of some element is needed in a certain anaerobic digester or otherwise. Energy content in a particular biogas system depends not only on environmental factors but also on anaerobic digestions and biomass loading (Schnurer and Javis, 2009).

Loading in an anaerobic digester indicates how much new material is added to the process per unit time. It is usually referred to as organic loading or organic loading rate (OLR). Biological decompositions of organic matter occur continuously in a biogas process. If no new material is added, the process gradually stops. However, excessive loading causes process instability by accumulating of volatile fatty acid (VFA) which acts as an inhibitor and lowers biogas yield. It may also cause proliferation of acidogenesis, decrease pH, and mass death of methanogenic bacteria (Igoni *et al.,* 2007; Ray *et al.,* 2013).

Loading should be tailored to the active microbial flora in anaerobic digester. Each anaerobic digester (AD) has its specific loading rate and depends on types of feedstock and anaerobic condition. Angelidaki *et al.* (2006) established that loading in a new digester should start with low biomass and gradually increase as the microorganisms grow. The authors added that sometimes it might take several months before the desired load can be achieved. This attributed to the slow growth rate of microorganisms. Schnurer and Jarvis (2009) reported that methane microorganism required several days to grow; therefore, if a large amount of substrate is added at the start may lead to the process disruption and determine the right loading rate for a particular digester with a specific substrate is a key to stable anaerobic digestion of that digester.

Typha is among the fifth most invasive species in West Africa (Noba *et al.,* 2017). The grass is commonly called “cattail” and the family is represented worldwide by the genus Typha. The weed is a perennial aquatic herbaceous plant with a world-wide distribution in freshwater habitats. It is an erect perennial that can grow to two or three meters in height (Bender and Bender, 2018). The typha family has a higher growth rate than any other aquatic plant and the family is characterized by having rhizomes, extensive fleshy stems, and tall leaf blades (Yakubu, 2015). An abundance of wind-dispersed seeds that allow typha to colonize wetlands across a great distance, and its rapid growth rate and large stature enable aggressive colony propagation. It is especially aggressive where water nutrient levels are elevated from agriculture. Over recent decades, the distribution and abundance of typha in wetland ecosystems around the world has caused great concern particularly in Africa. In sub-Saharan Africa, the grass is a menace to the optimal use of channels, rivers, and agricultural lands. Mshandete (2009) reported that Typha infestation in Tanzania Lake Jipe River was 65-80% of 30 km2. It was estimated to contain 193.3 tons of fresh typha, which is equivalent to 42.46 tons of dry material. A similar report from Senegal showed that there was 3.05 million tons of fresh typha material (approximately 520,000

tons in dry form) in the left bank of Delta area (Diouf *et al.,* 2015). In the Dakar area, typha covered 12,058 hectares with 800 tons of fresh material. Another report from Nigeria estimated that Typha infestation in the Hadejia Valley Irrigation Scheme alone contained 600,000 tons of fresh biomass (TRIMING, 2020).

The grass threatens the economic activity, health, and welfare of many communities in Irrigation Schemes in northern Nigeria. Because by its nature, it regenerates fast and re-establishes within a short time and colonized were ever it exist (TRIMING 2020). The conventional methods of controlling the grass consume time, labor, and cost thus found not to be effective. Therefore, recent efforts have been made to find alternative methods of typha management so that the threats posed by the grass will be converted to opportunities. The grass has been considered as a renewable energy source and a feedstock for livestock (Gijzen *et al.,* 1988; Hu and Yu, 2005). But appropriate nutrient composition and organic loading rates for good digestion were yet to establish in anaerobic digestion with rumen microorganism as the source of inoculant. Therefore, the research aimed to determine the chemical composition and organic loading rate of a typha biomass for anaerobic digestion to produce biogas for cooking.

2. MATERIALS AND METHODS

2.1 Sampling

The typha samples were harvested at different growth stages from Hadejia Valley Irrigations Scheme (HVIS) in Nigeria Figure 1 shown. Approximately 500 g was harvested at each growth stages and put in a vacuum bag, freeze, label, and recorded dry matter percentage. When the samples are about to send to the Department of Animal and Avian Science, University of Maryland United States, ice was added and put in Styrofoam insulated box. After the sample arrived in the United States it removed from the box and then dried with electric oven. The dried biomass was then milled to a powder form of 1- mm particle with a Wiley milling machine (Model 4, USA).

