



ORIGINAL RESEARCH ARTICLE

COMPOSITION AND BIOCHEMICAL METHANE POTENTIAL FROM
DIFFERENT TYPHA COMPONENTS

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ABSTRACT

Invasive species are recognized as one of the main causes of erosion of global biodiversity which represent one of the major environmental challenges of the 21st century. Typha is among the fifth most invasive species in West Africa, Nigeria included. Presently in Hadejia Valley Wetlands of Nigeria, typha grass has been perceived as a threat to the surrounding communities. Although some research efforts have shown it can be used as a resource material for renewable energy or animal feed, its use has not yet become a commercial practice. This study investigates compositional characteristic using in vitro digestibility and biogas production from different Typha botanical fractions using anaerobic digestion inoculated with rumen microorganisms. Treatments considered were single plant components as well as combinations. The results show that spike (seed) had the highest soluble crude protein and the leaves had the highest energy content (cellulose and hemicellulose). The leaf and stem at full maturity had a lignin content of 18-20%. Rhizome and stem contained higher ash content while root had greater total volatile solid. The spike yielded the most biogas (457 ml/g) at 60 days retention period. The root component yielded the least gas (259 ml/g) over the same period. The seed pod produced the most methane gas per weight of VS (255 ml/gVS). Blending leaf and stem or root and rhizome increased the volume of biogas generated (399 ml/g and 442 ml/g respectively) while a combination of all components of the plant decreased the volume of gas (388 ml/g – 289 ml/g). The combination of the root and the rhizome produced a higher volume of methane (254 ml/g) than either component by itself (root with 160 ml/g while rhizome 226 ml/g). Different typha components has different compositional characteristic which influence the activity of anaerobic microorganism and affect the volume and quality of biogas produced. Rumen microorganisms digest typha component and spike gives the highest gas volume per grams of typha biomass while rood produce the least volume. Combination of different typha component affect the volume of gas generation depending of which component of plants were mixed.

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1.0 Introduction

Invasive species are recognized as one of the main causes of erosion of global biodiversity. They represent one of the major environmental challenges of the 21st century (UICN, 2004). Many countries have studied the problem but have not found an ideal solution (Obiri, 2011). Indeed, when invasive species become well established in the environment, the control

becomes nearly impossible and requires considerable financial and human investment. For example on a continental scale, the United Nations Environment Program (UNEP) estimated that alien invasive species cost the global economy \$1.4 trillion annually (Obiri, 2011). In Nigeria, the annual cost estimated for invasive species is about \$50 million (Kasulo, 2000).

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 (NAERLS, 2020).

This aquatic weed threatens the economic activity, health, and welfare of many communities including the Hadejia Valley Irrigation Schemes in northern Nigeria. For example in Marma channel and Nguru Lake in Hadejia Valley the invasion was more severe with over 35,000 hectares of potential farming and grazing lands having been taken over by the typha grass. *Typha* infestation has also contributed to the desiccation of Burum Gana channels where about 60% of dry season irrigation farms have been affected (Babagana *et al.*, 2018). These situations increase the poverty level of communities around the wetlands and also often lead to conflicts among farmers (Babagana *et al.*, 2018). The average income of fish catch per fishermen has been reduced from about \$8 per day to less than \$3 per day (Sabo *et al.*, 2010). Indirectly, this weed decreases the productivity of rice and other related crops (Sabo *et al.*, 2016; Salako *et al.*, 2016) wherever it exists. Ahmed *et al.* (2017) and Goes (2004) established that the obstructions of canals and water infrastructure caused by *Typha* have increased the risk of flooding in farmlands. Ringim *et al.* (2015) investigated the implication of invasive typha plants on biodiversity in Hadejia-Nguru wetlands. The authors recommended the overall holistic utilization of the grasses as it had negative impacts on the community around them. In the past, most of the effort spent was on eradication and destruction of the grass without direct economic use. Because of its nature, it regenerates fast and re-establishes within a short time. These conventional methods of controlling the grass consume time, labor, and cost. 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.

