Fjrs.futa.edu.ng
FUTA Journal of Research in Sciences
ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 13 (1) 2017:25-38

EVALUATING THE ASSOCIATE MICROBIAL ORGANISMS, FISH FEED UTILIZATION POTENTIAL AND FEEDSTOCK IN BIOGAS PRODUCTION OF WATER-HYACINTH

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ABSTRACT

The nuisance constituted by water-hyacinth (*Eichhornia crassipes* (Mart.) Solms) in water ways has generated global concern which has poised challenges to aquatic organisms and man. This study therefore, seeks to understudy the potentials of the plant with the objective to provide an alternative control measure for its proliferation. The plant (feedstock) was obtained from Jabi Lake, Abuja, Nigeria. The microbial colonization of the different parts of the plant was determined, thereafter, the determination of proximate compositions of non-fermented and fermented samples with lactic acid bacteria. Samples of the plant were chopped into bio digesters of varying sizes (12.620 and 30 litres) with an inoculum for the batch reaction mode during the 21-day retention period. Seven (7) bacteria species, four (4) fungi were isolated and identified to be associated with the plant. Some proximate compositions of the fermented and non-fermented plant were significantly different ($p<0.05$). After the 21-day retention period, there was substantial increase in biogas yield that correlates with the rate of dilution. The presences of inoculum reduced the lag phase period of biogas production. There was over 30% increase in biogas production with increase in dilution factor that corresponds with 93.38% (maximum) increase in biogas production with inoculum. The formulation of 1:3 with 10g of inoculum gave the highest value of total biogas yield of 4066.40ml within the 21-days retention period. The proximate compositions of the fermented and non-fermented water-hyacinth revealed crude protein content and carbohydrates increased insignificantly ($P > 0.05$) while energy value was recorded to increase significantly ($P < 0.05$) with fermentation. Ipso-facto, water-hyacinth, the nuisance plant can be utilized effectively for man's benefit.

Keyword: *E. crassipes*, Bio-energy, proximate composition, microbial, cow-dung

INTRODUCTION

Water-hyacinth is an invasive alien species in waterways. According to Tellez *et al.,* (2008) the International Union Conservation of Nature (IUCN) identified the plant as one of the 100 most aggressive invasive species. Whilst the study conducted by Sheneb *et al.,* (2010) and Gichuki *et al.,* (2012) had recognised the plant as one of the top ten (10) worst weed in the world. The plant is also listed by law in Africa as a pernicious weed with the widest spread damaging aquatic plant species. UNEP (2012), reported that the plant is difficult to manage and control, thereby threatening economic development (Alimi and Akinyemiju, 1991; and Kunle 2014), human wellbeing (Toft *et al.,* 2003; Hellman *et al.,* 2008; Varshney *et al.,* 2008; Pysek and Richardson, 2010 and Borokini and Babalola, 2012) and biodiversity (Giraldo and Garzon, 2002, Mangnus-Ramirez and Elias-Gutierrez, 2004 and Villamagna and Murphy, 2010); thereby flourishing continuously in all surfaces of freshwater, wetlands and estuaries, appearing in cluster population and forming a heavy dense mat.