Figure 1: Harvesting of typha sample

2.2 Rumen Fluid Collection and Purification

Rumen fluid was sampled directly from a non-lactating Holstein cow at the University of Maryland College Park on-campus farm using an established protocol approved by the university International Animal Care and Use Committee (IACUC, 2011). Samples were collected before morning feeding of the animal and included both the solid and liquid portions from the rumen then it was transported via thermal cooler to the lab, where the fluid was processed. The sample was centrifuged for 15 min as adopted from Weimer and Kohn (2016) and allowed to cool before decant to removed fibre and other microbes. Figure 2 shows the centrifuged rumen fluid.

Figure 2: Centrifuged rumen fluid

2.3 Reducing Media

Media were prepared according to Goering and Van Soest (1970). The medium contained water, sodium, and potassium salts of bicarbonate and phosphate, ammonium, magnesium, sulfate, peptides, reducing compounds cysteine and sulfide, and micro-minerals calcium, manganese, cobalt, and iron. The medium was bubbled with carbon dioxide while bringing to boil so that pH was near neutral after cooling to incubation temperature.

2.4 Experimental Method

2.4.1 Experimental treatment and data analysis

A single factor experiments in a completely randomized design (CRD) with four levels (0.2, 0.4, .0.6 and 0.8 g) and repeated three times were considered. Data collected were analysed using descriptive statistic, analysis of variance (ANOVA) and Duncan multiple range test (DMRT) with statistical analysis software (SAS, 9.0).

2.4.2 Experimental Set up

A bench-scale anaerobic digester was developed with a 250 ml vial. Typha samples were weighed 0.2, 0.4, 0.6, or 0.8 g and then poured into a vial. Media was prepared and transferred 100 ml to each bottle. Each bottle was inoculated with a 10 ml source of microorganisms from the existing typha digester. After inoculations, the bottle was cover with a rubber and aluminum cap to have an airtight system. Immediately the vials were moved to an incubator set at temperature 35-39 ^{o}C Figure 3 shown. Feeding was done after four to five days intervals in an anaerobic chamber or plastic bag. In either case, before feeding the digesters, the environment has to purge with CO_2 three to four times to minimize air movement into the vial. Biogas

measurement was done daily with a glass syringe. The gas was also analyzed daily using gas chromatography (GC; Agilent 6890 model, USA) to monitor its quality. The experiment lasted for 34 days.

Figure 3: Experimental treatments

2.4.3 Determination of Nitrogen Content

Nitrogen was determined using the Kjeldahl method. One gram of the sample (Typha grass) was put into a digestion flask and then added Kjedahl catalyst (selenium tablets). The samples were also added with 20 ml concentrated tetraoxosulphate (vi) acid (H_2SO_4) and then fixed in the digestion units (Kjeldahl apparatus in a fume cupboard) at 450 ºC. The digest, a pure yellow coloration, changes into a colorless liquid after cooling, which was then transferred into 100 ml of 4 % boric acid solution into a conical flask. Five drops of methyl red were added to each flask on the indicator. The amount of nitrogen in a sample was calculated from the quantified amount of ammonia ions in the receiving solution as shown in Equations 1 and 2.

$$N_c = \frac{moles\ of\ NH_3 \times 1\ moles\ N}{1\ mole\ of\ NH_3 \times 14.01 g/mole} \quad (1)$$

where:

N_c = nitrogen concentration (g)

To obtain the percentage of nitrogen Equation 2 was used

$$N = \frac{Wn}{Ws} \times 100 \quad (2)$$

where:

N = nitrogen (%),
W_n = weight of nitrogen (g),
W_s = weight of sample (g)

2.4.4 Determination of Carbon Content

Five gram (5 g) of typha sample was put into a 250 ml Erlenmeyer flask, 35 ml of 72% of tetraoxosulphate (vi) acid was added and swirled to dissolve. Ten mills (10 ml) of concentrated trioxonitrate (v) acid (HNO_3) was also added and allowed to stand for 30 minutes. Followed by gentle boiling for 30 minutes and allowed to cool to room temperature. Fifty mills (50 ml) of 72 % tetraoxosulphate (vi) acid was again added, followed by 20 ml of concentrated trioxonitrate (v) acid. The residue was filtered and placed in a crucible and transferred to a muffle furnace at 800 ºC for 2 hrs until the carbon content burns off. Later cooled in desiccators and re-weighed. The percentage of carbon content was then calculated as in Equation 3 from the weight of carbon in the crucible, as Willian (1980) stated.

