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Research Article

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Impact of incubation period of primary sludge inoculum on biomethanation of water hyacinth

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ABSTRACT

Water hyacinth was introduced as an ornamental crop species in many countries more than a century ago, because of their attractive blue, lilac to purplish flowers and round to oval leaves. Soon, it was realized to be most invasive and noxious weed. Due to its fast spread and congested growth, it clogs up rivers, hydroelectric plants, waterways and entire lakes, killing aquatic life, hampering river transport and fisheries, endangering the livelihoods of millions of poor people. Many efforts were made to eradicate this weed but it has successfully resisted all the attempts. When looked from a resource angle, it has high content of fermentable matter which makes it a potential source for biogas production. Hence possibility of converting water hyacinth to biogas has been an area of major interest for researchers. In the present work experiments were performed in 250 ml batch digesters with primary sludge inoculum of different incubation period to understand the impact of incubation period on biomethanation of water hyacinth. All the digesters were operated in mesophilic condition with detention time of 60 days. Results showed inoculums of different incubation periods have remarkable effect on biogas production. In particular the digester with primary sludge inoculum of 30 days incubation period produced 0.44 l/gVS which was 91% more in comparison with the control digester.

Key words: Water hyacinth, biomethanation, primary sludge inoculum, biogas, incubation period.

INTRODUCTION

In today's energy demanding life style, need for exploring and exploiting new sources of energy which are renewable as well as eco-friendly is a must [1]. Biomethanation is an environmental friendly biological process in which microorganisms work synergistically to convert organic wastes into biogas and a stable product (soil conditioner) for agricultural practices without any detrimental effects on the environment. In biomethanation complex polymers are broken down to soluble products by enzymes produced by fermentative bacteria which ferment the substrate to short-chain fatty acids, hydrogen and carbon dioxide. Fatty acids longer than acetate are metabolized to acetate by obligate hydrogen-producing acetogenic bacteria (Fig.1, Group 2). The major products after digestion of the substrate by these two groups are hydrogen, carbon dioxide, and acetate. Hydrogen and carbon dioxide can be converted to acetate by hydrogen-oxidizing acetogens (Fig.1, Group 3) or methane by carbon-dioxide-reducing, hydrogen-oxidizing methanogens (Fig.1, Group 4). Acetate is also converted to methane by acetoclastic methanogens (Fig.1, Group 5).

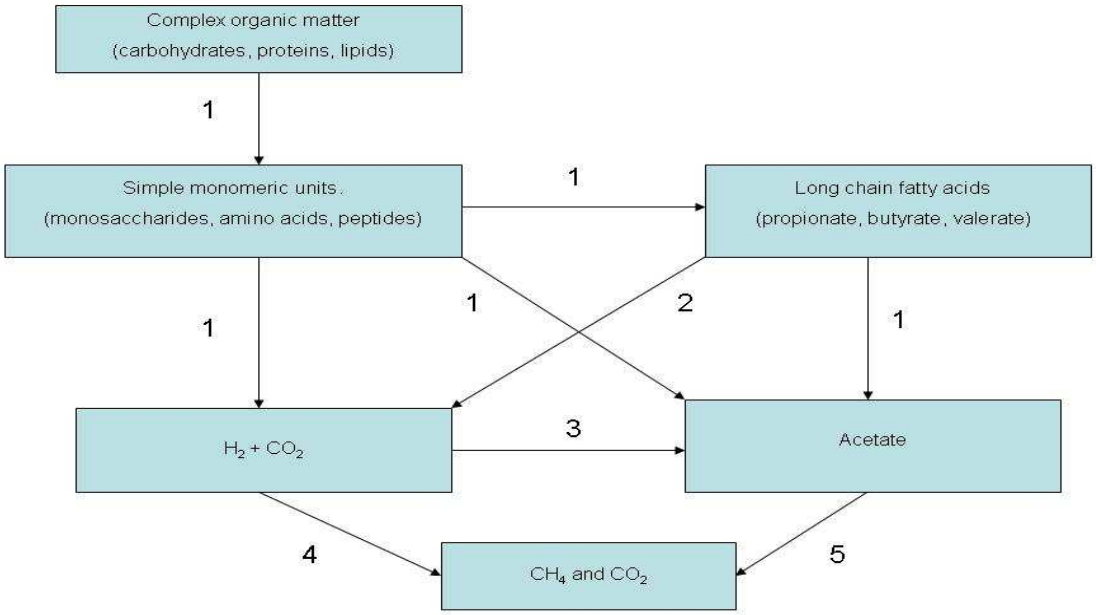

Fig. 1: Conversion of complex polymers by microbes

Biogas is a renewable source of energy with 20 MJ/m^3 calorific value and has the potential of supplementing other energy sources in a bid to encouraging the principles of sustainable development, and reduces the practice of total dependence on fossil oil that is finite [2] .

