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Anaerobic Digestion of Water Hyacinth, Giant Reed, Maize and Poultry Waste for Biogas Generation

Fayyaz Ali Shah, Qaisar Mahmood*, Naim Rashid, Arshid Pervez, Akhtar Iqbal and Mohammad Maroof Shah

Department of Environmental Sciences, COMSATS Institute of Information Technology, Pakistan

***Corresponding Author:** Qaisar Mahmood, Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottbad, Pakistan.

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Abstract

The current study explored the potential of three plants with different chemical composition (giant reed, water hyacinth and maize) and poultry waste for biogas generation. The substrates were analyzed for their chemical composition. A set of batch scale digesters (500 mL) was operated in triplicates to assess biogas generation from various substrates. The chemical composition of various assessed substrates was different from each other and maize had relatively high cellulosic, hemicelluloses and lignin contents as compared to other studied substrates. Water hyacinth had the highest biogas generation rate of 1000 mL/day followed by giant reed, maize and poultry wastes (850 mL, 800 mL and 650 mL per day, respectively). On the Volatile Solids (VS) weight basis, Water hyacinth generated 1200 mL/g VS, giant reed 1125 mL/g VS, Maize 1100 mL/g VS and poultry, 823.52 mL/g VS. Due to various chemical composition of water hyacinth, giant reed, maize and poultry wastes yield different quantity of biogas. At community scale it might be the potential substrates for biogas production and waste management

Keywords: *Mono-digestion; Biogas; Giant Reed; Water Hyacinth; Plant and Poultry waste*

Introduction

Renewable energy is one of the contributory energy resources in many countries which supplies up to 15% of primary energy [1]. Technological advancement and global warming are two main aspects of climate change which have led to increased application of renewable energies in modern world. Such form of energy has short carbon dioxide cycle, does not enhance the CO_2 in the atmosphere and can be produced using renewable resources. Biogas is a high quality renewable fuel, which can be utilized for various energy services such as heating, power generation, or transportation fuel instead of using fossil fuels [2]. The methane content of the generated biogas is usually variable and is dependent on the physical and chemical properties of the substrate employed [3]. The anaerobic digestion of solid waste not only treats solid waste, but also generates useful bio-fuel.

Biomass is an important renewable resource that may replace petroleum-based energy and chemicals [4,5]. However, global warming and depleting crude oils have compelled to switch towards the renewable energy. Some plants like water hyacinth (*Eichhornia crassipes*) quickly grow to very high densities (over 60 kg/m^2), thereby completely clogging water-bodies [6]. The mixture of animal waste and water hyacinth resulted in better biogas yields [7] and the sludge obtained from mixed feed had better nitrogen, phosphorus and potassium content which could serve as very good manure. Water hyacinth proved to be a promising substrate for anaerobic digestion with its digestion resulting in high biogas yields (267 L/kg VS) [8].

Giant reed has already been studied for bio-ethanol production, direct combustion, and other thermal transformations [9]. Giant reed is considered drought-tolerant species [9,10] and it can be grown in marginal or sub-marginal lands [11]. High solid contents of maize (about 30 tons of total solids - TS per hectare offers another potential biomass for anaerobic digestion [12,13] Due to high phosphorus content of poultry waste, it can be supplemented to enhance the C:N:P ratio for better anaerobic digestion. It was hypothesized that biochemical composition of substrates might influence the biogas output during anaerobic digestion. Currently, the biogas production from of these biomasses contains different biochemical composition has not been compared. The objective of the current study was to evaluate and compare the effect of differences in biochemical composition on biogas generation from giant reed (*Arundo donax* L.), water hyacinth (*Eichhornia crassipes* L.), maize (*Zea mays* L.) and poultry waste.

Materials and Methods

Sample collection and preparation

The plant biomasses were collected from various sites in Khyber Pakhtoon Khawa (KPK) Province in Pakistan. The leaf and stem parts of plants (Water hyacinth, giant reed, maize) and poultry waste were collected from Salhad, Abbottabad. The water hyacinth was collected from Charsada (KPK), Pakistan. All plants were spontaneous and abundant except maize which was cultivated. The poultry wastes were cleaned from hen feathers. After collection, the poultry waste was stored in a refrigerator at 4°C. The plant biomasses were allowed to dry at room temperature (25 ± 2°C) for about a week and then were chopped into pieces (3-5 cm long) in a chopping machine. The chopped biomass was ready to be fed in anaerobic batch reactors. The plant materials were analyzed chemically for nutrients and minerals content. No pretreatment was applied to plant biomasses during the experiment.

