



Investigation on Water Hyacinth in Anaerobic Co-Digestion for Biogas Production: A measure to Reduce Kosavampatti and Phoosur Lake Municipal Solid Waste Loading

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ABSTRACT

The main aim of this study was to evaluate the lab scale, microbe-enhanced biogas production from water hyacinth blended with poultry waste and cow dung. A mesophilic anaerobic two-stage continuous reactor was set up to study the co-digestion for enhanced biogas production. The optimized mixing ratio of cow dung, water hyacinth, and poultry litter (2:1:1; 4:3:1 and 5:2:1) was used along with the effective microbial solution in a two-stage continuous reactor. The biogas yield was maximum in the 2:1:1 blend than the blends with a mixing ratio of 4:3:1 and 5:2:1. The reactor-loading rate was 4 and 5 g.L⁻¹.day⁻¹ with a retention time of 30 and 60 days respectively. The two-stage anaerobic digestion helps in controlling toxicity by acidogenesis and enhances energy production, thereby proving to be a technology that prevents environmental deterioration and enhances energy recovery, both of which are twin issues that need scientific attention. Maximum specific biogas of 0.128 Nm.L.g⁻¹ of Volatile solid reduction (VSR) in hydrolyzer and 0.205 Nm.L.g⁻¹ of VSR in methanizer was obtained at an optimized Carbon/Nitrogen (C/N) ratio of 20.8. The co-digestion of protein-rich waste with a carbon-rich source offers a reduced acclimatization period in the hydrolyzer with an increased C/N ratio, thereby increasing the biogas yield as observed in the methanizer. Several other parameters including Ammonia level, Volatile Fatty Acids influenced by the reactor alkalinity also determine the biogas yield. With increased alkalinity, free ammonia increases and may be inhibitory for anaerobic fermentation and may be toxic for methanogenic bacteria, thereby contributing to the reduction in biogas yield. The present investigation has led to a novel biogas and power-producing clean development mechanism (CDM) for the first time from a heterogeneous mixture of animal excreta and plant waste (livestock droppings, cow dung, and water hyacinths). The waste samples have been collected from sewage and sullage polluted Kosavampatti Lake and Phoosur lake in Namakkal district of Tamilnadu, India therein ending up in a high quantity of CO₂ mitigation.

INTRODUCTION

Tropical and subtropical regions are encountering increasing abundances of invasive floating species (Gopal 1987, Arya et al. 2016, Portielje & Roijackers 1995). Moreover, aquatic species perform important ecosystem functions, particularly in shallow ecosystems, where they may act as engineer species, changing the structure of the ecosystems that they colonize (Bartoli et al. 2010). Most of India's water bodies are small (<1 km²) water-harvesting ponds and lakes that are often characterized by high nutrient inputs and substantial floating species coverage (Murthy et al. 2012). A common floating species in India is the invasive water hyacinth present since 1890 (*Eichhornia crassipes*), which is native to the lowlands of South America (Barrett & Forno 1982). Because of its rapid growth rate, which can double

the biomass within five days, and its ability to successfully compete with other aquatic plants, water hyacinths now cover more than 2,000 km² of the freshwater bodies in India, which corresponds to 10% - 15% of the total area covered by aquatic vegetation (Venugopal 2002, Attermeyer et al. 2016). Reports are evident that the invasive weed is a very good source of renewable energy for the biosynthesis of biofuel (Antai et al. 2014) and hence, biogas production by anaerobic digestion would provide a means for their disposal as well as an added benefit of energy production.