Cossil *et al.,* (2001) documented that the plant proliferation resulted in the reduction of maritime business, irrigation, house boat rental services, access to water for riverine settlement and recreational activities; thereby, increasing evapo-transpirational as well as fish losses (Irving and Beshir, 1982). Studies have revealed that human activities (Dagno *et al.,* 2012) have led to the spread of the plant as well as the lack of naturally occurring enemies that are capable of suppressing their spread (Mujigni, 2012). Shoeb and Singh (2002), ascertained that when conditions are favourable the plant can flourish well and can reach a mass density of 17.5 metric tonnes per hectare per day, and by conversion this value is equivalent to 6387.5 metric ton per hectare per annum thus its need in biogas production. Studies have also revealed that to keep the plant at an unproblematic level, the control methods that are often used includes physical – mechanical (Patel 2012), biological (Simberlof and Sliling, 2003; Barokoni and Babalola, 2012; Dagno *et al.,* 2012 and Venter *et al.,* 2012) and chemical (UNEP, 2013) methods. Therefore, the plant has found application in fire board production, organic fertilizer production, rope making, paper production, annual fodder, mat and basket making, water purification, fish feed formulation, charcoal briquetting, remediation of crude oil contaminated soil and bio fuel production such as biogas, bio-ethanol and biodiesel (Jayaweera *et al.,* 2007; Almoustapha *et al.,* 2009; Ndimele *et al.,* 2012 and Kunatsa and Mufundirwa, 2013). According to Wang and Calderon (2012), water hyacinth can be a potential resource to produce biogas and bioethanol as supported by other researchers (Bolenz *et al.,* 1990; Akinwande *et al.,* 2013; Gunnarsson and Petersen 2007; Anjanabha and Kumar, 2010; Cheng *et al.,* 2010 and Kunatsa and Mufundirwa, 2013). According to Zulu and Richardson, (2013), it can therefore, be a good alternative for the production of energy as more than 80% of urban households in sub-Saharan Africa use charcoal as their main source of cooking energy.

Researches are on-going on the possibility of bio-conversion of water hyacinth to yield biogas which may solve the problem of water hyacinth management as well as provide solution to the energy and power

shortages using different inoculum. Therefore, this study was conducted to determine the microbial colonization of the plant, the proximate analysis of fermented and non-fermented sample of the plant as well as the effect of inoculum (cow-dung) and dilution rate on biogas production yield from the plant during the 21-day retention period with the intent to determine the lag phase period in the biogas production.

MATERIALS AND METHODS

Sampling Site

Samples of water-hyacinth were identified and collected from Jabi Lake, Federal Capital Territory; Abuja, Nigeria located between latitude $9^{0}04^{1}17.88^{11}$ N and longitude $7^{0}25^{1}32.87^{11}$ E. (Plate 1). Alongside the plant samples, fifty (50)ml of water from the plant's root region was collected in a clean sterile bottle; while cow-dung was collected from the Ibrahim Badamasi Babangida (IBB) University Animal farm, Lapai, Niger State situated in between (latitude $9^{0}03^{l}17.60^{ll}$N and latitude $9^{0}05^{1}07.22^{11}$N) and (longitude $6^{0}33^{1}49.53^{11}$E and longitude $6^{0}35^{1}38.47^{11}$E). These were transported to the Department of Biology laboratory, IBB University Lapai, Niger State where the experiment was conducted.

A

B

Plate 1: Water-hyacinth sampling site. A= View of Jabi Lake, Abuja; B=Samples of water-hyacinth sampled around the water bank.

Experimental Procedure

The bacteria isolation, identification in the sampled water, plant's leaves, stolon and roots were done using serial dilution techniques in accordance with the standard procedure reported in Cheesebrough (2000). The isolation and identification of fungi from the plant's leaves, stolon and root with Potato Dextrose Agar was in accordance with the procedure of Fawole and Oso (1998).

Lactic Acid bacteria used for the fermentation of the plant were obtained from natural product (locally fermented African locust bean – *Dadawa:* a soup recipe). They were isolated based on the study reported by Mohammed and Ijah (2013), following the procedure of Cheesebrough (2000). Samples of shred plant's leaves and stolon were fermented with the isolated bacteria for 72 hours. Thereafter, the fermented and non fermented plant parts were subjected to proximate compositions (crude protein, crude lipids/fats, crude fibre, carbohydrate, ash, moisture and energy) in accordance to the standard procedure of AOAC (2007).

Some samples of the plant were shred (Larsen *et al.*, 1991 and Moorhead and Nordstedt, 1993) to improve its density to a higher value as well as to increase the active site for higher microbial action on it during digestion. The shredded water hyacinth and cow dung in small amount (10%wt) were co-digested at a specified proportion. Anaerobic digesters were designed and constructed as supported by the study of Batstone and Keller (2003).

the 21 days retention time. Biogas was produced and the sludge was thereof collected.