Water hyacinth is a free floating perennial herb of pickerel weed family (*Pontederiacea*e) which propagates itself profusely and has constituted a menace by clogging water bodies, which in turn may have negative effects on the environment, human health and economic development [3]. It is listed as one of the most productive plants on earth and is considered as one of the world's worst aquatic weed [4]. Attempts to control the weed have caused high costs and labor requirements, leading to nothing but temporary removal of the water hyacinths [5]. Therefore experts in the field believe that it is difficult to eradicate the water hyacinth, since the conditions that allow it to proliferate are not being controlled [6]. A better approach to solve the problem is by using water hyacinth for energy generation as it is rich in nitrogen, fermentable matter and other nutrients. However, plant materials are more difficult to biodegrade than animal manures. This is because hydrolysis of cellulose materials of crop residues is a slow process and can be a major rate determining step in anaerobic digestion process [7]. Optimization of the biogas process can be in the form of blending, size reduction, pre-decaying in water, chemical treatment (NaOH / KOH) addition of inoculum and metals (CO, Ni, Fe, Ca, Mg) to the wastes at required levels [8]. Earlier work carried out by [9] attributed the poor yield of biogas from water hyacinth to absence of seeding material, sheathing of biodigestible materials by a relatively thin impervious outer layer on the plant and the presence of lignin in the cell wall. The present study was undertaken to increase the biogas yield from biomethanation of water hyacinth using primary sludge inoculum of different incubation period.

EXPERIMENTAL SECTION

2.1 Sample collection

Water hyacinth used for the study was obtained from silver lake at HBR layout (Bangalore, Karnataka, India). Thickened primary sludge was collected from primary clarifier from Vrishabhavathi sewage treatment plant at Vrishabhavathi valley, Nayandanahalli (Bangalore, Karnataka, India).

2.2 Sample analysis

Water hyacinth and primary sludge were analyzed for the following parameters

1. pH analysis: pH was measured by pH meter which consists of a potentiometer, a glass electrode, a reference electrode and a temperature compensating device. Electrodes were connected to the pH meter and were calibrated

using buffer solutions before pH analysis.

2. Total solids (TS) and total volatile solids (VS): TS were determined at 104 ^{0}C to constant weight (Standard method part 2540 B) and VS were measured by the loss on ignition of the dried sample at 550 ^{0}C. (Standard method part 2540 E)

3. Biogas analysis: Gas chromatograph (Chemito 1000) equipped with a thermal conductivity detector was used to analyze the biogas sample. Hydrogen was used as carrier gas (25 ml/min) with Porapak Q column. Standard calibration gas mixture was used for calibration. Biogas samples were collected in rubber bladders; the sample and standard were injected using a gas tight syringe into the gas chromatograph. The parameters were set at oven temperature of 40^{0}C, detection temperature of 80^{0}C and the detector current of 180 mA. The concentrations of different components were calculated using equation (1):

$$\% \text{ of } X = \left(\frac{\text{Area of } X \text{ in sample}}{\text{Area of } X \text{ in standard}} \right) \times \% \text{ of } X \text{ in standard} \quad (1)$$

2.3 Biomethanation unit

A schematic diagram of biomethanation unit is shown in Fig. 2. The unit consists of a temperature controlled thermo bath which is maintained at the mesophilic temperature range of 30°C to 35°C. It has a battery of bio-digesters. Each bio-digester is connected to a graduated gas collector by means of a connecting tube. Each of the gas collectors are in turn immersed in a trough of water to ensure complete sealing. A stand holds all the gas collectors. Biogas evolved is collected by the downward displacement of water.

Fig. 2: Biomethanation unit

2.4 Inoculum preparation

In a 2.5 L glass bottle, 913 gm of primary sludge was mixed with 1087 gm of water to obtain a slurry of 7% TS. About 200 ml of the slurry was distributed in 6 glass bottles of about 250 ml capacities. The bottles were maintained at 35^0C and were fitted with a rubber cork having one hole. A glass tube was inserted in the hole which remained above the layer of the slurry. The other end was connected with Teflon tubing, the outlet of which was dipped in a container filled with water. The gas produced during the incubation period could bubble through the water but no air would enter the slurry thus, maintaining the anaerobic condition. After the expiry of the desired incubation period the bottles were opened and the contents were filtered through 60-mesh sieve. The filtrate was used as inoculums of 0, 10, 20, 30, 40 and 50 day's incubation period.