$$C_{per} = \frac{A-B}{S_{wt}} \times 100 \quad (3)$$

where:

C_{per} = percentage of carbon (%),
A = Weight of sample after digestion (g),
B = Weight of sample after the removal of carbon (g),
S_{wt} = weight of the sample (g)

2.4.5 Determination of Phosphorous and Potassium Content

Ten gram (10 g) of typha sample was incinerated to white ash at 550 °C in a muffle furnace for one hour, cooled, and the ash washed into a 250 ml beaker with 30 ml of concentrated trioxonitrate (v) acid. It evaporated to dryness on a steam bath, and the residue was further heated for 30 minutes. After that, the sample was dissolved in 40 ml of hydrochloric acid (HCl) and digested for 2 hrs on a hot plate magnetic stirrer. Later it was filtered while hot using Whitman Number 4 filter paper, washed with HCl, and the volume made up to 100 ml with distilled water. The minerals (potassium and phosphorous) were then determined using atomic absorption spectrophotometer (AAS-3110, USA). The concentration of each element was calculated as given by Akinnuli and Olugbade (2014) in Equation 4.

$$E_{con} = \frac{S_{con} \times S_{ab}}{St_{ab} \times S_{wt}} \times 100 \quad (4)$$

where:

E_{con} = Element concentrated (mg/100g),
S_{con} = Standard concentrate (mg/100g),
S_{ab} = Sample absorbance (mg/100g),
S_{tab} = Standard absorbance (mg/100g),
S_{wt} = weight of the sample (g)

2.4.6 Dry Matter Content (DMC)

Dry matter content was established by drying in a Koster^{TM} crop forage tester (BHT- 6071, USA, accuracy 0.1%) and weighed with an electronic balance before and after drying. The dry matter content was calculated using Equation 5 below.

$$W_d = \frac{W_f - W_p}{W_i - W_p} \text{ X } 100 \quad (5)$$

where:

W_d = dry matter percentage (%), W_f = final weight of the sample (g),
W_i = initial weight of the sample (g), W_p = weight of the pan (g)

2.4.7 Sequential Fibre Analysis

After 48 hours of incubation with rumen fluid, samples were subjected to sequential fiber analysis with crude fiber extractor (Model 304 with 230V, USA) to determine the quantity of remaining fibre fractions after digestion.

i) Neutral Detergent Fibre (NDF)

NDF Procedure. After 0 or 48 hrs incubation, each sample was transferred to a 600-ml beaker then diluted with 100 ml of NDF solution. The samples were refluxed in a beaker for 60 minutes. Furthermore, the contents of the beakers were filtered through pre-warmed Gooch crucibles. After rinsing with hot water, the crucibles' residues were washed in a fume hood with acetone and then dried in an oven at 100 °C for at least 8 hours. Crucibles were then weighed, and NDF residue was calculated from Equations 6 below.

$$\boldsymbol{NDF = \frac{W_{NDF}}{W_s} \times 100} \quad (6)$$

where:

NDF = percentage of NDF (%),
W_{NDF} = weight of NDF residue (g),
W_s = weight of the sample (g)

ii) Acid Detergent Fibre (ADF)

Experimental Method of ADF. Gooch crucibles with fibre content from initial NDF experiments were placed in a 600 ml beaker, and 100 ml ADF solution was added. The beaker with crucible was heated to boiling, the fibre was separated from the crucible with a rubber spatula and added to the solution, and fibre in solution was refluxed for 60 minutes. Samples were removed, and contents were returned to crucibles in the filtering unit. After removing liquids under suction, each sample was rinsed with hot water, acetone in a fume hood, and dried in an oven at 100 °C. Crucibles were weighed, and ADF was calculated from Equations 7.

$$\boldsymbol{ADF = \frac{W_{ADF}}{W_s} \times 100} \quad (7)$$

where:

ADF = percentage of ADF (%),
W_{ADF} = weight of NDF residue (g),
W_s = weight of the sample (g)

iii) Acid Detergent Lignin (ADL)

Seventy two percent (72%) Sulfuric acid (H_2SO_4) solution was prepared with distilled water (818 ml), sulfuric acid (H_2SO_4, 96%, 1332 ml). Each crucible from ADF residues was filled to 2/3

with 72% H_2SO_4 solution then stirred thoroughly with a stir rod. After three incubation hours in the room, the temperature crucible was filtered and rinsed thoroughly with hot water. Samples were dried overnight at 100 °C. The weight was recorded and the percentage of lignin calculated as in Equations 8.