Typha has been considered as a renewable energy source and a feedstock for livestock (Gijzen et al., 1988; Hu and Yu, 2005). Although this weed is high in lignin, the literature shows it can be degraded by fiber-degrading microorganisms to produce biogas (Thanacoses et al., 2003). Weimer and Kohn (2016) used ruminant microbes for rapid lignocellulose degradation and short-chain fatty acid production for conversion to methane Sale et al. (2019) reported the adaptation of rumen microorganisms to typha biomass. Mshandete (2009) demonstrated the use of cockroach gut microorganisms with different Typha botanical fractions for improved biogas production. Adeneran and Onothoja (2017) investigated biogas production from constructed wetland (Typha grass, water hyacinth, and cyperus papyrus). Diouf et al. (2015) studied the fermentability of typha domengensis with rumen content and compared the gas production potential with other biomass. Similarly, Hu and Yu (2005) studied the anaerobic digestion of typha by rumen culture from small ruminant animals. However, the effects of different Typha components such as leaves, stalk, root and different combination using rumen fluid from a cow has not been reported. Therefore, the present study investigates compositional characteristic and biochemical methane potential of typha biomass from different plants components inoculated with rumen microorganisms.

2.0 Materials and Methods

2.1 Sample collection

Typha *latifolia* was obtained from a pond at the University of Maryland, United States. This species is similar to the one in Hadejia-Nguru wetland Northern Nigeria (Yakubu, 2015). Therefore, it is assumed to have similar characteristics. The mature plant was harvested at 16% dry matter (DM). The plant was separated into different components (spike, leaf, stem, root, and rhizome) as shown in Figure 1. Samples were chopped with an electric 15-amp shredder and sun-dried to a dry matter content of 93, 91, 91, 92, and 90% for spike, leaf, stem, root, and rhizome respectively. The second plate in Figure 1 similarly shows the dried Typha. The samples were then ground with a Wiley mill through a 1-mm screen.



Figure 1: Different components of Typha are shown as wet, dry, and chopped forms.

2.2 Rumen fluid

In this study, all procedures including the use of animals were approved by an International Animal Care and Use Committee at the University of Maryland. Rumen fluid was collected anaerobically before daily feeding from a fistulated non-lactating Holstein cow fed a Timothy hay-based ration. Immediately, the rumen fluid was moved to the laboratory and transferred to a blender under CO₂. After blending, rumen fluid was squeezed through four layers of cheesecloth and then glass wool, into an air-free flask under CO₂ (Figures 2).



Figure 2: Collection of the rumen fluid and filtration of fibers

2.3 Reducing media

Media were prepared using the method of Kohn and Kim (2015) to minimize the adjustment period. The medium contained water (400 ml/L, micromineral (0.1 ml/L), digested Casein (2 g/L), macromineral (200 ml/L), IV buffer (200 ml/L), rumen fluid (200 ml/L) and resazurin (1 ml/L). It was bubbled with CO₂ while bringing to boil and subsequently cooling. The pH was measured at 39°C and adjusted using NaOH or HCl to 6.8.

2.3 Analytical methods

2.3.2 Dry matter content (DM)

The dry matter content was determined by the method adopted from Kohn and Kim (2015) drying in a Koster™ crop forage tester for an hour and weighing with an electronic balance before and after drying at 15 minute interval. The dry matter content was calculated using the equation below.

$$\text{Dry matter percentage} = \frac{\text{Final weight-Pan}}{\text{Initial weight-pan}} \times 100 \quad (1)$$

2.4 Experimental method

2.4.1 Experimental factors and treatment

Total volume of gas and methane production were determined for the whole-plant, different vegetative part (spike, leaf, stem, root and rhizome), and the combinations of all components as similarly reported by Mshandete (2009). A total of eight treatments were considered. The treatments were: spike, leaf, stem, leaf and stem, root, rhizome, root and rhizome, and the whole plant (spike, leaf, stem, root, and rhizome). The combinations of leaf and stem or root and rhizome were 50:50 ratios by weight while the whole plant came out in 20:20:20:20:20 ratio by weight for spike, leaf, stem, root, and rhizome accordingly. The experiment focus on biochemical methane potential from different plants component and no control was considered.

2.4.2 Experimental Set up

Each anaerobic digester was developed using a 1,000ml flask fitted with a 2-hole rubber stopper. Each stopper had glass tubing attached to Luer-lock connectors and valves for sampling. A 1-L mylar gas collection balloon was attached to a valve on each of the flasks Figure 3 showed diagram of the experimental setup.

