

Synthesis of biomethane from obnoxious weed *Parthenium hysterophorus* using biocatalyst in semi-batch anaerobic digester

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Abstract:

In today's world due to rapid urbanization and industrialization the global energy demand is increasing rapidly and about 88% of this demand is ruled over at present by fossil fuels. The dependence on fossil fuel as primary energy source has led to environment degradation and human health problems. To mitigate these problems, an alternative energy resource which can meet the sufficient demand is Biogas production from wastes. A promising alternative raw material for the accomplishment of Biomethanation is utilization of renewable lignocellulosic biomass. *Parthenium hysterophorus* L, an obnoxious flowering plant, offers a big challenge to all attempts of control because of its high regeneration capacity, production of huge amounts of seeds, high seed germinability and extreme adaptability to wide range of ecosystems. An experimental study on biomethanation using bio-waste – *Parthenium hysterophorus* was utilised to optimize the yield of methane gas with cow urine used as a catalyst. The experimental study was carried out under anaerobic condition in a semi-batch digester over the influence of pH (6-

7.5), temperature (30-40 °C). In this study, pH, temperature, total solid (TS) and volatile fatty acid (VFA) and chemical oxygen demand (COD) were measured during the experiment. In the experiment it was found that using different parametric range like pH 6.5-7.5, temperature 35-40 °C and total solid (TS) 7.5 - 8.1%, the volatile fatty acid (VFA) yield was 128-942 mg/L and the maximum amount of methane produced was 62%.

Keywords: Bio-waste, Catalyst, Biomethanation, Anaerobic condition, Semi-Batch Digester.

1. INTRODUCTION:

The country's economy mainly depends upon availability of energy resources. In today's world rapid urbanization and industrialization has lead to the increased rate of energy consumption. The global energy demand is increasing rapidly and about 88% of this demand is met at present by fossil fuels[1]. The dependence on fossil fuel as primary energy source has led to global climate change, environment degradation and human health problems [2]. In order to minimize related global warming and climate change impacts , green house gas emission must be reduced to less than half of global emission levels of 1990[3]. So, the ideas to identify the strategies of reducing the rate of green house gas emission have led the researchers to investigate alternative energy resources during the last two decades[4].The currently utilized renewable energy resources, including solar, wind, hydro, biomass accounts for the approximately 14% of the primary energy consumption in the world. Among them biomass is a major contributor to renewable energy[5]. Biomass can be defined as "all renewable organic matter including plant material, whether grown on land or water, animal products and manure, food processing and forestry by-products; and urban wastes"[6]. New research ideas for biogas production are simply based on different types of bio-wastes such as parthenium, water hyacinth canteen waste etc. These are considered to be attractive raw material because of its availability at low cost. Most of the developing countries, producing a huge amount of biomass in different forms are facing different ecological problems due to the disposal of those wastes.

The process of Biomethanation can be expressed in terms of Anaerobic digestion (AD) which offers a very attractive route to utilize certain categories of biomass for meeting partial energy

needs. Anaerobic digestion is a biological process where organic material is decomposed by anaerobes in absence of air to yield methane rich biogas[7]. The anaerobic digestion of solid waste leads to high degree of waste stabilization, low production of excess biological sludge, low nutrient[8]. Anaerobic digestion has the advantage of biogas production and can lead to efficient resource recovery and gives contribution to the conservation of non-renewable energy sources. AD is the controlled degradation of biodegradable waste in absence of oxygen and presence of different consortia of bacteria that catalyze series of complex microbial reactions[9]. Anaerobic digestion involves a series of metabolic reactions such as hydrolysis, acidogenesis and methanogenesis for the production of biogas. The production of biogas through anaerobic digestion offers significant advantages over other forms of waste treatment including-

- Less biomass sludge is produced in comparison to aerobic treatment technologies

[10-13].