$$\boldsymbol{ADL} = \frac{\boldsymbol{W_{ADL}}}{\boldsymbol{W_s}} \times 100 \quad (8)$$

where:

ADL = percentage of ADL (%),
W_{ADL} = weight of NDF residue (%),
W_s = weight of the sample (%)

iv) ADL-Ash Analysis

The Crucibles containing lignin residues were placed in a muffle furnace at 500 °C. The muffle furnace was turned off the next day and allowed to cool to room temperature, and then the weight of crucibles was recorded. The percentage of ADL-ash contents was calculated as in Equations 9.

$$\boldsymbol{Ash} = \frac{\boldsymbol{W_{Ash}}}{\boldsymbol{W_s}} \times 100 \quad (9)$$

where:

Ash = percentage of ash (%),
W_{Ash} = weight of ash (%),
W_s = weight of the sample (%)

3. RESULTS AND DISCUSSION

3.1 Mineral Compositions of Typha

Table 1 shows the mineral composition of typha grass at different maturity levels. The result showed that nitrogen ranged from 0.34 to 1.58% while organic carbon ranged from 52.42 to 55.78%. Calcium was found to be in the range of 0.69 to 1.36%. Phosphorous, Magnesium, Potassium and Sodium ranged 0.03 to 0.29, 0.07 to 0.23, 0.73 to 3.13 and 0.13 to 1.21%, respectively. It was observed that for all the different maturity levels evaluated, nitrogen was concentrated more in the leaf and the spike. Calcium which is a pH adjusting mineral was higher in stem while organic carbon which is the main source of energy for microorganisms shows irregular patterns. Phosphorous and magnesium observed to be more concentrated in seed at senescence growth stages. These showed the nutrients concentration varies on the plants component which might be due to morphological characteristic of the plants and the medium of which its growth.

Potassium was concentrated more in stem at first growth stage (0.5 m height) and leaf at third growth stages (1.5 m height). The results obtained from this study also showed a different pattern from that of Nacaroglu *et al.* (2009). They reported more concentration of potassium, sodium, calcium, and manganese in the stem, and magnesium, zinc, iron, and copper were concentrated more in the root. These might be due to using different typha species. Matheri *et al.* (2015) reported potassium ranged of 6 - 18 mg/l for pig waste and cow dung, the study recorded

maximum in pig waste and minimum in cow dung. The maximum potassium level obtained from this research (31000 ml/l) is above what was reported by Avfall (2010) 3000 ml/l. This is due to higher ability of the typha plant to absorbed and stored potassium from the medium of which is growth. Also the results indicates that the sodium concentration was higher in the stem at some growth stages (0.5, 1.5 m, and senescence stage). However, when the plant was at 1 m height and early bloom sodium concentrations were more in the leaf. A reason for having a difference in sodium concentrations would be due to the different medium characteristics for which the plant was grown (Avfall, 2010).

The results obtained in this study also showed that iron was within the range of 196 to 705 ppm while manganese was 235 to 857 ppm. It was also observed, zinc concentrations in typha grass ranged from 7 to 26 ppm while copper ranged 2 to 9 ppm. The result indicates that iron was higher in a stem at the early bloom stage and leaf at senescence stages. However, the concentrations of iron in the second and third maturity stage were higher in a leaf than the stem. When the results were compared with the recommended concentrations in literature it was observed iron, manganese, and zinc obtained from this study were above the recommended threshold of 10, 50 and 1 mg/l for Iron, Manganese, and Zinc respectively as reported by Bozym *et al.* (2015) and Takashima *et al.* (1990). This is due to the higher ability of the plant in absorbing heavy metal from its medium. Zinc was mostly more in the stem at 0.5 m, early bloom, and senescence growth stage. However, at 1 and 1.5 m height plant (second and third maturity stages) Zinc concentrations were more in leaf. Senescence seed has higher zinc than early bloom seed. The stem has higher concentrations of copper at 0.5 m and early bloom growth stages while at 1 m and 1.5 m height, showed the same Cu concentration in both the leaf and stem. Copper concentrations were within the threshold (400 ppm) as reported by Matheri *et al.* (2015).