A total volume of 330 ml media and sample was prepared with 30 g at 90% dry matter content of Typha biomass and 300 ml of media. The mixture has a total solid content of 2% with organic loading rate of 1g/30 ml. After mixing the samples, carbon dioxide was used to purge the air since it's heavier than air and can easily replace it. Next, the digesters were moved to a thermostatically water bath in a hood at a temperature between 35 - 40°C with water volume in the bath maintained to replace evaporation. Feeding of the material into the digester was done in three phase's at 10 g each. The first feeding was the initial seeding of the digester. The second feeding was done after ten days while the third was carried out after twenty-three days of the first feeding. Volumes of gas produced from the Typha degradations were measured three times a week with a 100 ml glass syringe. Balloons were used to collect the generated gas over time and then the syringes were used to measure the accumulated gas in the balloons. The pH was tested using the pH meter and a piece of litmus paper at certain intervals. This is to monitor and maintain the pH at 6.5-7.5 by adding NaOH or HCl as the case may be.

Methane, CO₂, and other gasses were analyzed by wet chemistry method which is simple, cheap and affordable. The method involved the use of alkaline base (NaOH, or KOH) that absorbed CO₂ and H₂S leaving the CH₄ and some water vapour. The method is an alternative to the used of GC and other biogas analysers especially in places where that equipment were not available (Pham et al., 2013). For this research 50 ml of collected biogas were injected into a bicarbonate/sodium hydroxide solution into a sealed Wheaton bottle with a glass syringe. The bottle was shakes for 5 minutes and the sample absorbed the CO₂ and other gasses such as H₂S while the methane pushed back the syringe and the volume is read from the graduated syringe. The experiment used Completely Randomized Design (CRD) with eight treatments and repeated three times (8X3=24). The treatments were T₁= Whole plant; T₂ = Spike; T₃ = Leaf; T₄ = Stem; T₅ = Leaf and Stem; T₆ = Root; T₇ Rhizome; T₈ = Root and Rhizome. Biogas and methane volume were measured as an indicator of the performance of each treatment. Data collected were analysed using statistical analysis software (SAS, version 20).

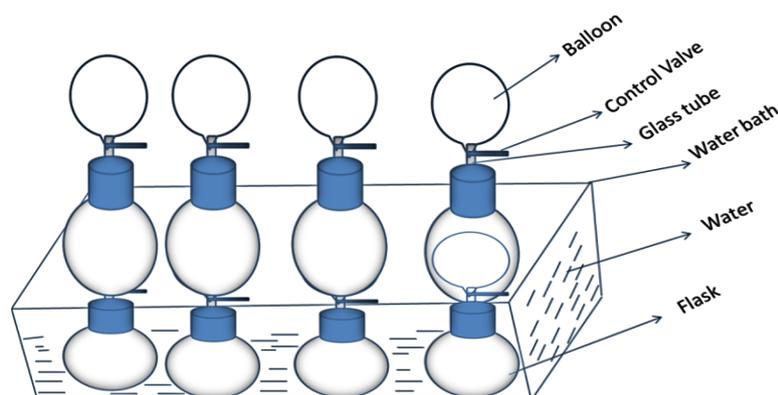


Figure 3: Schematic arrangement of an experimental treatment.

3 Results and Discussion

3.1 Fiber Component of Typha at Different Maturity Levels

Table I shows the compositional properties of the *Typha latifolia* at different maturity levels. These records were established to guide the selection of material at appropriate growth stages and provide necessary information to improve the digestibility of Typha biomass in anaerobic conditions. The maximum crude protein of 9.9 and 9.8% was observed in a leaf when the plant

was at a tender age (0.5 m height) and senescence seed. However, the minimum crude protein of 2.1 and 2.3 % was recorded when the plant was at the second maturity up to the senescence stages. The result did not agree 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 was obtained in the early bloom of the seed while the lowest soluble protein was recorded in the stem at the second stage of maturity. Neutral Detergent Fiber (NDF) varied from 68.2 to 79.2% while the maximum was observed in the early bloom leaf. The minimum detergent fiber (NDF) was recorded at 0.5 m leaf. The Acid Detergent Fiber (ADF) ranged from 34 to 60% with the maximum recorded in the senescence leaf and minimum found in the early bloomed seed. This indicates more fiber concentrate in the mature leaf than any part of the plant. The results obtained are within the range reported by Hu and Yu (2005) and Nuntiya *et al.* (2009). The authors obtained the NDF to be 64% and ADF 42%. The Lignin was found to increase with an increase in maturity but it varies slightly within the plant components. The maximum lignin obtained was in the senescence leaf, and the minimum was found from the stem at 0.5 m height plant; the range for the lignin was recorded at 11.5 to 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), and Nuntiya *et al.* (2009), this could be due to the age of the plants considered. The implication of this result is that as the plant mature it developed resistant to digestibility under conventional method of anaerobic digestion due to higher lignin content. A higher carbon to nitrogen ratio was also observed within the range of 34 to 162. The stem produced a higher ratio at all growth stages. 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, senescence seed and stem have minimum ash content (5.05, 5.92, 5.97%, respectively) while stem at first maturity growth stage showed the maximum (10.74%). 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. These have an influence of the activity of anaerobic microorganism and affect the biogas and methane volume.