- Successful in treating wet wastes of less than 40% dry matter[14].
- Minimal odour emission as 99% of volatile compounds is oxidatively decomposed upon combustion. , e.g. H_2S from SO_2 .
- The slurry produced is an improved fertilizer in terms of both its availability to plants and rheology[15].

Once produced biogas through AD is generally composed of 48-65% of Methane, 36-41% of Carbon di-oxide and traces of other gases[16]. Carbon di-oxide and Methane are potent green house gases. To reduce the impact of the rate-limiting step, pre-treatment is required. Several works has already been done on the effects of different pre-treatments of different substrates on the production of biogas [17-19].

2. MATERIAL AND METHODS

2.1 BIOMETHANATION PROCESS:

The biomethanation process can be divided in four conversion and degradation phases- hydrolysis, acidogenesis, acetogenesis and methanation.

- **Hydrolysis Phase-** The hydrolase enzyme secreted by facultative and obligate anaerobes break down cellulose, carbohydrates, protein and fats into monomers.
- **Acidogenic Phase-** The monomers produced on hydrolysis are degraded further, by those bacteria, into short-chain organic acids, C1–C5 molecules (e.g., butyric acid, propionic acid, acetate, and acetic acid), alcohols, hydrogen, and carbon dioxide. Intermediate hydrogen affects the fermentation products. Intermediate fermentation products are formed if the partial pressure of hydrogen is high enough.
- **Acetogenic Phase-** Acidogenic products of the previous phase serve as substrates for bacteria in the acetogenic phase. Homoacetogenic microorganisms of the acetogenic phase use H_2 and CO_2 to form acetic acid. Methanogenic bacteria grow concurrently with acetogenic bacteria. Short- chain organic acids and alcohols are converted to acetate. In the conversion of ethanol to acetate, carbon dioxide is used and acetate and hydrogen are produced. Acetate production decreases if hydrogen partial pressure is great enough.
- **Methanogenic Phase-** The methane production takes place under strict anaerobic conditions. Not all methanogenic bacteria degrade all substrates.

It can be divided into acceptable substrates acceptable for methanogenesis into the following three groups:

- (I) Acetoclastic Methanogenesis : $\text{Acetate} \rightarrow \text{CH}_4 + \text{CO}_2$
- (II) Hydrogenotrophic Methanogenesis: $\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4$
- (III) Methyltrophic Methanogenesis: $\text{Methanol} \rightarrow \text{CH}_4 + \text{H}_2\text{O}$

2.2 OPERATIONAL PARAMETERS FOR BIOMETHANATION:

The anaerobic digestion process needs some basic requirements depending on feed characteristics and environmental conditions. The optimum conditions that are required are mentioned below.

Retention Time

Higher retention time leads to higher production of biogas, as the production is cumulative. At the initial stage of biomethanation, the gas production rate is high but then it gradually declines as the digestion approaches completion[20]. Digestion of volatile fatty solids is not significant, even when the retention time is more than 12 days.

pH

It is an important parameter for biomethanation. It affects the growth of the organisms involved in this process. The optimum pH range is from 6.0-8.5[20]. A pH below 6.0, inhibits the whole process. Sodium hydroxide can be used sometimes to adjust the pH.

Temperature

The optimum range of temperature for methane forming bacteria varies between 30-35 °C for mesophilic bacteria, 50-60 °C for thermophilic bacteria and 10-20 °C for psychrophilic bacteria. Methane-forming bacteria are not active at temperatures between 40 and 50 °C.

Biomethanation in the psychrophilic range is generally not very productive[20]. A temperature below 30 °C inhibits the process as bacteria are not active at this point. The thermophilic range has an advantage over other ranges as the degradation time is shorter.

Organic Loading Rate

The ratio of amount of substrate present in the process to the amount of inoculums added is known as organic loading rate. The specific carbon loading affects the growth response of methanogens. Normally the loading ratio should be 1:1, 1:2 and 1:3. However, conversion of organic material to biogas is based upon the retention time[20] and optimal pH is dependent on optimum loading rate.