Anaerobic digestion is one such energy-efficient technology in which microorganisms utilize carbon as a sole energy source which subsequently gets converted by oxidation and reduction to its oxidized state (CO₂) and reduced state (CH₄) (Ahring et al. 2003, Ahring & Angelidaki 1993). The merits

of this anaerobic digestion process from agricultural biomass are of growing importance as it offers considerable monetary and environmental benefits (Chen et al., 1980, Chynoweth 2004) and becomes an additional source of income to farmers. Anaerobic digestion of lignocellulosic substrates is a much more complex process, requiring the syntrophic and cooperative interaction between several types of microorganisms. It is a multi-stage, complex, natural process in which a consortium of microbes degrades organic molecules into methane and carbon dioxide via a variety of intermediates (Gujer & Zehnder 1983, Gyllenberg et al. 2002, Healy 1979 Tadrup 1994, 1995). It's a four-step process with various trophic groups participating at each stage. In the first (hydrolysis) stage, organic macromolecules are broken down into monomers like sugars, fatty acids, and amino acids. In the second, the acidogenesis stage, these components are further broken down into VFAs (volatile fatty acids: short-chained fatty acids like acetate, butyrate, or propionate), organic acids, and alcohols, along with small amounts of hydrogen. The largest fraction of H_2 and acetate comes from the third step, the acetogenesis stage, in which bigger VFAs and other organic acids from the previous stage are converted into the two substances. After the final methanogenesis stage, methane and carbon dioxide are formed as the final products (Miyamoto 1997, Murugesan et al. 2020).

Among the different substrates exposed to anaerobic processing (Gyllenberg et al. 2002) for biogas production, better yields of biogas are achieved utilizing a combination of animal waste and lignocellulosic waste. Animal waste especially cow dung has a huge syntrophic system upgrading microbes preferring biogas production. Since the plant has plentiful nitrogen content, it tends to be utilized as a substrate for biogas production. For enhancing the C/N proportion of agricultural waste, co-digestion with sewage slime, animal excrement, or poultry litter is suggested. Then again, the high nitrogenous mixes in the livestock and cattle manure upgrade the ammonia toxicity in the anaerobic digestion and thereby hinder the methane formation (Heinrichs et al. 1990, Addink et al. 1988). However, these nitrogenous compounds were decreased by the co-digestion with different carbonaceous wastewater which could reduce the toxic effect for methane formation (Tadrup 1995, Catharina et al. 2015). Various examinations exhibit the process stability, control, smaller reactor volumes, and high tolerance to toxicity effect and shock loads, as an advantage of anaerobic digester over regular anaerobic digester (Borja et al. 2009a, Chen et al. 2010, Borja et al. 2009b, Koutrouli et al. 2009, Chen & Demirel 2004, Demirel & Yilmaz 2008, Demirel & Othman 2008).

Generally, animal manures contain high protein which on degradation releases ammonia which is a potent inhibitor of methanogenesis (Heinrichs et al. 1990). To meet the

problem, co-digestion technology has been followed to provide nutrient balance (Lebioccka et al. 2008), relatively high methane yield, dilution of a toxic substance (Borja et al. 2009), thereby providing better digester performance and maximum biogas production. In rural and semi-urban areas, handling and utilization of agricultural waste and livestock droppings is a major problem. Many existing solid waste management methods such as landfilling, incineration, composting, and pyrolysis always trigger primary, secondary, and tertiary environmental impacts such as odor problems, Green House Gases (GHG) emissions, and groundwater contamination, etc Abbasi et al. 2005. Alternately, the waste to energy technology through biomass has attracted the solid waste managers for the production of biogas and power coupled with a high quantity of CO_2 mitigation as an initiative to clean development mechanism (CDM). In this study, a solution to the water hyacinth menace in the water bodies that are ultimately dumped into the landfills has been attempted. A dual-stage biogas production technology was developed using livestock droppings, cow dung, and water hyacinths as raw materials collected from sewage and sullage polluted Kosavampatti Lake and Phoosur lake in Namakkal district of Tamilnadu, India.