2.5 Fermentation slurry

Fresh water hyacinth (leaves, stem and root) on collection was chopped to small sizes of about 2 cm allowed to dry under the sun for a period of 7 days, after which they were dried in an oven at 60^0C for 6 hours [10]. This oven-dried water hyacinth was then ground to fine powder. A series of laboratory experiments using 250 ml biodigesters were performed in batch operation mode. Each biodigester was fed with 4 g of finely dried and ground water hyacinth. These were mixed with 25 g of water and 75 g of primary sludge inoculumof 0, 10, 20, 30, 40 and 50 day's incubation period resulting in six different fermentation slurries IPS-0, IPS-10, IPS-20, IPS-30, IPS-40 and IPS-50 respectively. Digester WHB fed with 4 g water hyacinth and 100 g water was considered as blank. All digesters were given 0.3 ml of 10% by volume of acetic acid as it catalyzes the biometanation process. Table 1 presents detailed contents of digesters. Biomethanation of these digesters were carried out in duplication with a retention time of 60 days in the mesophilic range (30-40^{o}C). Cumulative biogas production, slurry temperatures were monitored throughout the study.

Table 1: Contents of digesters

Digester	Water hyacinth (g)	Water (g)	PSI (g)	Acetic acid 10% by vol.(ml)
IPS- 0	4	25	75 (0 day incubation period)	0.3
IPS-10	4	25	75 (10 day incubation period)	0.3
IPS-20	4	25	75 (20 day incubation period)	0.3
IPS-30	4	25	75 (30 day incubation period)	0.3
IPS-40	4	25	75 (40 day incubation period)	0.3
IPS-50	4	25	75 (50 day incubation period)	0.3
WHB	4	100	-	0.3

RESULTS AND DISCUSSION

3.1 Solids and pH analysis

Total solids are the sum of suspended solids and dissolved solids. Total solids analysis and pH are important for assessing anaerobic digester efficiencies. TS analysis was done using (standard methods, 1995) while pH was measured using pH meter (Systronics). The TS are composed of two components, volatile solids (VS) and fixed solids. The VS are organic portion of TS that biodegrade anaerobically. TS and VS are calculated as given bellow.

$$\text{TS, \%} = \frac{(A-B)}{(D-B)}\times 100 \quad \text{and} \quad \text{VS, \%} = \frac{(A-C)}{(A-B)}\times 100$$

Where

A is weight of dish + dried sample at 103^0C to 105^0C (grams)

B is weight of dish (grams)

C is weight of dish + sample after ignition at 550^0C (grams) and

D is weight of dish + wet sample (grams)

Table 2 gives the solid analysis and pH data of primary sludge and water hyacinth

Table 2: Solid analysis and pH data

Material	% TS	% VS	pH
Primary sludge	15.33	51.84	6.8
Water hyacinth	16.89	82.85	6.4

3.2 Impact of incubation period of primary sludge inoculum

The cumulative biogas produced with time for all the digesters are given in Table 3. The specific biogas production is presented in Fig. 3 which, shows biogas production tend to obey sigmoid function (S curve) as generally occurred in batch growth curve.

Table 3: Biogas production

Digester → Time ↓(days)	WHB (liters/g VS)	IPS-0 (liters/g VS)	IPS-10 (liters/g VS)	IPS-20 (liters/g VS)	IPS-30 (liters/g VS)	IPS-40 (liters/g VS)	IPS-50 (liters/g VS)
0	0	0	0	0	0	0	0
5	0.002	0.006	0.015	0.01	0.015	0.01	0.01
10	0.01	0.02	0.04	0.04	0.06	0.05	0.05
15	0.03	0.09	0.11	0.15	0.18	0.12	0.12
20	0.04	0.14	0.19	0.25	0.27	0.22	0.22
25	0.07	0.21	0.26	0.29	0.32	0.3	0.3
30	0.11	0.25	0.29	0.33	0.35	0.34	0.34
35	0.16	0.27	0.31	0.35	0.37	0.36	0.35
40	0.18	0.3	0.33	0.37	0.39	0.37	0.36
45	0.2	0.32	0.35	0.39	0.41	0.39	0.37
50	0.21	0.33	0.36	0.4	0.43	0.4	0.38
55	0.22	0.34	0.38	0.41	0.44	0.4	0.39
60	0.23	0.34	0.38	0.41	0.44	0.4	0.39