Three (3) basic mixing formulation ratios used were reached after preliminary investigation where each formulation had two portions; the seeded and the un-seeded portion, the seeded portion was catalysed with 10g of cow-dung as inoculum, while the un-seeded portion contains no inoculums in replicate setup thus;

i. F_1 (Un-seeded) = 1:1 (water hyacinth: water), labelled F1
ii. F_1 (Seeded) = 1:1 (water hyacinth: water) + 10g cow-dung, labelled as F1-S.
iii. F_2 (Un-seeded) = 1:2 (water hyacinth: water), labelled as F2.
iv. F_2 (Seeded) = 1:2 (water hyacinth: water) + 10g cow-dung, labelled as F2-S.
v. F_3 (Un-seeded) = 1:3 (water hyacinth: water), labelled as F3.
vi. F_3 (Seeded) = 1:3 (water hyacinth: water) + 10g cow-dung, labelled as F3-S.

The digesters were thereafter, closed air tight, agitated, allowed to rest and digest anaerobically to produce biogas. The volume

Six (6) single 12.620 litres and one (1) single 30 litres of improvised steal cylinder cans were used as anaerobic digesters. The biogas digester consists of a control valve for biogas flow regulation, and a one inch socket-pipe combination that can be closed and opened, for lodging and dislodging of formulation (slurry) and sludge respectively at the top. 1.0kg (1000g) of the shredded biomass was fed to the single stage digester. The equivalent amount of water was added for

of biogas produced daily was collected and measured over water in a measuring cylinder and burette (Plate 2). The experiment was conducted at room temperature, which is within the mesophilic temperature range (Batstone *et al.*, 2003). The pH of each medium before the digestion was determined using a pH meter, and found to fall within the range of 6.8 and 7.2.

Plate II: Experimental set-up for studying the biogas yield from water hyacinth

Data Analyses

The data obtained from the identified bacteria, fungi and proximate compositions were presented in tables. Single and double factor(s) analysis of variance was used to test for significance difference using Minitab software version 16.1. The daily and

cumulative biogas yield recorded for each formulation over the 21 days retention period was collated. The percentage difference was calculated; average biogas yield in millilitres per day was also computed. A line graph of the volume of biogas produced in (ml) each day against the retention period in days was also presented.

RESULTS

Microbial Studies

Seven (7) bacteria were isolated and identified in water samples obtained from the root region of the sample plant as presented in Table 1. In addition to the seven isolated bacteria from the roots, two additional bacteria were isolated and identified from the plant's leaves. In addition to the above, *Pseumona aeraginosa*, *Salmonella typhi* and *Staphylococcus aurens* were isolated from the stolon. In the root were *Salmonella typhi*, *E. coli* and *Proteus vulgaris* as presented in Table 2. Similarly, the fungi associated with the plant's leaves, stolon and roots are presented in Table 3. *Aspergillus niger* and *Penicillum digitatum* were most dominant.

Table 1: Cell Morphology and Biochemical Characteristics of Isolates from water-hyacinth, habitat (water) sampled from Jabi Lake, Abuja

Isolate code	Cell Morphology	Gram Reactivity	Motility	Catalase	Oxidase	Citrate	Urease	Indole	Glucose	Sucrose	Lactose	Dextose	Organism
WS2	Rod	G-	M	+	-	-	+	+	G	A	AG	AG	*Escherichia coli*
WS4	Rod	G-	+	+	+	+	-	-	-	-	A	A	*Pseudomonas aeraginosa*
WS5a	Rod	G-	+	+	-	+	+	+	G	A	-	A	*Salmonella aurens*
WS5b	Rod	G-	+	+	-	+	+	-	G	A	-	A	*Shigella dysenteriae*
WS6a	Rod	G-	+	+	-	+	-	-	G	A	-	A	*Salmonella typhi*
WS6b	Rod	G-	+	+	-	-	+	-	G	A	A	A	*Proteus vulgaris*
WS6c	Rod	G-	+	+	-	-	+	+	-	A	A	A	*Klebsiella ozaenae*