3.2 Fibre Component at different maturity

Table 2 shows the compositional properties of typha at different growth stages. The maximum crude protein of 9.9 and 9.8% was obtained from leaf when the plant was at a tender age (0.5 m height) and seed at early bloom and senescence growth stages. However, the minimum of 2.1 and 2.3% was recorded in the stem when the plant was at second maturity and senescence stages, respectively. The result did not agreed with Mshandete (2009) that reported the maximum crude protein of 29.19% from a combination of root and rhizome while the minimum (10.63%) from a combination of stem and leaf. This was due to the combination of different typha botanical fractions and the present study analysed the typha plant at different growth stages. The highest soluble crude protein (SCP) was obtained from early bloom seed while the lowest was recorded in the stem at the second stage of maturity which was due to different concentration of nitrogen from different component.

Neutral detergent fiber (NDF) varies from 59 to 75 and the maximum was observed in senescence leaf (75%) and the minimum of 59.3% was recorded from the early bloom seed. Acid detergent fiber (ADF) ranged from 33.6 to 60.4% with the maximum recorded in senescence leaf while the minimum in early bloom seed. The results obtained are within the range reported by Hu and Yu (2005); Nuntiya *et al*. (2009). The authors obtained the NDF to be 64% and ADF 42%.

Table 1. Mineral compositions of typha in a different stage of maturity

Plant	N	C	Ca	P	Mg	K	Na	Fe	Mn	Zn	Cu
	- - - - - - - - - - - - - % of DM - - - - - -							- - - - - - ppm - - - -			
0.5 m Stem	0.86	55.74	0.98	0.22	0.18	3.13	0.85	458	498	26	7
0.5 m Leaf	1.58	55.43	0.84	0.20	0.20	2.32	0.72	283	700	12	5
1 m Stem	0.34	54.37	0.95	0.06	0.10	1.50	1.02	196	354	14	3
1 m Leaf	0.83	54.92	0.82	0.07	0.16	1.74	1.16	452	857	21	3
1.5 m Stem	0.64	54.25	0.97	0.15	0.12	2.05	0.69	237	270	14	4
1.5 m Leaf	1.20	55.75	0.74	0.13	0.17	2.38	0.66	389	478	15	4
Early Bloom Stem	0.37	55.78	0.96	0.04	0.07	0.73	0.74	705	261	12	3
Early Bloom Leaf	0.91	54.83	0.86	0.09	0.16	0.98	1.21	258	752	8	2
Early Bloom Seed	1.57	54.40	0.69	0.25	0.22	1.80	0.13	223	288	20	9
Senescence Stem	0.37	54.47	1.06	0.03	0.09	0.91	0.62	354	235	9	4
Senescence Leaf	0.50	52.42	1.36	0.05	0.15	1.12	0.50	667	348	7	3
Senescence Seed	1.57	54.06	0.63	0.29	0.23	1.90	0.19	285	301	22	8

N: nitrogen; C: carbon; Ca: calcium; P: phosphorous; Mg: magnesium; K: potassium; Na: sodium; Fe: Iron; Mn: manganese; Zn: zinc; Cu: copper, % of DM = Percentage of dry mater, ppm = Part per million

Table 2: Fibre analysis of typha biomass

Typha at different maturity level	CP	SCP	NDF	ADF	Lignin	C/N	Ash
	- % of DM -						
0.5 m Stem	5.4	1.5	68.0	49.5	11.5	64.5	10.7
0.5 m Leaf	9.9	2.9	67.8	49.0	12.1	35.0	8.6
1 m Stem	2.1	0.4	63.2	46.7	12.3	161.8	7.7
1 m Leaf	5.2	1.8	69.5	49.5	12.8	66.0	8.3
1.5 m Stem	4.0	1.2	68.5	49.1	11.8	84.8	8.2
1.5 m Leaf	7.5	2.0	64.7	52.0	11.7	46.5	8.2
Early Bloom Stem	2.3	0.7	66.3	48.1	13.4	151.6	7.7
Early Bloom Leaf	5.7	1.7	70.9	50.4	13.7	60.1	7.5
Early Bloom Seed	9.8	4.8	59.3	33.6	12.1	34.7	5.0
Senescence Stem	2.3	0.8	70.1	57.9	18.2	148.0	6.0
Senescence Leaf	3.1	1.1	75.0	60.4	20.8	105.7	7.1
Senescence Seed	9.8	4.6	63.9	40.0	12.0	34.5	6.1

DM: dry matter; NDF: Neutral deter gent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; C/N: carbon-nitrogen ratio; Ash: ash content