Table 1: Compositions of Typha at different stages of maturity

<i>Plant maturity</i>	<i>DM</i> %	<i>CP</i>	<i>SCP</i>	<i>NDF</i>	<i>ADF</i>	<i>Lignin</i>	<i>C/N</i>	<i>Ash</i>
-----% of DM-----								
0.5 m Stem	0.102	5.4	1.5	75.3	49.5	11.5	64.51	10.74
0.5 m Leaf	0.181	9.9	2.9	68.2	49	12.1	34.99	8.63
1 m Stem	0.23	2.1	0.4	74.0	46.7	12.3	161.82	7.71
1 m Leaf	0.242	5.2	1.8	72.2	49.5	12.8	66.01	8.3
1.5 m Stem	0.14	4	1.2	75.7	49.1	11.8	84.77	8.19
1.5 m Leaf	0.187	7.5	2	69.6	52	11.7	46.46	8.17
Early Bloom Stem	0.094	2.3	0.7	72.7	48.1	13.4	151.58	7.71
Early Bloom Leaf	0.135	5.7	1.7	79.2	50.4	13.7	60.12	7.52
Early Bloom Seed	0.149	9.8	4.8	75.1	33.6	12.1	34.69	5.05
Senescence Stem	0.386	2.3	0.8	75.9	57.9	18.2	148.02	5.97
Senescence Leaf	0.198	3.1	1.1	78.5	60.4	20.8	105.69	7.06
Senescence Seed	0.138	9.8	4.6	73.7	40	12.0	34.47	5.92

DM= Dry Matter; CP= Crude Protein; Scp = Soluble Crude Protein; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber; C/N= Carbon to Nitrogen Ratio; and Ash =Ash Content.

3.2 Proximate properties of the Typha components

Typha components contained 80 to 90% water before air drying (Sale et al., 2018). The total solids of the plants before drying ranged from 8 to 11% as reported by Sale et al. (2018). Such a high amount of moisture was a challenge in the process of using the plant as feedstock for biogas production. Furthermore, the plant anatomy is such that it conserves the moisture during drying as each stem is wrapped with concentric layers of tissue. However, Sale et al. (2018) reported that typha could be air dried once the tissue was disrupted by slicing or chopping. The root and the stem were found to have the highest total solid at (> 10%) while the rhizome and the spike had the lowest solid concentration at (< 8%). The maximum volatile solid was observed in a spike at 92% and in the leaf at 90%. However, the minimum value was recorded with the stem and the rhizome components. The ash content from different botanical fractions ranged from 8 to 12%. As expected, the higher the volatile solids the more digestible the material while the higher total solid indicates higher energy content. Therefore, this data suggests the spike might have a higher gas potential than any part of the plant component on a wet basis.

The rhizome and the stem components had the highest ash content at > 12%. The spike showed the lowest value of ash at < 8%. Interestingly, it was observed that as the ash content increased, the volatile solid decreased as shown in Table 2.

Table 2. Proximate properties of the Typha components

Plant components	TS (%)	VS (%)	Ash (%)
Rhizome	7.84	87.67	12.33
Root	10.78	88.89	11.11
stem	10.78	87.72	12.28
leaf	8.91	90.44	9.56
spike	6.93	92.37	7.63
whole plant	10.68	88.79	11.21

TS = Total Solid. VS= Volatile Solid. Ash= Ash Content

3.3 Gas productions per Volatile Solid (VS)

Figure 4 show gas production per volatile solid obtained the highest gas was obtained using the single botanical fractions of the spike at 455 ml/g VS. The lowest value was recorded with the root component at 292 ml/g VS. The records obtained might be due to lower fiber content found in the spike and higher volatile solid as against any other parts of the plant. The root contained a lot of ash which could have affected the volatile solid. When the plant components were blended, the composition of root and rhizome produced the highest gas per volatile solid at 442 ml/g VS. The whole plant produced less than the combinations of the leaf and the stem at 313 ml/g VS as shown in Figure 4. Getahu *et al.* (2014) obtained gas production from different wastes to be 0.15, 0.17, and 0.08 m³/kg VS fruit, food, and paper respectively. The authors conclude that paper waste produces the least gas.