Key: + = Positive; - = Negative; A= Acid production; G= Gas production; G- = Gram negative; AG=Acidic and gas production; M = Moderate

Table 2: Cell Morphology and Biochemical Characteristics of Isolates from water-hyacinth sampled from Jabi Lake, Abuja

Plant Part	Cell Morphology	Gram Reactivity	Motility	Catalase	Oxidase	Coagulase	Citrate	Urease	Indole	Glucose	Sucrose	Lactose	Dextose	Organism
Leaves	Rod	G-	M	+	-	-	-	+	+	G	A	AG	AG	*Escherichia coli*
	Rod	G-	+	+	-	-	+	+	-	G	A	A	-	*Proteus mirabilis*
Stolon	Rod	G-	M	+	-	-	-	+	+	G	A	AG	AG	*Escherichia coli*
	Rod	G-	+	+	+	-	+	-	-	-	-	A	A	*Pseudomonas aeraginosa*
	Rod	G-	+	+	-	-	+	-	+	G	A	-	A	*Salmonella typhi*
	Rod	G-	+	+	-	-	+	+	-	G	A	A	-	*Proteus mirabilis*
	cocci	G-	-	+	-	+	-	+	-	G	A	A	A	*Staphylococcus aureus*
Root	Rod	G-	+	+	-	-	+	-	+	G	A	-	A	*Salmonella typhi*
	Rod	G-	M	+	-	-	-	+	+	G	A	AG	AG	*Escherichia coli*
	Rod	G-	+	+	-	-	+	+	-	G	A	A	A	*Proteas vulgaris*

Key: + = Positive; - = Negative; A= Acid production; G= Gas production; G- = Gram negative; AG=Acidic and gas production; M = Moderate

Table 3: Fungi Isolates from water-hyacinth sampled from Jabi, Lake, Abuja

Plant Part	Code	Macroscopic examination	Microscopic examination	Organism
Leaves	WHL2	Black colonies, white-white edges	Conidial head are large globase	*Aspergillus niger*
	WHL4	Smooth green colonies	Hyphae is colourless	*Penicillium digitaum*
	WHL5	-	Thalus is multinucleated	*Mucor mucedo*
Stolon	WHS5	Smooth green colonies	Hyphae is colourless	*Penicillium digitaum*
	WHS7	Black colonies, white-white edges	Conidial head are large globase	*Aspergillus niger*
	WHS8	Light yellow green	Conidial head are radicate	*Aspergillus flavus*
	WHS9	Colonies	Thalus is multinucleated	*Mucor mucedo*
Root	WHR1	Whitish-sponge	Threadlike structure	*Mucor mucedo*
	WHR2	Thread-like structure	Rhizoids	*Aspergillus niger*
	WHR3	Light yellow green	Conidial head are radicate	*Aspergillus flavus*
	WHR6	Smooth green colonies	Hyphae is colourless	*Penicillium digitaum*

The morphology, cultured and biochemical characteristics of lactic acid bacteria isolated from the locally fermented African locust bean (*Dadawa*) is presented in Table 4.