Volatile Fatty Acids (VFA)

The intermediate VFA products (acetic acid, propionic acid and butyric acid) in the biomethanation process are capable of inhibiting methanogenesis at high concentrations[20, 21]. A high organic loading rate causes accumulation of VFAs. This occurs since acetogens grow more slowly. The rate of acidogenesis is reduced at high VFA concentrations because of inhibition by acid producing bacteria. Total VFA concentrations above 4 g/l inhibit fermentation of sugars. VFA levels above this level inhibit methane production[20].

Chemical Oxygen Demand(COD)

The Chemical Oxygen Demand is a measurement of the amount of material that can be oxidized (combined with oxygen) in the presence of a strong chemical oxidizing agent. Since the COD test can be performed rapidly, it is often used as a rough approximation of the water's BOD,

even though the COD test measure some additional organic matter (such as cellulose) which is not normally oxidized by biological action. Generally it reduces with time. It is because due to growth of microorganisms the oxygen demand decreases gradually with time.

C/N ratio

High amount of nitrogen (>80 mg/l), as undissociated ammonia, at low C/N ratios can cause toxicity. In addition, low levels of nitrogen at high C/N ratios can slow the rate of digestion. It is important to keep the C/N ratio in the desired range. The microorganisms in biomethanation processes utilize 25-30 times more carbon than nitrogen. The type of reactor selected is partially determined by the C/N ratio of the intended feedstock. A two-stage reactor is reliable with C/N ratios less than 20:1[20].

Uniform Mixing

Mixing can create uniformity in fluids and eliminate concentration and temperature gradients[20]. Intimate contact between microorganisms and substrate while stirring the digester can enhance the biomethanation process. Excessive mixing can reduce biogas production. In addition, excessive mixing causes disruption of granular structures. It reduces the rate of oxidation of VFA leading to digester instability. In case of large scale production, an average mixing time of 3-4 h/day with 10-20 rpm is useful for high solid contents as per the studies conducted earlier[22].

Catalytic Activity

Catalysts are used to enhance the rate of substrate digestion by the microorganisms. It influences the rate of reaction. The rate of bio gas production varies with different conditions and

parameters like temperature, stirring speed, feed concentration, catalyst concentration, etc. It has been found that the catalyst mainly increases the production rate of biogas.

2.3 Substrate Preparation:

The substrate required for the methane gas production should be in the form of shreds. In order to produce the *Parthenium hysterophorous* shreds, the fresh plant is taken, chopped finely and the product is grinded to obtain the substrate.

2.4 Characteristics of Parthenium Hysterophorous :

The characteristics of the parthenium biomass revealed the following characteristics as described in the Table 1a & Table 1b.

Table 1.a Proximate analysis of parthenium hysterophorous

Parameters	Results (%)
Moisture Content	80.26
Ash Content(dry basis)	3.31
Volatile Matter(dry basis)	12.63
Fixed Carbon	3.80

Table 1.b Average biomass composition of *Parthenium hysterophorus* L.

Components	Percentage (%)
Lignin	13.9
Cellulose	27.8
Hemicellulose	21.01

All the chemicals that are used are not in the pure form. Inoculums used are of two different types, Gobar gas plant slurry and Sewage Plant sludge. Cow urine is used as a catalyst along with water to prepare different solutions. Sodium hydroxide is used in case, to adjust the pH. Concentrated sulphuric acid is used with a measurement of 2.5 ml, 400 ml distilled water and 5-6 drops of phenolphthalein indicator each time for measuring the VFA concentrations. Ferrous sulphate and urea are used as additives plastic bottles of beverages, rubber pipes, corks, caps of syringe , measuring cylinder are also used in this experiment.