Lignin was found to increase with an increase in maturity and varies slightly within the plant component. The maximum lignin obtained was at senescence leaf and minimum in 0.5 m height plant. The range was between 11.5 and 21%. Hu and Yu (2005) reported the maximum lignin of 10.5% while Nuntiya *et al.* (2009) reported the maximum lignin of 8.86%. The lignin content obtained from this research was higher than what was reported by Hu and Yu (2005); Nuntiya *et al.* (2009). This could be due to the age of the plants considered. Higher carbon to nitrogen ratio (C/N) was obtained (161.8:1). The stem has the highest C/N ratio at all growth stages which is due to the low nitrogen content from the stem compared to other plant`s components. This revealed that if single stem component is use the microorganisms will have nitrogen deficiency which inhibits methane production. Mshandete (2009) reported the minimum and maximum carbon to nitrogen ratios of 12 and 23, respectively from typha *domingensis.* Early bloom seed and senescence seed have minimum ash content (5.0 and 6.1%, respectively) while stem at first maturity growth stage showed the maximum (10.7%). Mshandete (2009) obtained the maximum ash at root components (27.68%) and the minimum (8.84%) with the stem. These indicate that different growth stages, components, and typha species affect the fiber composition.

3.3 Gas Productions from Different Organic Loading

Maximum gas productions were obtained one day after seeding the digester (62, 70, and 79 ml for 0.2, 0.4, and 0.8 g respectively) for all the treatments except 0.6 g of which maximum was recorded after 29 days (100 ml). One of the reasons for having maximum gas production on the second day would be due to media in the supernatant that can easily release CO_2. It was observed that gas production increased a day after feeding and gradually decreases before the next feeding. This could be due to the deflation of the most hydrolyzable portions of biomass by the activity of microorganisms. And therefore, when new material is added to the system, it increases the activity of microorganisms which enhances gas productions.

From Figure 4, it was understood that gas production increased the next day after loading 0.2 to 0.8 g within three weeks. However, for the fourth treatment (0.8 g) after twenty one (21) days, the gas production gradually decreases due to the accumulation of volatile fatty acid (VFA) and low buffering capacity of system. The pH was below 6 ppm which affects microorganisms' activity especially methane producing organisms that are sensitive to acidic conditions. When feeding was adjusted to five days, all the treatments show an irregular pattern except 0.6 g which indicates a rapid increase in gas production, while 0.8 g continued to decrease. This indicates that the accumulation of VFAs in the samples was above the consumption level of the microorganisms, therefore, the microorganism cannot recover the shock absorbed and adopt the new environment.

Gas productions from 0.2 and 0.4 g were also improved due to the adjustment of time for microorganisms to utilize the produces VFAs before addition to the new substrates. These results affirmed what the previous researchers reported such as Hu and Yu (2005); Magdalena *et al.* (2019), they all agreed that accumulation of VFAs affects biogas yield. The ANOVA results showed that there was significance difference between the different loading rates evaluated at 1% probability level (Table 3). Furthermore, the Duncan Multiple Range Test (DMRT) revealed that in the first week, mean volume of gas produced using loading rates of 0.2 and 0.4 g were significant while that of 0.6 and 0.8 g were not significant with each other. These indicate that at

first week 0.2 and 0.4 have different effects while 0.6 and 0.8 g statistically have the same effects on the gas yield. However, in the second week, the result showed that the loading were significant with each other but in the third week, the 0.2 g was significant with 0.4, 0.6, and 0.8 g, while in the fourth week 0.4 and 0.6 g were not significant. For the fifth week the entire loading rate were significant with each other (Table 4).