MATERIALS AND METHODS

Sample Collection and Feed Stock Preparation

Water hyacinth used for the study was obtained from Kosavampatti lake and Phoosur lake; Fresh poultry litter was collected from Municipality and cow dung was obtained from the livestock farm all located in Namakkal District, Tamilnadu, India. The inoculum was prepared using 150 grams crushed jaggery, 100 grams curd, and 1 L pure water. It was kept for 6 days under anaerobic conditions to ferment. After the fermentation period, three different substrates viz., cow dung, water hyacinth, and poultry waste were mixed in three different ratios (2:1:1, 4:3:1, and 5:2:1) for processing.

Characterization of Feedstock and Digester Material

The samples prepared in three different ratios were analyzed for total solids (TS), volatile solids (VS), volatile fatty acids (VFA), pH, and Alkalinity using a standard protocol (APHA 1998). The moisture content (MC) in the test samples was determined according to ASTM- D 3173-87 (ASTM 2002). The pH was recorded daily using a digital pH meter Eco-Scan (Eu-tech instrument Singapore) and adjusted to neutral by the addition of either 6 N NaOH or 1 N HCl.

Batch and Dual-Stage Continuous Reactor Operation

The study was carried in two different reactor arrangements i) anaerobic Biochemical Methane Potential (BMP)

batch reactor and ii) mixed continuous dual-stage anaerobic reactor to evaluate the maximum stable biogas yield.

Biogas Generation in BMP Reactor

The serum bottles of capacity 500 mL considered equivalent to batch BMP reactors were used. The mixing ratios of the three proposed waste were decided based on the Mariotee principle (Tadруп 1995). The study was carried to evaluate the gas yield potential of co-digested slurry containing cow dung, water hyacinth, and poultry waste in three different mixing ratios 2:1:1, 4:3:1, and 5:2:1 in three different anaerobic BMR. The pH was checked daily and adjusted by the addition of either 6 N NaOH or 1 N H₂SO₄. The Biogas generation was measured; the biogas yield and the effect of substrate mixing ratios on it is shown in Fig. 3a.

Biogas Generation in a Two-stage (Hydrolyzer and Methanizer) Anaerobic Reactor

The waste mixed in optimal proportions was subsequently fed into the dual-stage anaerobic reactor (hydrolyzer and methanizer) shown in Fig. 1, the configuration of which is given in Table 1. Both the digesters were initially fed with inoculum which was collected from Common Effluent Treatment Plant (CETP), Pallavaram, and mixed with substrates cow dung, poultry waste, and water hyacinth for the acclimatization of the anaerobic organism for a period of one month. The prepared feedstock of 50% TS was used as an influent for the first stage hydrolyzer reactor after being purged with nitrogen gas for 10 min to achieve strict anaerobic conditions. The effluent of the hydrolytic reactor was used as an influent for the second stage methanogenic reactor after adjusting the pH to 7 with 5N NaOH. The volume of biogas production was measured by a water displacement system at STP (Standard Temperature and Pressure).



Fig. 1: Schematic representation of two-stage continuous anaerobic digester.

RESULTS AND DISCUSSION

Characterization of Waste and Composition

Primarily, physico-chemical properties of cow dung, water hyacinth, and poultry waste were determined, and the results are given in Fig. 2(a) and Table 2. It is evident from Fig. 2(a), TS and Moisture of both cow dung and water hyacinth are in proximity while in the poultry waste the TS is predominantly higher than the Moisture. The predominance of Carbon in the slurry makes them suitable for Biogas production (McCarty & McKinney 1961). The elemental analysis of the cow dung, water hyacinth, and poultry waste is taken in the slurry in different proportions is given in Fig. 2(b). A higher percentage of Oxygen, zero % Sulphur, lower ash content makes water hyacinth a suitable waste material that can be potentially blended with the cow dung used in biogas production (EI-Mashad & Zhang 2007).