2.5 Methods used:

Determination of Total Solids:

The total solids in influent and effluent slurry were measured using Standard Methods (Anonymous, 1989). Influent was checked before starting the experiment and effluent was tested after terminating the experiment. About 5 gm of each sample was weighed in a pre-weighed porcelain dish using an ADAIR DUTT make, MJ 500 series electronic balance having 0.001 g

least count. The samples were dried first at 60 °C for 24 h and then at 103 °C for 3 hours using a Wiswo make hot air oven having a range of 0 – 240 °C. The dried samples were again weighed using the same electronic balance. The total solids in a sample were calculated using the following equation:

$$TS = (W_d/W_w) \times 100$$

Where, TS = total solids, percent

W_d = weight of dry sample, gm

W_w = weight of wet sample, gm

Determination of Methane :

To estimate the Methane (CH₄) content of the biogas, the gas was taken with syringe from that digester and pushed into the Methane gas analyzer to obtain the methane yield.

$$\text{Percentage of yield of methane} = \frac{\text{volume displayed by gas analyzer}}{\text{volume of water displaced}} \times 100$$

2.6 Experimental Details:

The batch digestion units were made by plastic bottles, cork, and gas pipes. In total 2 bottles of 1000 ml capacity were used along with same number of drilled corks, pipes, thermometer, pH paper, distillation unit, APHA analyzer, Methane Gas analyzer. Each sample was co digested with cow dung at water ratio of 1:1. The 2 bottles were distributed into two categories.

- One incorporated with gobar (cow dung) gas plant sludge with parthenium where cow's urine used as a catalyst.

- Other incorporated with sewage plant sludge with parthenium where cow's urine used as a catalyst.
- **Experimental Procedure:**

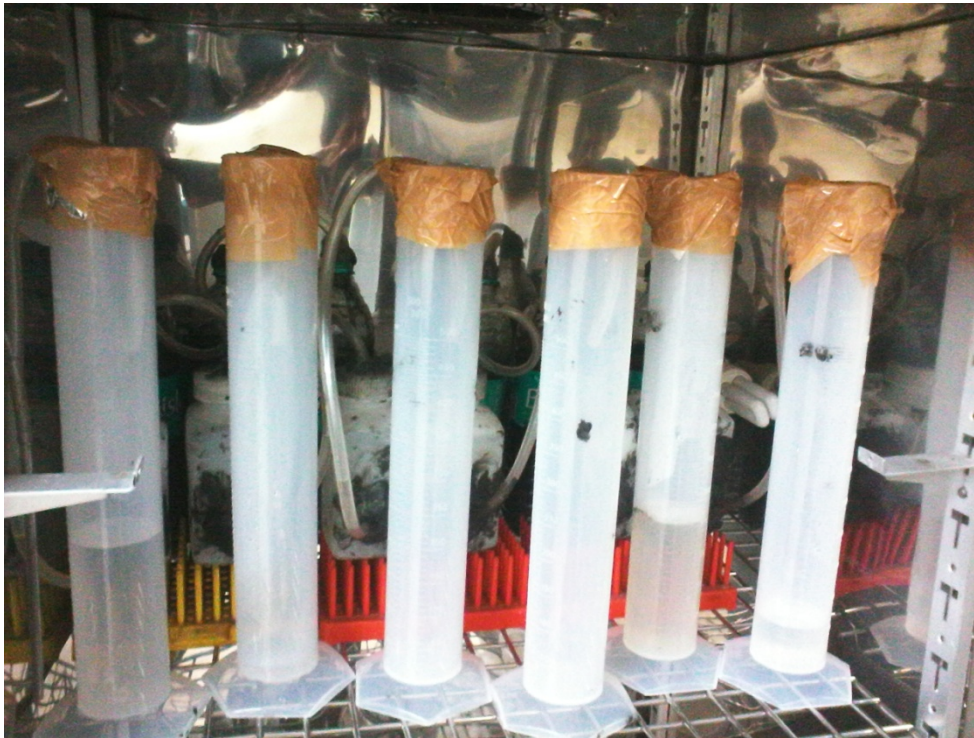


Fig.1. Experimental set up using *parthenium hysterophorus*

Gas production was observed in all digesters for 1:1 water ratio. Then parthenium was collected as before, co-digested with cow-dung, pre-treated with different chemicals and effluent sludge, collected from biogas plant. Some additives like urea and Ferric sulphate were added to catalyse the reaction procedure. Then the digesters were kept in the hot air oven at 37 °C as shown in Fig.1.