Seed Inoculum

The inoculum for the experiment was obtained from a biogas plant located near Abbottabad three days before the experiment. The biogas plant was 10 cubic meters capacity and it was fed with animal waste generated at nearby farm houses. There was no defined hydraulic retention time (HRT) and it operated based on the activity of microbial species present in the digester. The maximum care was taken to avoid the air exposure to the inoculum during collection. The inoculum which was in the form of slurry was allowed to adapt at 35°C in a closed container and was employed to the test bottles as slurry. The inoculums were chemically analyzed and the pH, ammonium content, Total solids (TS), volatile solids (VS) and VS/TS of the inoculums were found to be 7.9, 1250 mg L^{-1}, 5.6 mass %, 28 g L^{-1} and 0.89, respectively.

Experimental Design

All experiments were conducted in batch cultures in volumetric bottles of 500 ml capacity for 30 days. A schematic diagram of the experimental set up is shown in Figure 1. The headspace was 100 mL and working space was 400 mL which comprised of substrates, inocula and deionized water containing various ions in tolerable limits such as calcium (0.01 mg/L), magnesium (0.01 mg/L) and potassium (0.02 mg/L) to prevent inhibition of growth of microbial communities in the anaerobic reactors. The temperature of the reactors was kept at 35 ± 2°C in a water bath. The substrates were added to the reactor with inoculum to substrate ratio (I/S) of 2:1 (Figure 1). The amount of inoculum used in the test bottles was determined on the basis of the amount of organic substrate available for degradation, i.e. an inoculum-to-substrate (I/S) ratio, which was equivalent to the inverse value of the food-to-microorganisms (F/I) ratio. The I: S ratio of 2: 1 was selected on the basis of TS content in the substrates. The constant volume of inoculum (100 mL) was added to the substrate (50 g/L) in the bioreactors under a stream of nitrogen gas to create anaerobic conditions. The reactor flask/bottle was shaken to mix the substrates. The initial pH was kept at 7 by adding phosphate buffer in the reactors. The organic loading was 50 g/L. The reactors were flushed with nitrogen for 5 minutes to create a feasible anaerobic environment for microbes and then immediately sealed with butyl rubber stoppers. An outlet in the stopper was used for collecting biogas in inverted graduated cylinder of 500 mL capacity and biogas was measured daily by water displacement. Biogas was removed through second outlet through a valve from the graduated cylinder. Syringes were used to remove biogas collected in the graduated cylinder. The liquid in the inverted cylinder was kept at pH 2 by adding 0.01N HCl to avoid carbon dioxide absorption from biogas [14]. A blank reactor was also set up containing 100 mL inoculum in 100 mL of deionized water and incubated at the same temperature to measure the background biogas produced from the inoculum.

The most important factors affecting the precision of biogas volume measurement and sensitivity are errors due to varying temperatures, vapor content, solubility and pressure [15]. Temperature was controlled at 35°C to create uniform thermal conditions. All the experiments were run in triplicates and monitored daily for biogas production.

Analytical procedures

The biogas was collected in an inverted measuring cylinder (500 mL capacity) in water as shown in Figure. 1 & 2. Biogas volume was measured at 35°C under standard pressure and biogas production was calculated by subtracting the amount of the biogas produced by residential substrates in the control reactor. The cellulose, hemicelluloses and lignin were analyzed using proximate analysis [16]. TOC was calculated by dividing VS with 1.8 [17]. Carbon (C) was measured by combustion method in a Muffle furnace where plant materials were incinerated at 550°C for 4h [18]. Nickel, Phosphorus, potassium, copper, Iron, Zinc, Manganese and Nickel were analyzed through atomic absorption Analyst 700; Perkin Elmer; Flame furnace Auto sampler. TS and VS were determined according to the methods described by equations 1 and 2 [19].

$$TS = \frac{Weight\ of\ total - weight\ of\ dish}{Weight\ of\ sample - weight\ of\ dish} \times 100 \quad (1)$$

Weight of total: weight of dried sample and dish
Weight of sample: Weight of wet sample and dish

$$VS = \frac{Weight\ of\ total\ \text{-}weight\ of\ volatile}{Weight\ of\ sample\ \text{-}weight\ of\ dish} \times 100 \quad (2)$$

Weight of total: Weight of dried residue and dish
Weight of volatile: weight of residue and dish

Statistical Analysis

All determinations were performed in triplicates and mean values were presented in the results as described by [20]. Statistical comparisons of the mean values were performed by two way analysis of variance (ANOVA) using Sigma Plot™ v.12, where One way was performed for 4 substrates and the other for the days in triplicates, followed by Duncan's multiple range test ($p < 0.05$), using SAS 8.3 software (SAS Ins. Inc., Cary, USA). Graphical presentations were done using Sigma Plot™ v. 10.