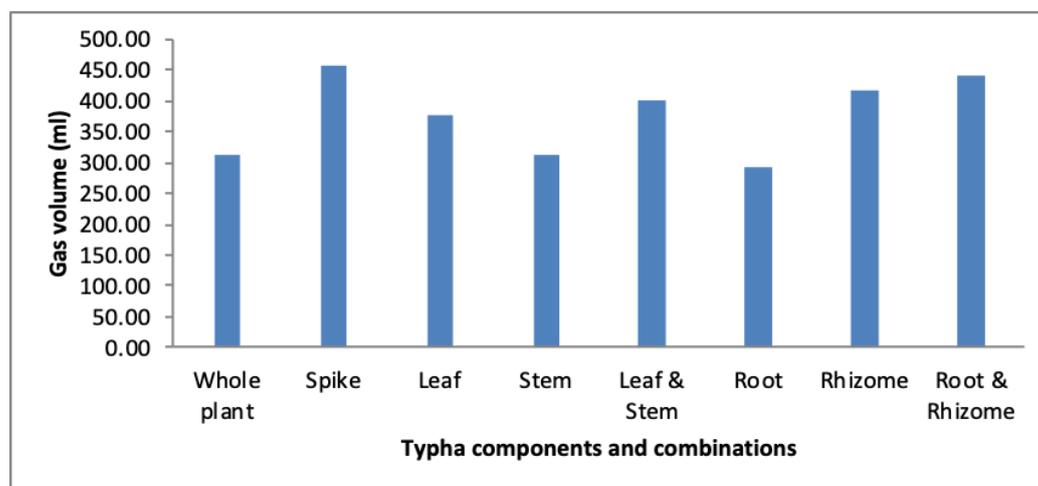


Figure 4: Gas productions per gram of volatile solid for single plant components and combinations

3.4 Methane yield from different vegetative parts of the plant

Figure 5a indicates the methane yield obtained from the different Typha botanical fractions. The maximum methane yield was obtained for the treatment with only a spike (255 ml/g of biomass at sixty (60) days retention time). The lowest yield was observed from the root (161 ml/g of biomass over the same period). One of the possible reasons might be due to the higher soluble crude protein content in the seed which enhances the growth and development of methanogen that are responsible for methane production. Another reason was oil content in the seed which has higher potential methane content. Leaf has the second-highest volume of methane generation (231 ml/g) over the same retention period. This is because leaf has a

lower carbon to nitrogen ratio before the plant mature (0.5-1.5m height) A good carbon to nitrogen ratio enhances methanogen development and thereby increases the activity of microorganisms. Higher cellulose and hemicellulose were also obtained from the leaf as indicated in Table I. Biogas generation from typha grass has a little number of publications around the world. The available literature indicates that only one similar study was conducted by Mshandete (2009). Therefore, the comparison was not much. However, the result obtained was not in agreement with Mshandete (2009) that obtained the maximum yield of methane using single leaf botanical fractions and minimum with the spike. One of the reasons might be due to the difference in inoculant source and typha species used. Mshandete used inoculant from cockroach gut microbial source with typha *domingensis* while the present study used rumen microbes with typha *latifolia*.