Before sealing the corks, little amount of samples from each digester were collected and the tests for influent (pH, Total solid, COD) were done. The digesters were then incubated in the hot air

oven at 37 °C for maintaining a uniform temperature. After day one the gas was generated and from the gas holding water filled bottles water displacement started. The amount of water displaced was the amount of gas produced every day. The gas production increased day by day and on day 14th, it reached its highest amount and then a decreasing trend was observed and the gas production continued up to 23 days. Production of gas, Methane and Carbon di-oxide content were measured daily for 23 days. Tests for effluent (pH, Total Solid, COD) were done using standard methods.

- The optimum pH range and temperature range is (6.5-7.92) and (30-40 °C) respectively, for mesophilic bacteria responsible for bio methanation[20]. The pH of the experiment varied between 6.5 to 7.0 and the temperature varied between 31-33 °C.
- The normal concentration of VFA must not exceed 4g/l [20]. The VFA concentration for each set-up that was measured once a week varied between 120 mg/l to 550 mg/l. The whole VFA procedure was carried out in reference to[21].
- The COD was measured once in every week.

3 RESULTS AND DISCUSSIONS:

3.1 pH Variation:

Variation of pH for each sludge are given in the Fig 2. The pH and temperature of the samples were measured in everyday for continuous 3 weeks. The average pH remained between 6.5 to 7.5.

The pH variation could be categorized into three main zones. The first zone started from the first day till fifth day, which showed a drastic drop of pH. This is due to high development rate of

volatile fatty acids and amino acids from lipids and protein breakdown during hydrolysis stage. The second zone started from the sixth till the eleventh day of the experiment. In the second zone, the pH was in the range of 6.8 to 7.2 as shown in Fig 2a & Fig.2b.