Results and Discussion

Characterization of Substrates

The characteristics of the substrates used in the anaerobic digestion (water hyacinth, giant reed, maize and poultry litter) were presented in the Table 1. Giant reed had the highest TS followed by water hyacinth, maize and poultry. There was a great variation in the volatile solids. Water hyacinth had highest content of VS of 20 mass % followed by poultry (17 mass %); giant reed (16 mass %) and maize (12.5 mass %). Water hyacinth had highest TOC (11.11 mg/kg) followed by poultry wastes (9.44 mg/kg).

Biogas production from organic substrates mainly depends on their organic substances that are degraded to CH_4 and CO_2 [21]. Water hyacinth is the leading substrate in terms of biogas production with 21.34 mg/kg N and 13.45 mg/kg K. The highest amount of P was present is the Poultry waste (Table 1). Cu was less than 0.05 mg/kg in all biomass except poultry (0.06 mg/kg). Maize had the highest Fe content of 3.81 mg/kg followed by giant reed (1.80 mg/kg). The concentration of Zn was around 0.05 mg/kg in all substrates. The concentrations of Mn and Ni were highest in giant reed (2.86 mg/kg, 5.55 g/kg, respectively).

General	Water Yacinth (WH)	Giant Reed (GR)	Maize	Poultry
TS (mass %)	88.23	89.28	85	43.5
VS (mass %)	20	16	12.5	17
Total Soluble (mass %)	70.27	56.36	28.97	ND*
Cellulose (mass %)	4.50	15.45	15.91	ND
Hemicelluloses (mass %)	12.91	19.09	39.79	ND
Lignin (mass %)	12.31	9.09	15.33	ND
N (mg/kg)	21.34	9.81	11.93	16.12
P (mg/kg)	18	21	19	510
K (mg/kg)	1345	300	510	616
Cu (mg/kg)	< 0.02	< 0.02	< 0.02	0.06
Fe (mg/kg)	0.75	1.80	3.81	0.13
Zn (mg/kg)	< 0.05	< 0.05	< 0.05	< 0.05
Mn (mg/kg)	0.52	2.86	1.19	< 0.01
Ni (mg/kg)	3.14	5.55	2.52	5.05
TOC (mg/kg)	11.11	8.88	6.94	9.44
Carbon (mg/kg)	33.88	17.84	17.6	5.66

Table 1: *Chemical and minerals composition of WH, GR maize and poultry waste (Dry matter basis).*
**ND = Not Determined.*

Biogas production rate of Water Hyacinth Giant Reed, Maize and poultry waste

The daily biogas yield of various biomasses is presented in Figure 2. Biogas production started immediately on the first day. Overall, water hyacinth and giant reed had the highest biogas production during first week. During first week, the biogas generation of water hyacinth and giant reed was statistically similar ($p > 0.05$) but were significantly different from other substrates. From first week onwards, the biogas production rate of water hyacinth was greater than giant reed and rest of the substrates. A general look at the Figure 2 reveals that overall biogas production from water hyacinth was statistically greater than all other substrates during 30 days of the experiment. The highest rate of biogas production from water hyacinth was around 1000 mL/g VS/d on 11th day which subsequently dropped to around 650 mL/g VS on 30th day (but still statistically higher than rest of substrates).

The rate of biogas production was significantly different ($p < 0.05$) for all treatments as compared to control throughout the experiment. Water hyacinth showed the significant results throughout the experiment as compared to all other treatments. While maize and poultry showed an irregular patterns of significance. As far as the biogas production rate for maize and giant reed was concerned, giant reed showed highly significant biogas production rate as compared to maize till day 6. Giant reed produced significantly higher biogas as compared to poultry from day 1 to 16.