Effect of Mixing Ratio on Biogas Yield in BMR

The study was carried to evaluate the gas yield potential of co-digested slurry containing cow dung, water hyacinth, and poultry waste in three different mixing ratios 2:1:1, 4:3:1, and 5:2:1 in three different anaerobic BMR. From Fig. 3(a) it is evident that biogas production in all three digesters with respective mixed substrates starts without any lag. Surprisingly, the 2:1:1 substrate ratio produces the most biogas and is stable for longer periods. On the contrary biogas generation potential and its stabilization while compared to other co digested slurry (4:3:1 and 5:2:1) shows slight variation because of ammonia release and VFA which is toxic to methanogenesis. The maximum biogas yield was obtained in a blend ratio of 2:1:1 (cow dung, water hyacinth, and poultry waste) in the pH of 6.5 in which the cumulative biogas yield was observed as 895 Nm.mL⁻¹ whereas, in the

Table 1: Configurations and operations of anaerobic lab-scale continuous reactors.

Sl. No.	Description	Dimension [cm]	Volume [mL]
1	Total height of Cylinder 1	10	
2	Inner diameter of cylinder 1	11.5	1038
3	Effective Liquid Volume	7.4	778
4	Effective liquid volume of cylinder 2	14.2	2255
5	Inner diameter of Cylinder 2	14.2	3007
6	Total height of Cylinder 2	18	
7	Sample Draining 1	½ inch pipe connected to similar valve (1/2 inch)	

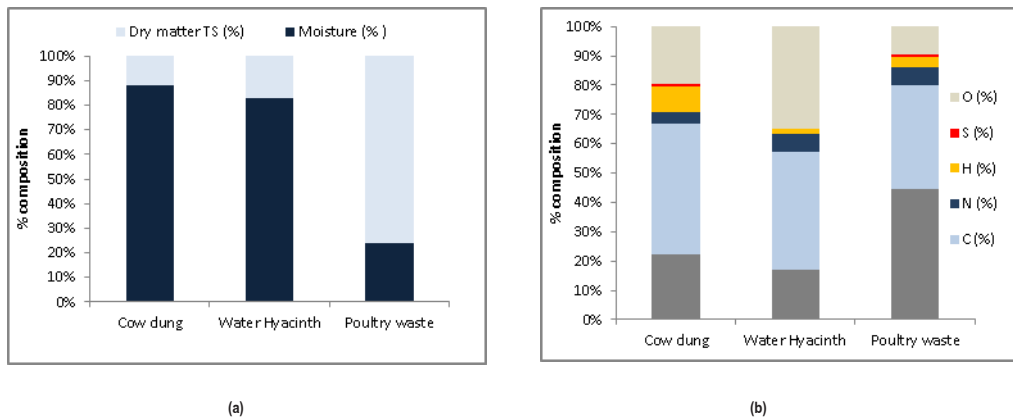


Fig. 2: Characterization of waste and composition. (a) composition analysis; (b) elemental analysis

ratio of 4:3:1, the same was reduced to 694 Nm.mL^{-1} . Dichtl et al. (2007) stated that ammonia levels exceeding 100 mM could inhibit methanogenesis (Manoharan et al. 2014, Samuel & Ukwuaba 2018).

Biogas Yield in the Dual-Stage Anaerobic Reactor

In the case of two-stage continuous reactors (Fig. 3b), the biogas yield was high when compared to the batch reactor. Cooney (2007) also reported that a dual-stage continuous reactor will provide a considerably better yield compared to the batch reactor. In hydrolyzer, the biogas yield was about 3605 mL and 5221 mL in methanizer (Fig. 3b). Since the hydrolyzing reaction is carried out separately, it produces a considerable yield of biogas.