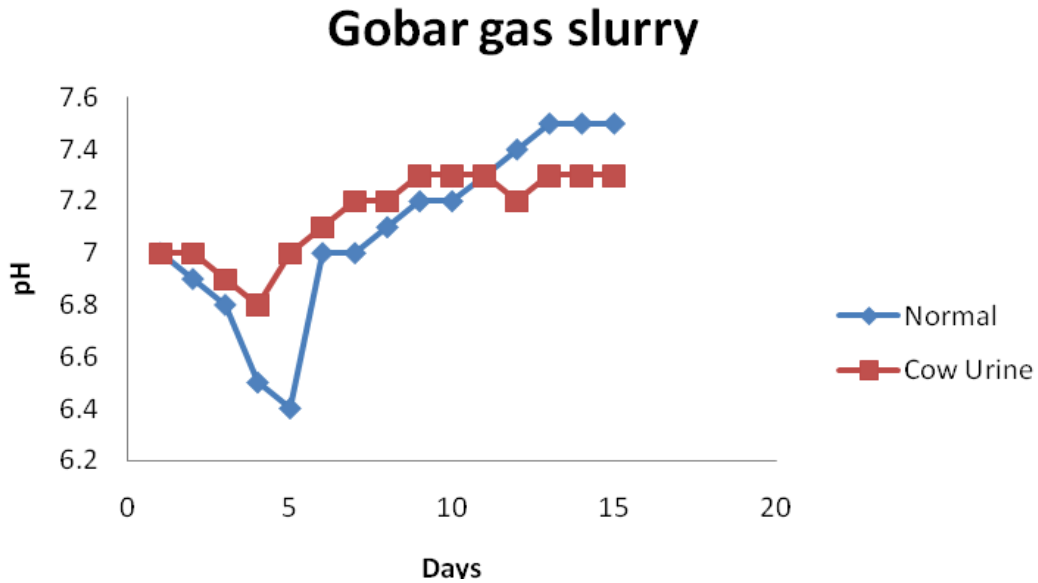


Fig.2.a) pH variation with time for gobar gas slurry

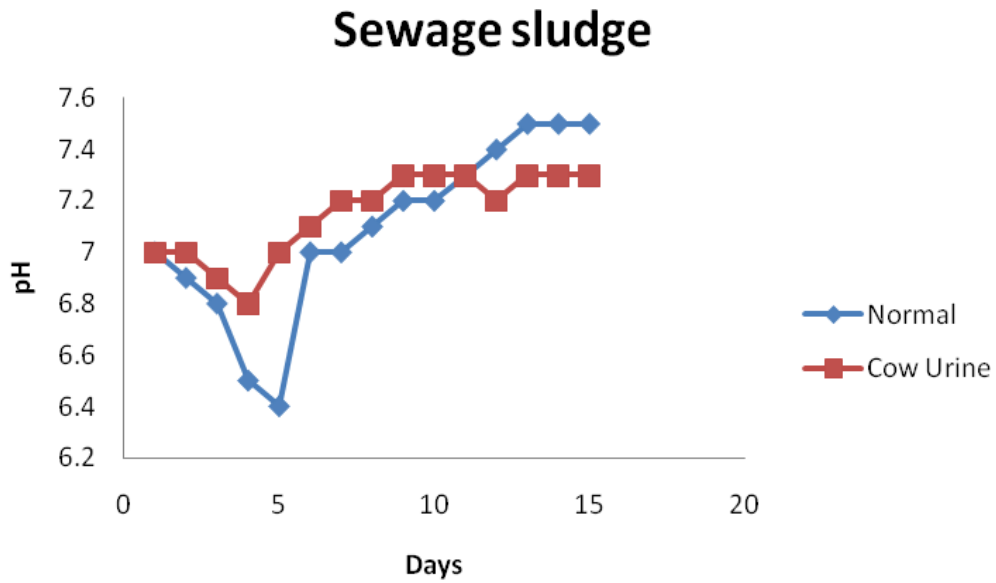


Fig.2.b) pH variation with time for sewage sludge

This is due to the development of $\text{CO}_3\text{H}\text{NH}_4$ from CO_2 and NH_3 , which were produced during the acidogenesis stage[23]. The percentage of $\text{CO}_3\text{H}\text{NH}_4$ caused the increase alkalinity of the samples. Due to this, any differences in the volatile fatty acid content did not affect the pH value. The third zone started on the twelfth till the last day of the experiment. In this zone, it was found that the pH value of the samples started to increase. This is due to the development of $\text{CO}_3\text{H}\text{NH}_4$ continuing while no more volatile fatty acid was produced. The pH observation results are in agreement with Feng et al.[24].

3.2 Temperature Variation:

The temperature of the samples varied between 30-33 °C [20]. This temperature range is the optimal for biogas production. The methanogens are mainly facultative. These microorganisms are active at temperatures 30 °C to 40 °C. A temperature below 30 °C would have inhibited the whole process, as the mesophilic bacteria are not active, as well as there would be a reduction in the methane gas production.

3.3 Volatile Fatty Acids:

The variation of VFAs with time for each of the two sludge's is given in the Fig.3a & Fig. 3b. The maximum limit of total VFA concentration should not exceed 4000 mg/l [20]. The VFA concentration of all the samples was within the limit.