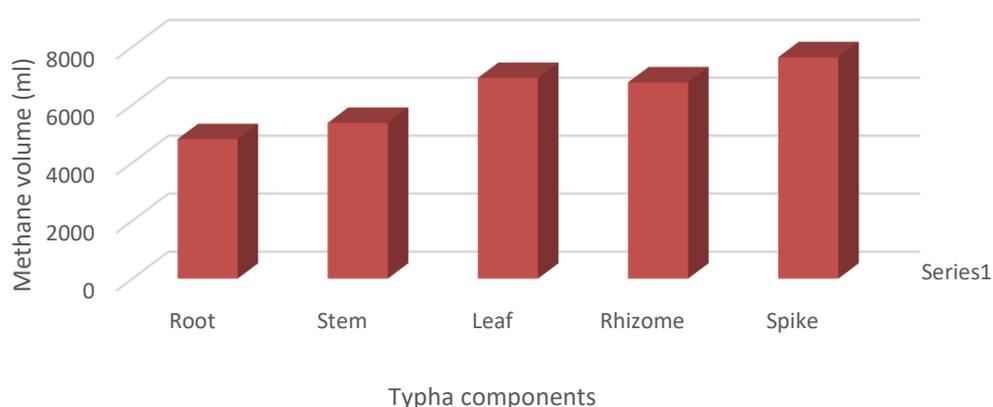


Figure 5a. Methane yield from 30 g of different Typha components

Figure 5 b show methane yield from the combination of different typha components. A combination of the root and rhizome produces the highest yield of methane (254 ml/g). This is due to higher carbohydrate content from the two components and higher total volatile solid from the root. Similarly, the lignin content from these two components was not much therefore, the components were easily degraded and produce higher gas yield. Also, the combination of these two components might produce a good balance of micro and other trace elements for the growth and activities of microorganisms. The combination of whole plant components produced the lowest methane volume (202 ml/g), which might be due to the production of inhibitory substance to some microorganisms. These inhibited the process and therefore led to a decrease in volume generation. The blending of the leaf and stem was the second after the root and rhizome with the gas yield of 206 ml/g Figure 5b shown. However, a similar result from combination of different typha component was obtained by Mshandete (2009). The author obtained the maximum methane generation from the combination of root and rhizome (75 ml/gVS) at 30% total solids.

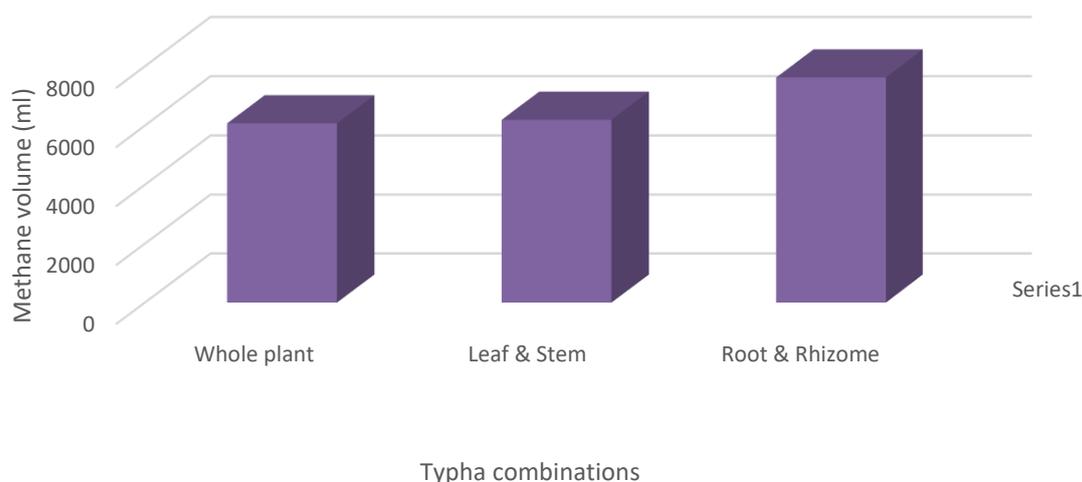


Figure 5b. Methane yield from 30 g blended Typha components

4. Conclusions

Typha compositional characteristics at different maturity and the effect of the different components for methane production has been evaluated. Plant growth stages affect the compositional characteristic which has an influence of the activity and growth of microorganisms that digest the plant and produce biogas. Seed (spike) had higher soluble crude protein (4.8%) which gives it a higher potential for methane production. Leaf had the highest energy content (cellulose and hemicellulose) (78%). Lignin is one of the limiting factors in the anaerobic digestion of lignocellulosic material obtained to increase as the plant's maturity increases. This affect the digestibility of the plant under conventional method and therefore some pretreatment is needed to improve biodigestion process. Rumen microorganisms improve digestion of typha component despite the higher lignin content. Root and stem have the maximum total solid among all the different components tested (10.78%). The spike had the highest volume of gas per gram of volatile solid at 455 ml/g VS and the highest methane generation (255 ml/g). Gas production increased with the composition of the leaf and the stem or the root and rhizome compared to single component production. The combinations of the entire parts of the plant decreased the cumulative volume of gas compared to a composition of only the stem and the leaf or that of the root and the rhizome. However, a combination of the root and rhizome increased the methane concentration compared to other combinations.

Conflict of Interest

The authors have no conflicts of interest

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