Table 3 shows the specific biogas yield in a dual-stage continuous reactor with a shorter period of HRT i.e. during

maximum gas production. In this study, the co-digestion of cow dung, water hyacinth, and poultry litter produced enhanced biogas with optimized conditions. The specific gas yield in the hydrolyzer was $0.128 \text{ L.g}^{-1} \text{ VSR}$ whereas in the methanizer was $0.205 \text{ L.g}^{-1} \text{ VSR}$. Several previous studies on the anaerobic co-digestion of water hyacinth with other organic waste like cattle dung were reported to increase biogas yield and COD removal (Tadруп 1995, El-Mashad & Zhang 2007, Koutrouli et al. (2009). Table 3 shows the process performance of a dual-stage anaerobic digester with 30.4 per cent of volatile solids removal in the hydrolyzer and 26.6 per cent in the methanizer (Fig. 4a and 4b). Patil et al. 2014 reported a biogas yield of $0.36 \text{ L.g}^{-1} \text{ VS}$ for an HRT of 60 days in a batch reactor of co-digestion of water hyacinth with sheep waste. Comparing the results obtained with reported literature provides a clear idea that the

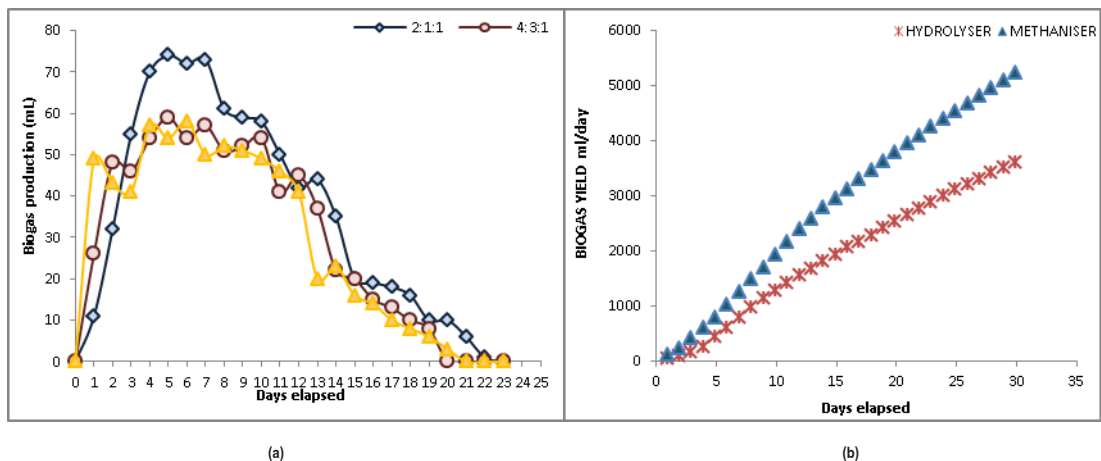


Fig. 3: Cumulative biogas yield for three different substrate ratios. (a) Biogas yield in BMR; (b) Biogas yield in two-stage continuous reactors

Table 2: Characteristics of the feedstock.

Parameters	Cow dung	Water Hyacinth	Poultry waste
Moisture (%)	88±2.3	83.1	26
Dry matter TS (%)	12±2.3	16.9	74
Organic matter VS (%)	77.9±4.5	82.8	72.3
Inorganic matter (% of TS)	22.1±2.5	17.2	27.7
C (% wt /wt)	44.7	40.2	33.3
N (% wt /wt)	4.1	1.8	3.2
P (% wt /wt)01	2.5	1.0	2.99
K (% wt /wt)	3.4	2.9	2.35

Table 3: Specific biogas per gm fed and VS removed in Hydrolyser and Methanizer.

Digester sample	OLR [g.L ⁻¹ .d ⁻¹]	HRT [days]	TS _{in} [%]	VS _{in} [%]	TS _{out} [%]	VS _{out} [%]	VS _r [%]	SPG [L.g ⁻¹ VS _r]
Hydrolyser	4	17	23.01	68.31	16.06	44.7	30.4	0.128
Methanizer	5	11	12.01	44.7	10.3	32.21	26.6	0.205

*SPG- Specific Gas yield, HRT- Hydraulic retention time, TS –Total Solids, VS- Volatile solids

dual-stage is more effective and provides better biogas yield when compared to the batch reactor within a shorter span of HRT.