From second week onwards, the biogas production from giant reed, poultry and maize were statistically non-significant ($p > 0.05$) although it was less than water hyacinth Figure 2. The highest rates of biogas production per day of the maize and poultry were 500 mL and 400 mL, respectively. Such differences in the biogas production can be explained on the basis of the biochemical content of the substrates used (Table 1). The biochemical contents clearly indicated that water hyacinth had higher VS and soluble contents as compared to rest of the plant substrates. Furthermore, water hyacinth had higher soluble solids in comparison to other materials. Another feature of the water hyacinth as better substrate for higher biogas yield was its better C: N ratio. All these features (low cellulose/hemicelluloses, greater soluble content, higher C: N ratio) rendered it superior substrate for biogas yield among materials used for the present investigation. Several factors are known to influence anaerobic digestion and biogas production including C/N ratio [22] C/P ratio, nature of organic matter constituting volatile solids, trace nutrients [23] retention times and physicochemical conditions. Previously, it was reported that water hyacinth could produce high biogas volume irrespective of its N content; however, VS concentration was critical factor high biogas yields [24]. Our results are consistent with previous results as water hyacinth had high VS concentration (Table 1) as compared to other substrates under trial. The difference in biogas production during later part of experiment might be caused by the lignin content, availability of the material for microbes, inoculum which plays a significant role as presented previously [25]. After the utilization of its soluble contents, slower lignin degradation by anaerobic consortia and its subsequent utilization might have resulted in decline of biogas production from water hyacinth during second half period of the experiment. Giant reed can be another potential candidate for anaerobic digestion after water hyacinth based on its low lignin but higher cellulose content (Table 1). However, some pretreatment can reduce lignin and cellulose content to release soluble solids to be utilized in the anaerobic digestion effectively. Previous studies on water hyacinth also supported the present results. Continuous mesophilic digestion of horse dung mixed with different bedding materials was conducted for 238 days [26]. The study observed highest biogas production by water hyacinth owing to suitable C:N ratio 25:1.

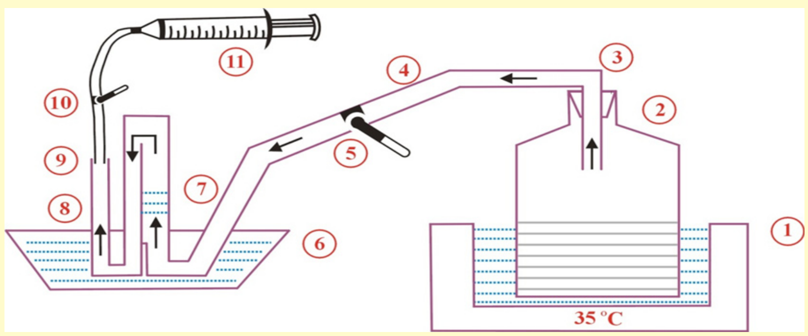

Figure 1: *Experimental set up, 1.Thermostat 2.Bioreactor 3.Rubber bung 4. Gas pipe from reactor to graduated cylinder, 5. Valve from reactor to Gas collector (500 ml), 6. Acidified water, 7. Gas collector, 8. Bucket containing Graduated cylinder (500 ml), 9. Removal pipe for Biogas 10. Valve in the removal pipe, 11. Syringe 60 ml.*

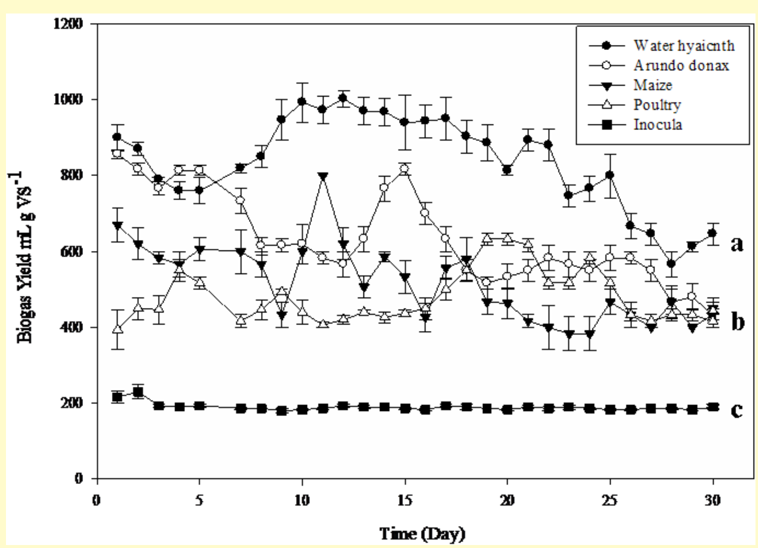

Figure 2: *Biogas production rate from Mono-digestion of Water hyacinth (WH), Giant Reed (GR), Maize, Poultry waste and control. Lowercase letters represent the statistical significance (< 0.05).*

Cumulative biogas Yield of Water Hyacinth, Maize, Giant reed and Poultry waste

The cumulative biogas produced by the studied biomasses is shown in Figure 3. The cumulative biogas production was highest for water hyacinth (25780 mL), followed by giant reed (18845 mL), maize (15900 mL) and poultry wastes (14670 mL). The cumulative biogas production by various substrates was significantly different from each other ($p < 0.05$). The biogas production started immediately on day 1 and kept increasing until last day of the experimentation. The immediate production of biogas might be due to the use of inocula originating from the community scale digester. Low cumulative biogas production from poultry waste might be due to its low TS, VS, carbon and nitrogen contents (Table 1).