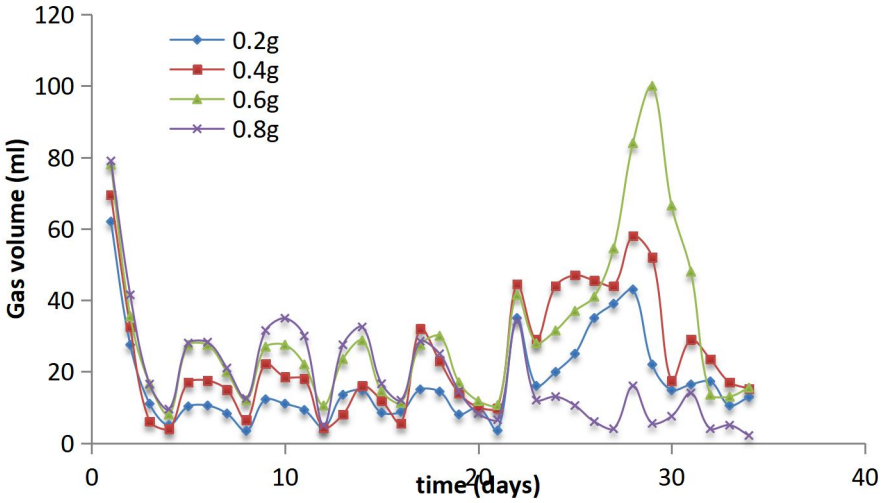

Figure 4: Gas production from different loading rates

Table 3: Analysis of variance (ANOVA) for a completely randomized design on the effect of biogas production at different organic loading rate

Sources of variation	Degree of freedom	Sum of squares	Mean squares	Computed F	Tabular F (5%)	(1%)
OLR	3	323.0682781	107.6894260	59.15**	4.07	7.59
Experimental Error	8	14.5650000	1.8206250			
Total	11	337.6332781				

Organic loading rate (OLR), ** Significant at 1% level

Table 4: Duncan Multiple Range tests of weekly biogas production at different organic loading

Organic loading rate (g/100ml)	Week 1. Gas volume (ml)	Week 2. Gas volume (ml)	Week 3. Gas volume (ml)	Week 4. Gas volume (ml)	Week 5. Gas volume (ml)
0.2	19.357^{c}	10.4235^{d}	11.343^{b}	24.771^{b}	19.550^{c}
0.4	23.057^{b}	14.3582^{c}	16.057^{a}	37.679^{a}	30.300^{b}
0.6	30.414^{a}	21.8378^{b}	20.107^{a}	34.871^{a}	48.643^{a}
0.8	31.964^{a}	24.7663^{a}	19.621^{a}	12.286^{c}	7.729^{d}
SE±	0.671	0.452	0.832	1.053	0.915

Mean values with different letters are statistically significant while mean with same letters are not significance (a, b, c, and d)

3.4 Analysis of Biogas and Methane Yield from Appropriate Organic Loading

The result of gas yield from different loading was presented in Figure 5a. It was observed that 0.6 g produced the highest cumulative volume of gas (1,061 ml) for 34 days while 0.2 g produces the lowest (578 ml). However, the gas yield increases with an increase in loading (0.2 to 0.6 g) and decreases when loading was increased to 0.8 g/100 ml. The study showed that 0.6 g loading rate has the highest biogas yield. Although for methane productions, the results were different.

Figure 5b shows methane yield at different loading rates. The results indicate that methane generations were increased with an increase in loading from 0.2 to 0.4 g and decrease after increasing the load to 0.8 g. Maximum methane yield was observed using 0.4 g/100 ml loading rate while the minimum was at 0.8 g/100 ml. In terms of methane generations, 0.4 g loading found to be the highest. However, 0.2 g was found to have the highest volume of gas per volatile solid (437 ml/g VS) while 0.8 g show the lowest (122 ml/g VS). Also, it was observed for design considerations it's advantageous to operate at 0.2 g since it required low biomass and a smaller digestion tank to provide average performance for gas and methane yield.

The analysis of variance from methane production showed that there was significant difference between the different loading rates evaluated at 1% probability level (Table 5). Furthermore, the Duncan Multiple Range Test (DMRT) in Table 6 revealed that for week one and two the mean value of the gas from all the treatments (0.2 to 0.8 g) were significant with each other while that of week three and four showed 0.2, 0.4, and 0.6 g were not significant. However, in week five 0.4 g and 0.6 g were not significant while 0.2 g and 0.8 g were significant with each other. The results obtained also agreed with Tin-Sun *et al.* (2017) that reported growing organic loading rate in an appropriate reactor could improve biogas yield while higher organic loading rate could cause system instability. The authors obtained the average gas yield of 438.9, 477.3, 480.1, and 188.7 ml/g from the digestion of macroalgae at 1.37, 2.74, 4.12, and 6.85 $kg/m^3.d$ organic loading rate, respectively.

Eslami *et al.* (2017) evaluated organic loading (1.04 to 19.65 gCOD/L.d) of leachate material in a batch reactor, they observed that with the increase in organic loading rate (OLR), the biogas and methane production also increases at 18.52 gCOD/L.d, sudden increase in organic loading rate resulted to decrease in methane and biogas yield. Mel *et al.* (2015) obtained the highest methane quality (70.1%) and gas yield of 38.1 l/d at 50 g/l from three organic loading rate (OLR) evaluated (25, 50, 75 g/l). The author explained that as the organic loading rate increased from 50 to 75 g/l the chemical oxygen demand (COD) degradation increases and biogas yield decreases.