Effect of Ammonia and Alkalinity on Biogas Yield

The minimum and maximum level (Table 4) of NH₃ in the batch was observed as 1500 mg.L⁻¹ and 5500 mg.L⁻¹. The average NH₃ level in the whole of the batch experiment was 4500 mg.L⁻¹. The observed minima and maxima NH₃ level was correlated with an alkalinity level of 6500 mg.L⁻¹ on the same day, which confirms that the formation of high NH₃ was

triggered by the digester alkalinity conditions. Similar results were also observed by Sung & Liu et al. (2003) which caused the methane production rate to drop approximately 39% and 64%. It was clear that the initial ammonia concentration was high in the three mesophilic batch reactors due to the protein substance present in animal manure.

The initial concentration of NH₃-N in reactor 1 containing the 2:1:1 of Cow dung: poultry waste: water hyacinth slurry is 2732 mg.L⁻¹. Despite this, researchers have shown that as ammonia is introduced to the digester, the pH rises until it reaches equilibrium, after which it drops, resulting in VFA

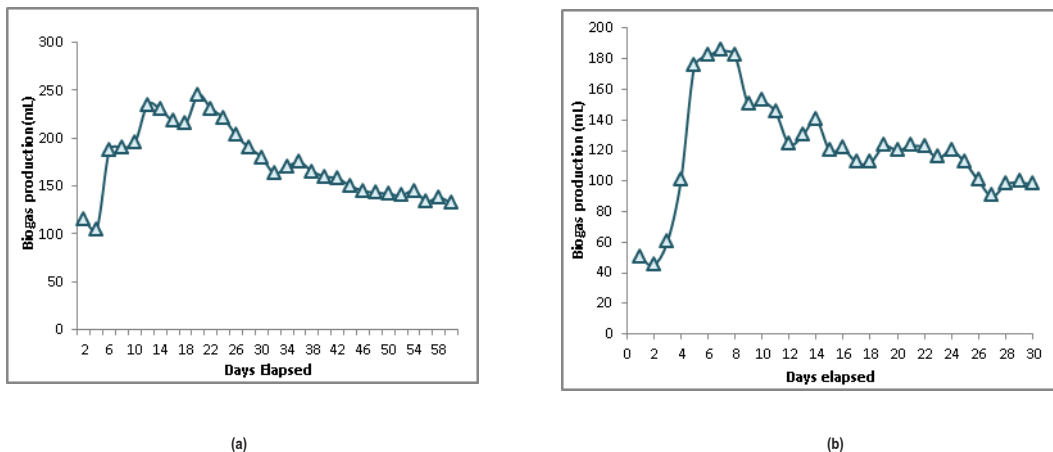


Fig. 4: Biogas production in two-stage continuous reactor (a) Hydrolyser (b) Methanizer

Table 4: Process performance evaluation for three different mixing ratios.

Parameters	2:1:1		4:3:1		5:2:1	
	[Max]	[Min]	[Max]	[Min]	[Max]	[Min]
TS (%)	11	5	16.5	3	21	7
VS (%)	89	40	52.7	50	42.8	35.7
pH	7.2	6.2	7.5	5	7.1	8.3
VFA (mg. L ⁻¹)	1200	5600	1500	7600	800	5950
Alkalinity (mg. L ⁻¹)	2500	3200	3200	2100	1500	5500
NH ₃ -N (mg. L ⁻¹)	2530	2100	3210	1820	2520	4500
C/N	20.8		23.3		24.4	

formation as a result of the pH drop (Engler et al. 2001). In contrast, the waste (cow dung) containing the methanogenic bacteria are used in mixing ratio 5:2:1 the unionized ammonia on the 10th day was too high (5200 mg.L⁻¹) would be attributed to the drop in biogas production. For instance, free ammonia may be inhibitory for anaerobic fermentation and may be toxic for methanogenic bacteria (Gyllenberg et al. 2002, EI-Mashad & Zhang 2007, Demirer & Yilmaz 2008).