Various physic-chemical factors are responsible for the variable biogas yields during anaerobic digestion.

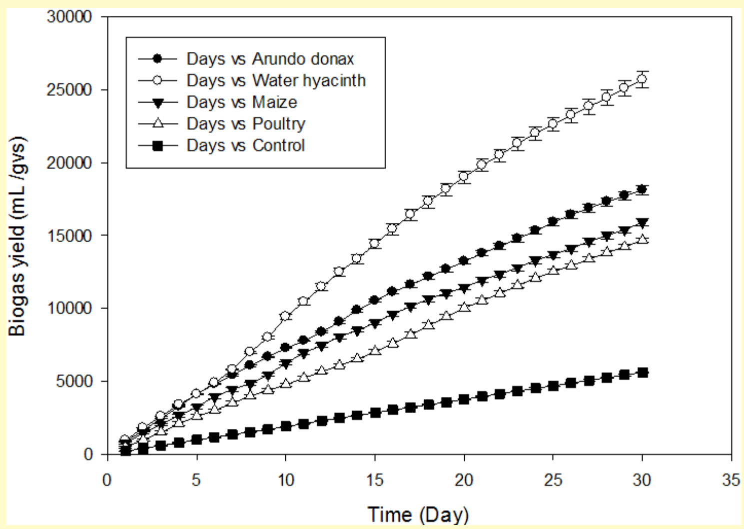

Figure 3: *Cumulative biogas generation from mono-digestion of water hyacinth, maize, giant reed, poultry waste and Control.*

The amount of lignin and cellulose is another influencing factor of biogas production which was observed during the present investigation. Recent studies revealed that lignin in herbaceous plant and/or non-woody portion of softwood and hardwood contains hydroxycinnamic acids (ferulic acid and p-coumaric acid), which has ester bonds and are cross-linked to polysaccharides and lignin polymer [27,28]. Previous studies on the mono-digestion of water hyacinth and giant reed biomasses produced variable results [29,30]. For example, giant reed was considered to possess good potential to be used as a feedstock for methane production via anaerobic digestion. The biogas produced during that study was in range of 129.7~150.8 L-CH_4/kg-VS at F/E of 2.0. Overall organic components were degraded by 17.7–28.5 mass % to 24.0–26.6 mass %, among which cellulose showed the highest degradation rate and the highest contribution to methane production. This study also showed that giant reed had a higher methane yield potential [29]. Another study explored the potential of giant reed for biomethane production by examining the influence of harvest time and frequency on the biochemical methane potential, the kinetics of biomethane accumulation in batch reactors and the expected methane yield per hectare [30]. Methane generation from cabomba and water hyacinth was more than three times the highest yields from Salvinia. The small scale tests yielded biogas volumes of 292 ± 43 L/kgVS, 322 ± 21 L/kgVS and 52 ± 55 L/kg VS for water hyacinth, cabomba and salvinia, respectively. Another investigation observed biogas production of 15.4–23.65 L/kg dry weight from water hyacinth during 21days digestion period [31]. The presence of high lignin and cellulosic contents in the studied biomasses suggested that some pretreatment can be useful to enhance the biodegradability and subsequent high biogas volume from these substrates [32].

Conclusion

The current study analyzed the biochemical composition of giant reed, water hyacinth, maize and poultry waste with reference to their utilization in anaerobic digestion. Owing to better C: N ratio and biochemical composition, water hyacinth was a successful substrate for mono-digestion which resulted in its highest biogas production rate among the four investigated substrates. The cumulative biogas production during 30 days was highest for water hyacinth (25780 mL), followed by giant reed (18845 mL), maize (15900 mL) and poultry wastes (14670 mL). So, we recommend the utilization of substrates like water hyacinth and giant reed produced on marginal lands for biogas production to overcome the energy crisis in developing countries. Further studies on the pretreatment of studied biomasses to enhance the availability of the readily available substrates for biogas production are recommended.

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