4. CONCLUSIONS AND RECOMMENDATION

Typha plant contained higher amount of most of the required nutrients for building blocks, vitamins and provide energy sources for the microorganisms. Carbons which provide more than 50% of the energy had obtained to be 56%. However, higher fibre content was observed especially at the mature leaves and stem. Lignin content was obtained to be around 21% while cellulose and hemicellulose had 40 and 15% respectively. Moreso, the different loading rates evaluate for anaerobic digestion, 0.6 g turned to have a higher gas yield (1061 ml), while 0.4

produces the highest methane yield (122 ml). However, 0.2 g has the highest volume of gas per volatile solid (437 ml/g VS). However, it is advantageous to operate with 0.2 g due to the higher volatile solid.

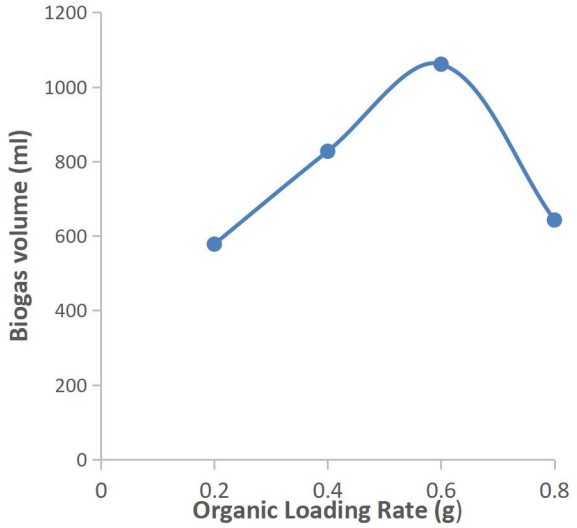

Figure 5a: Gas yield from different loading loading

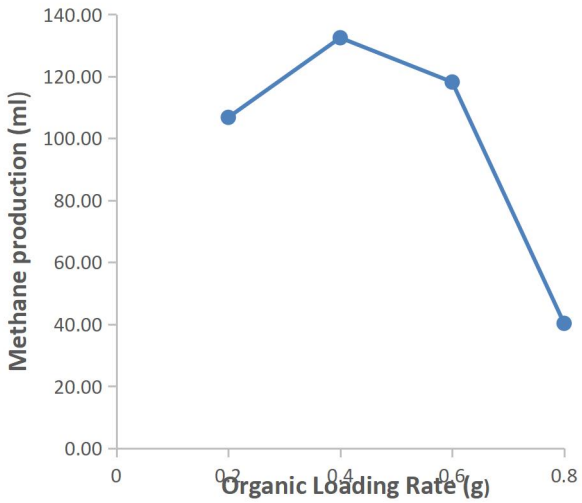

Figure 5b: Methane yield from different

Table 5: Analysis of variance (ANOVA) for a completely randomized design on the effect of methane production at different organic loading rate

Sources of variation	Degree of freedom	Sum of squares	Mean squares	Computed F	Tabular F (5%)	(1%)
OLR	3	56.32165244	18.77388415	15.63**	4.07	7.59
Experimental Error	8	9.61006344	1.20125793			
Total	11	65.93171588				

Organic loading rate (OLR) ** Significant at 1% level

Table 6: Duncan Multiple Range tests of weekly methane production at different organic loading

Organic loading rate (g/100ml)	Week 1. Methane volume (ml)	Week 2. Methane volume (ml)	Week 3. Methane volume (ml)	Week 4. Methane volume (ml)	Week 5. Methane volume (ml)
0.2	4.0849^{c}	5.0848^{c}	8.838^{b}	29.9328^{a}	42.673^{a}
0.4	6.3936^{b}	6.8070^{b}	14.555^{a}	30.4629^{a}	33.989^{b}
0.6	7.6095^{b}	7.9610^{a}	10.118^{ab}	31.1802^{a}	31.447^{b}
0.8	10.0895^{a}	6.7684^{b}	2.257^{c}	4.5493^{b}	6.968^{c}
SE±	0.604	0.448	0.922	0.526	1.051

Mean values with different letters are statistically significant while mean with same letters are not significant (a, b, c, and d)

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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