Table 4: Cell Morphology and Biochemical Characteristics of Lactic acid bacteria isolated from locally fermented African Locust Bean (Dadawa) obtained from Lapai Market

Isolate Code	Colony Morphology	Cell Morphology	Gram Reactivity	Oxidase	Manitol	Catalase	NH_3 from Arginine	Gelatin Liquifaction	Glucose	Sucrose	Lactose	Fructose	Organism
FALB1	Circular	Cocci chain	G+	-	-	-	+	-	AG	A	AG	A	*Lactococcus latis*
FALB2	Slightly convex	Rods	G+	-	-	-	-	-	G	A	A	A	*L. lactobacillus fermentum*
FALB3	Circular convex	Rod	G+	-	-	-	-	-	AG	-	AG	AG	*Lactobacillus bulgarius*

Key: + = Positive; - = Negative; A= Acid production; G= Gas production; G+ = Gram positive; AG=Acidic and gas production

Proximate Composition

The proximate compositions of the fermented and non-fermented water-hyacinth are presented in Table 5. The crude protein content and carbohydrates increased insignificantly ($P > 0.05$) while energy value was recorded to increase significantly ($P < 0.05$) with fermentation. However, other determined parameters were observed to decrease significantly ($P < 0.05$) and insignificantly ($P > 0.05$).

Biogas Production

The amount of biogas produced per day was presented in Fig 1. It was observed that the rate of production increased as the retention period increased with seeded set-up recording the highest values of daily production yield. F3-S recorded the first biogas production yield of 16.80ml at day one, while F1 recorded its biogas production yield of 0.08ml at day 12 which therefore recorded the highest and least biogas production yield at the end of 21-day retention period respectively.

Table 5: Mean ± Standard error of proximate composition of fermented and non-fermented water-hyacinth obtained from Jabi Lake, Abuja.

Status	Plant part	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy value (Kcal)
NF	Leaves	62.57±2.34	14.41±0.31	2.02±0.08	10.90±1.12	14.80±1.32	9.31±0.44	451.92±23.57
	Stolon	61.82±1.45	13.72±1.01	1.80±0.02	13.51±1.41	13.40±1.89	9.04±0.39	413.28±29.38
F	Leaves	20.44±1.28*	18.69±1.26	1.62±0.14	5.37±0.42*	2.27±0.03*	57.05±8.18*	1206.162±112.21*
	Stolon	17.83±1.92*	17.71±2.21	17.71±1.21*	7.66±0.63*	24.02±2.46*	55.12±6.98*	1220.72±125.03*

NF=non-fermented; F=Fermented, * $p<0.05$ when compared with its corresponding parameter in non-fermented form

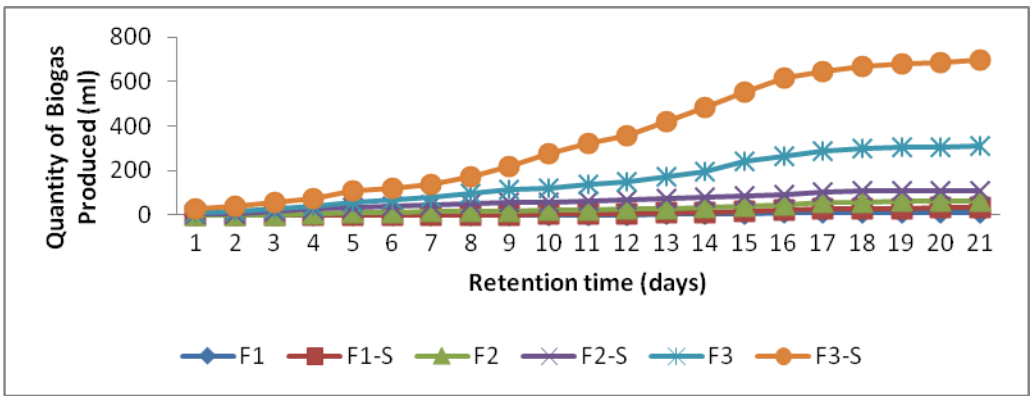


Fig 1: Daily Biogas production as a function of reaction time for the various water hyacinth formulations during the 21-day retention time

The average value of biogas produced per day of 3.17, 6.62, 17.33, 35.61, 93.46 and 193.64 ml was recorded for F1, F1-S, F2, F2-S, F3, and F3-S set-ups respectively. The percentage changes in biogas production in relation to the inoculum and dilution are presented in Table 6. The dilution factor increased as the rate of biogas yield increased (Fig 2). The total biogas produced after the 21 days retention time was recorded as 66.50, 139.10, 364.00, 747.90, 1962.60 and 4066.40ml for digesters F1, F1-S, F2, F2-S, F3, and F3-S respectively showing that the seeded formulation recorded higher values compared to the un-seeded and F3-S recorded the highest value.