Effect of VFA and alkalinity on Biogas Yield

Cessation of biogas production could be attributed to the accumulation of volatile fatty acids, whereas high protein substrate leads to a high ammonia concentration. Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which again leads to a decrease in pH and thereby a declining concentration of ammonia. Before digestion, the pH of the reactor is 7 and after 20 days

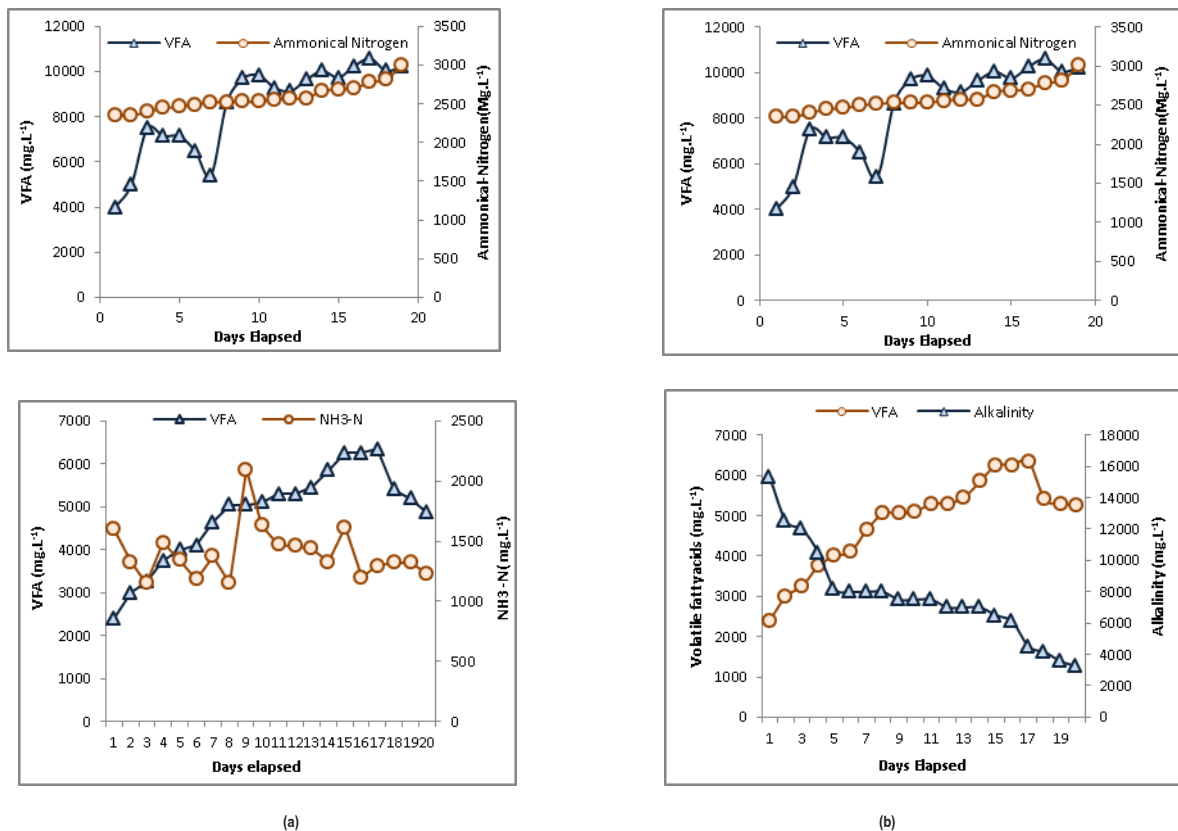


Fig. 5: Relationship between VFA, Alkalinity and Ammonical nitrogen in (a) Hydrolyzer (b) methanizer.

Table 5: Performance indicators of Hydrolyser and Methanizer.