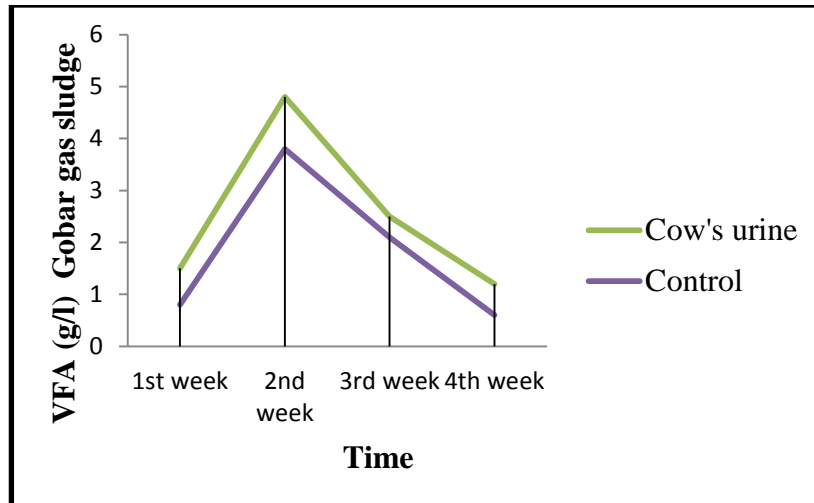


Fig.3.a) VFA conc. Variation with time for gobar gas slurry

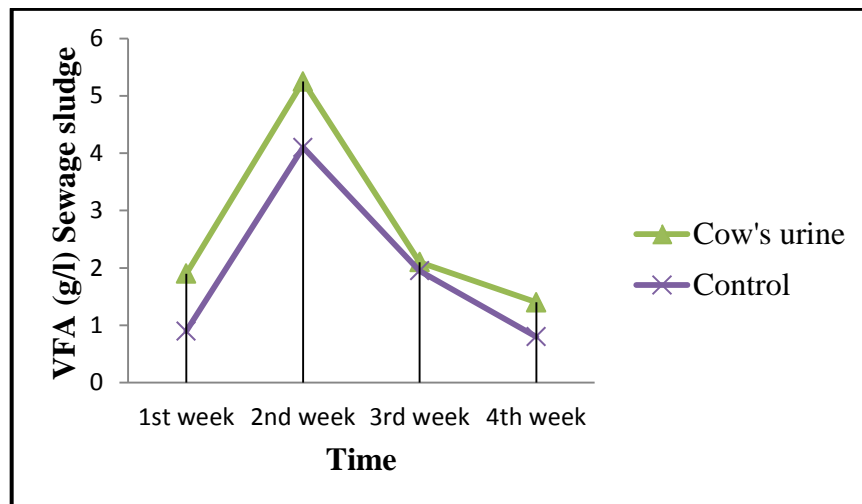


Fig.3.b) VFA conc. Variation with time for sewage sludge

It may be seen from the figures that, the yield of VFA increased in the second week and declined sharply in the third week.