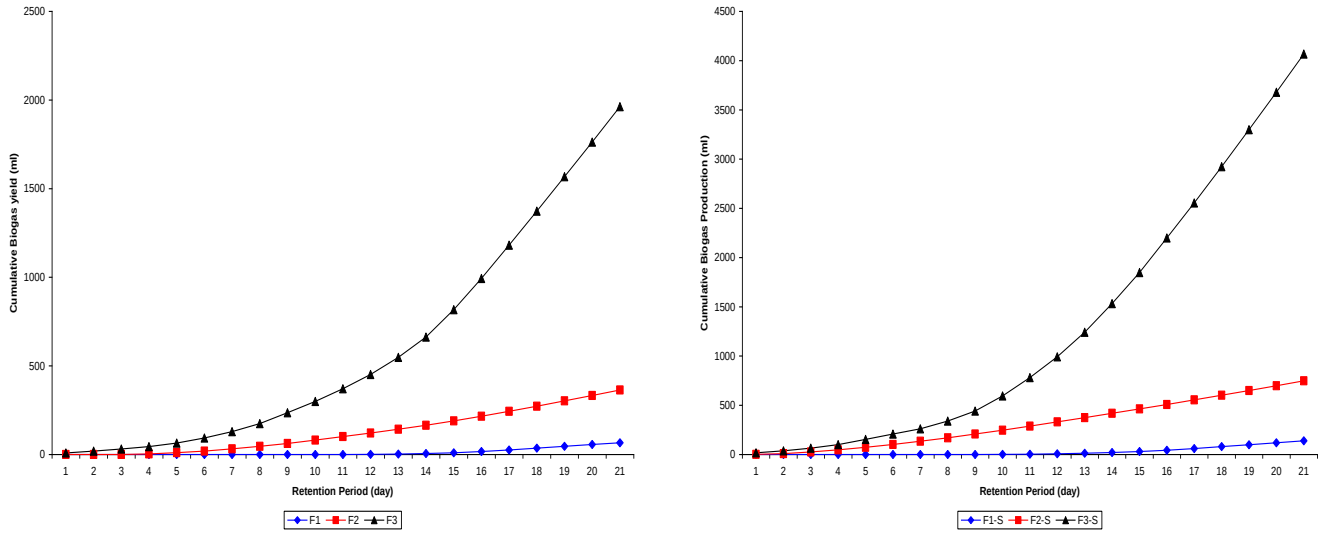
Table 6: Percentage Change in Total biogas production from water-hyacinth after the 21-day retention period

Change between seeded and Unseeded

Setup	Total biogas production (ml)	% Change
F1	66.50	-
F1-S	139.10	35.31
F2	364.00	-
F2-S	747.90	34.53
F3	1962.60	-
F3-S	4066.40	34.89

Change between dilution ratio

Setup	Total biogas production (ml)	% Change
F1	66.50	-
F2	364.00	69.11
F3	1962.60	93.45
F1-S	139.10	-
F2-S	747.90	68.65
F3-S	4066.40	93.38



A B

Fig 2: Cumulative Biogas yield from water-hyacinth during the 21-day retention period, A is Unseeded While B is seeded setup

DISCUSSION

The presence of these bacteria may be an indication that the plant can be utilized for the production of microbial cellulose as it serves to provide large surface area for microbial attachment that may stimulate biodegradation of organic matter and other nutrients. Some of the bacteria isolated have been reported by NBS (2010). UNEP (2013), had identified that water-hyacinth played role as breeding ground for many pests, vectors and causative agents of epidemic diseases (Feikin *et al.*, 2010) thus the resultant microbial diversity associated with the plant. The presence of these isolated bacteria is an indication of human and animal contamination of the water body.