Parameter/mixing ratio	Hydrolyser		Methanizer	
	Maximum	Minimum	Maximum	Minimum
TS [%]	23.1	16.01	12	8.2
VS [%]	68.3	81.5	40.3	45.23
pH	8.3	5.2	6.5	7.1
VFA [mg. L ⁻¹]	10050	4000	5450	3250
Alkalinity [mg. L ⁻¹]	6500	6000	5000	10500
NH ₃ -N [mg. L ⁻¹]	2400	2576	1600	1296
ORP	-250	-256	-270	-280

it dropped to acidic condition, where the process is made stable by adding NaOH at regular intervals 0, 5, 10, 15, and 20th day. It has been reported that a concentration of volatile fatty acids (VFA) of more than 2000 ppm inhibits methane formation in an anaerobic digester (Tadup 1995). Initially, the VFA and alkalinity of reactor 1 containing the 2:1:1 blend had 1500 mg.L⁻¹ and 2000 mg.L⁻¹. As the days progressed, the CO₂ evolution increased which showed a drop in biogas after the 9th day. A detailed picture is shown in Table 4.

Process Performance in a Two-Stage Continuous Reactor

The initial pH of the inoculum used for the seed microflora was 8.3 (Table 5). Once the feeding process started, the pH steadily decreased to 5.2 due to high acidification in the hydrolyzer. However, this sudden decrement due to acidification was balanced and maintained at the pH of 6 with the alkaline solution. Further, no corrections were undertaken until the next feeding process in the hydrolyzer. The alkaline solution was sufficiently concentrated to ensure that the volume added was insignificant relative to the amount of substrate fed into the system. The oxidation-reduction potential (ORP) of hydrolyzer and methanizer was -250 mV and -280 mV (Table 5) respectively, which showed the high reduction potential in methanizer due to the high biological conversion of organic matter into bio-energy. This was attributed to the high biogas yield (245 mL) on the methanizer.

Further, the high reduction potential by high activity of anaerobic bacteria was confirmed by the reduced pH of 5.2 in the hydrolyzer which evidenced the high acidification due to high VFA production of 10500 mg.L⁻¹. The gas production in the hydrolyzer started on 1st day and the maximum (185 mL) was observed on the 6th day and this was consistent with the average VFA of 5400 mg.L⁻¹ on the 6th day. After the systems started up, gas production reached maximum value over the first 5 days and then the production of biogas was slightly stable after the 10th day and continued until biogas

production ceases. After the 27th day the peak was observed to be stable and may be attributed to the acclimation of acidogens in the hydrolyzer.

Contemporarily, the increased total ammonical nitrogen with increased VFA was observed in the hydrolyzer (Fig. 5a and Fig. 5b). This may be because of increased total ammonical nitrogen due to the hydrolyzation of protein compounds in the hydrolyzer. However, the decreased alkalinity due to increased VFA (Fig. 6a and Fig. 6b) in the hydrolyzer confirms that there is no negative impact. On the other hand, the lower total ammonia in the methanizer indicates that the reactor is in good health, which is further supported by the lower alkalinity in the methanizer due to the high VFA.

CONCLUSION

The mesophilic anaerobic two-stage continuous reactor was designed to study the optimized mixing ratio of cow dung, water hyacinth and poultry litter of 2:1:1, 4:3:1 and 5:2:1 for the co-digestion for enhanced biogas production using the effective microbial solution. The reactor-loading rate was 4 and 5 g.L⁻¹.day⁻¹ with a retention time of 30 and 60 days. Maximum specific biogas of 0.128 Nm.L.g⁻¹ of Volatile solid reduction (VSR) in hydrolyzer and 0.205 Nm.L.g⁻¹ of VSR in methanizer was obtained at an optimized Carbon/Nitrogen (C/N) ratio of 20.8. In addition, it is observed that dual-stage anaerobic digestion helps in controlling toxicity by acidogenesis. Thus, the implementation of this technology would benefit the environment and play a major role in energy production.

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