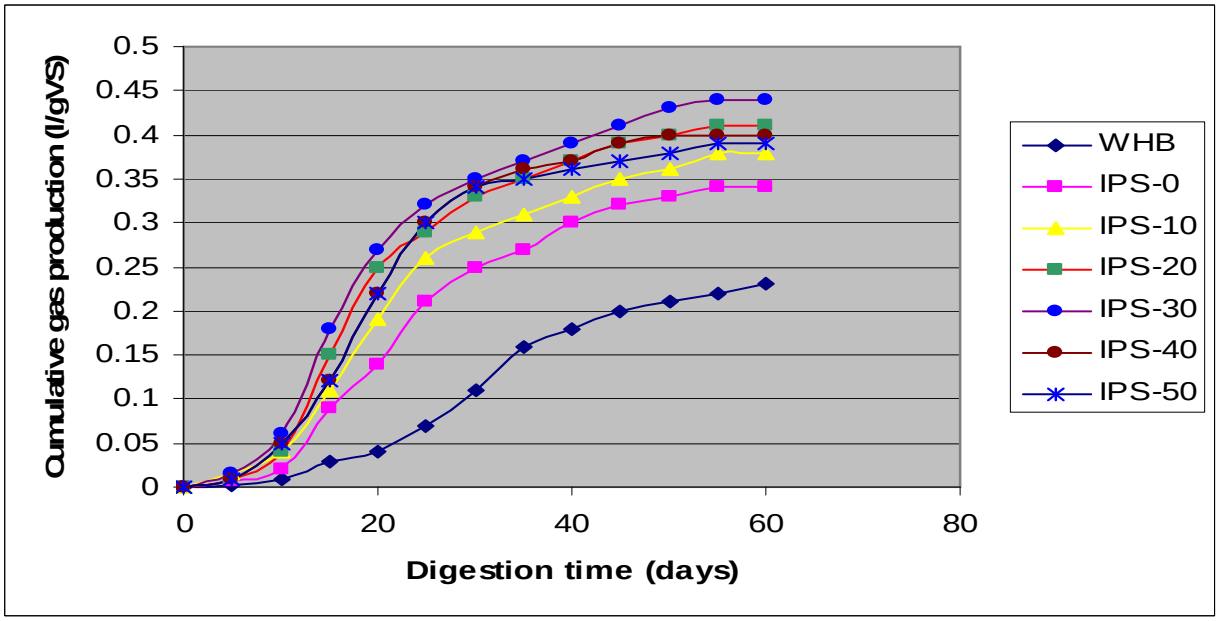

Fig. 3: Daily biogas production

From Fig. 3 it can be observed inoculums of different incubation periods have remarkable effect on biogas production. All the digesters with primary sludge inoculums produced more biogas than control digester WHB. This indicates that digester WHB does not have essential microbes for early start up of biomethanation process which is the reason for longer lag phase and lesser biogas production. The total amount of gas produced at the end of detention period was highest for digester IPS-30, which produced 91% more in comparison with the control digester WHB. This performance could be because inoculum with 30 days incubation period contains all the essential microbes (hydrolyzing, fermentative, acetogenic and methanogenic bacterial consortium) this could have optimized syntrophic interaction between acetogens and methanogens which is the most critical step in the biomethanation process [11]. The same reasons could be attributed for the performance of the digester IPS-20 which produced 78.3% more biogas than the digester WHB. The total amount of gas produced depends on the amount of volatile fatty acid decomposed, which in turn would depend on the number of methanogenic bacteria present in the system. If the inoculums are kept for a longer period the amount of substrate does not increase further. However, there is a

possibility of decreasing number of methanogenic bacteria in the inoculums of longer incubation period because they are active only during a particular time period [12]. The less the bacterial count the lesser would be the gas produced. The same effect is observed for digesters IPS-40 and IPS-50 which produced 73.9% and 69.6% more biogas than the control. Thus, it is inferred that inoculums of incubation period of 20 and 30 days are the best for the maximum production of biogas during a particular time period.

CONCLUSION

The effect of primary sludge inoculumof different incubation period on biomethanation of water hyacinth was studied by performing a series of laboratory experiments. The most important findings from this study are that water hyacinth is a very good biogas producer but needs enriched seeding (inoculum) to enhance biomethanation. Inoculums of different incubation periods have remarkable effect on biogas production. Biomethanation of water hyacinth with primary sludge inoculumof 30 days (IPS-30) produced maximum biogas (0.44 liters/g VS), followed by IPS-20 (0.41 liters/g VS). Primary sludge inoculum with incubation period longer than 30 days decreases the methanogenic bacterial count resulting in lesser biogas yield. Overall conclusion is that primary sludge inoculums of incubation period 20 and 30 days improve the syntrophic reactions of the process and produces maximum biogas yield.

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