The fear of transmitting the pathogen after biogas production has be alleviated by the findings of NBS (2010), that these isolated pathogens can only survive at the mesophilic fermentation for only seven days. Thereof, the 21-day retention study period, will allow for complete eradication of the pathogens. Nature has also provided for the control of biological system proliferation as reported in the study. The presence of isolated fungi that act affect the growth, spread of water-hyacinth is a biological control measure. Thereof, may be responsible for the decrease of the water-hyacinth mat as they are known to cause stunted growth affecting the production of new leaves and stolons.

The high ash content reported in this study is an indication that the plant has high mineral content (Sheob and Singh, 2002). The higher crude protein recorded in the leaves is indifferent with the report of Okoye *et al.*, (2002) that the leaves have higher crude protein content than other parts of the plant. The quantity of reported crude protein content that increases with fermentation is an indication that the plant can be used as supplementary protein source as Virabalin *et al.*, (1993) reported that the plant contains glutamine, asparagines and leucine which are of good importance to fish growth. The ash and crude fiber contents reported in this study were within the ranges reported by Kumar *et al.,* (2008). The study thereof revealed that the inoculum reduced the lag phase as production began on day one compared to the study of Almoustapha *et al*., (2009) that reported production after day 8. It can be deduced that the introduction of inoculum (cow dung) to any of the formulation had increased the total biogas yield by nearly 2 times, compared to similar formulation without cow dung. This result is comparable to the work reported by Jagadish *et al.,* (2012), where poultry litter was used as inoculum. The choice of cow-dung as inoculum to optimize the characteristics of the feedstock has yielded positive result as Gunnarsson and Petersen (2007), suggested that for any substrate to be an effective inoculum it must contain enough microorganisms that will aid digestion. The study also revealed that formulation with cow-dung greatly reduced or cancelled out the lag phase period of biogas production, and had promoted immediate and progressive biogas generation. Where there is a reduced or cancelled lag phase, the initial and cumulative biogas yield over the retention time of 21 days was greater than the formulation without innoculum. This finding is in concordance with the conclusion reached by Jagadish *et al.,* (2011) and NBS (2010). It was also recorded that increase in dilution increased, the total biogas yield nearly 34% which is comparable to that reported by Jagadish *et al.,* (2011). The retention time of 21 days adopted for this study was within the average value of the 14 days reported by Yadvika *et al*., (2004) and the 30 days reported by Van der Werf, (2014).

Similarly, the report of Almoustapha *et al.,* (2009) on daily production biogas was lesser than that reported in this study. These high values obtained may be related to the mesophilic temperature, inoculum and the reduced retention time of the substrate in the digester as supported by SERC (1994).

The maintenance of a pH range of 6.8 and 7.2 in this study was supported by earlier studies (Kaparaju and Angelidaki, 2008 and Demirci and Demirer, 2004) where Bailey and Ollis (1977), reported that for increased biogas

yield a pH of 6.6 to 7.6 is adequate. Similarly, temperature is an important parameter that affects biogas production as it affects the enzymatic activities of the micro-organism responsible for the bioconversion of substrates into gas (Tchobanoglous *et al.*, 2003) thus the temperature was maintained at room temperature (25 to 30°C) as supported by NBS (2010).

The biogas produced was flammable, combustible with odour and on combustion produced blue flame indicating the presence of methane. This odour can be attributed to the presence of other gases such as H_2S. The study thereof revealed that the higher the dilution level and presences of cow-dung, the higher the biogas production yields. Thence, the production of biogas from water hyacinth can serve as a sustainable control measure for managing the weed proliferation, additionally, the improved energy value, carbohydrate as well as crude protein contents after fermentation had also proved its potential for inclusion in fish feed. However, the presences of associated pathogens are threat that needs to be addressed appropriately.

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