3.4 Variation of total solid (% TS) of Influent and Effluent slurry:

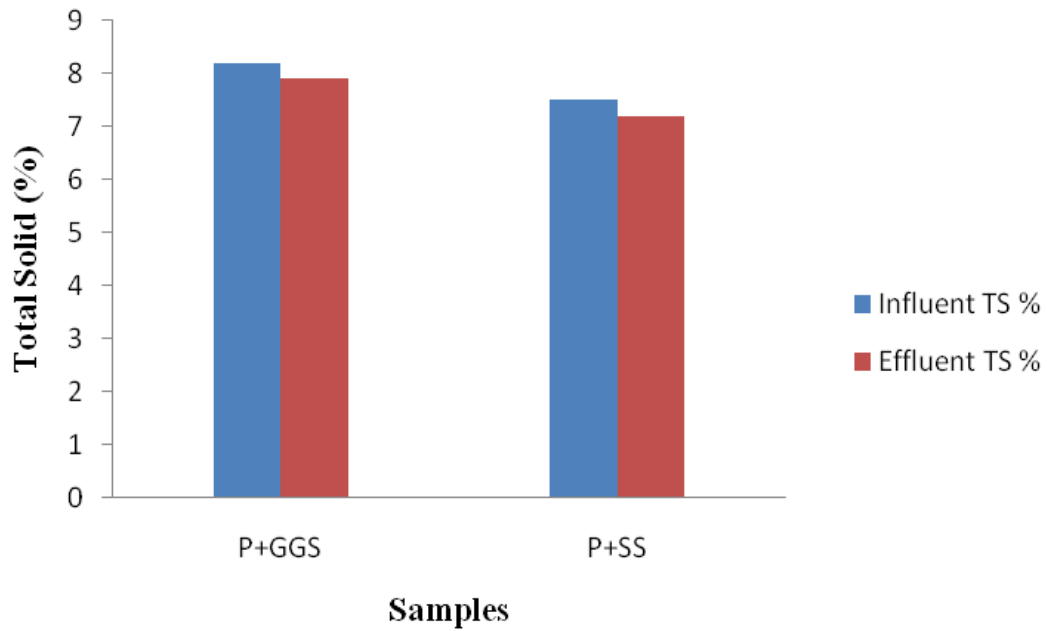


Fig. 4. Variation of total solid (% TS) of Influent and Effluent slurry

P = parthenium

GGS = Gobar gas slurry

SS = sewage sludge

Generally it is observed that after the production of biogas, the total solid content decreased. It denotes the anaerobic microbial activity, which resulted in satisfactory digestion of feedstock. The ideal TS (8-10%) for both the influent and effluent slurry was observed as depicted in Fig.4.

3.5 Variation of COD of Influent and Effluent slurry:

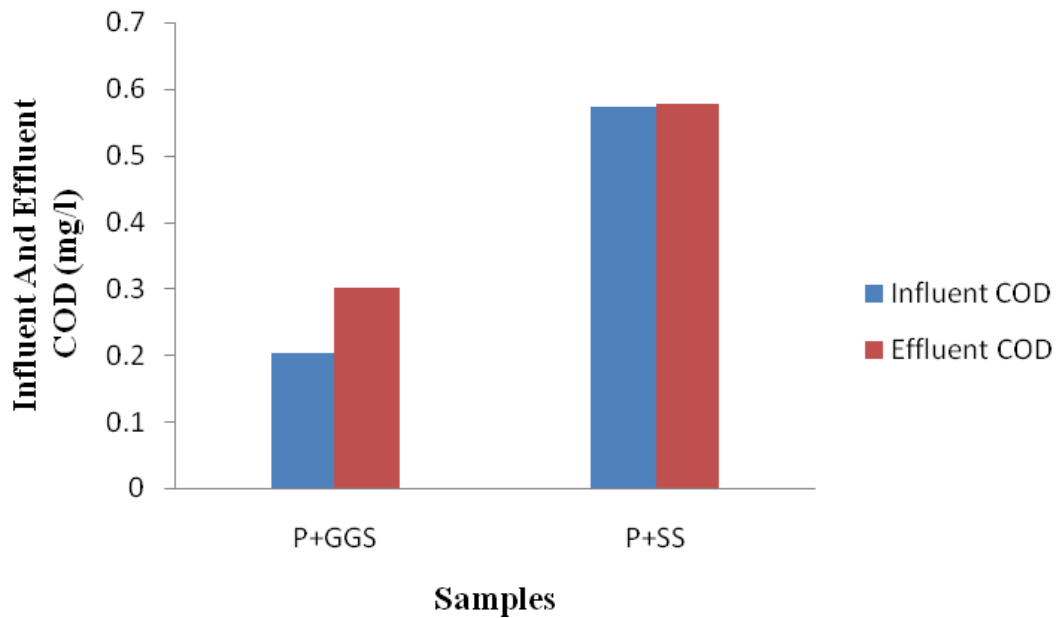


Fig. 5. Variation of COD of Influent and Effluent slurry

***P =Parthenium**

GGS =Gobar gas slurry

SS =sewage sludge

General it is observed that after the production of biogas COD value of the slurry increases as shown in Fig.5.The COD of influent slurry decreases because of the degradation of organic matter and due to high bacterial activity , the oxygen demand decreases gradually. COD of effluent slurry increases because of the addition of the substrate[25].

3.6 Variation of Methane (%) with time:

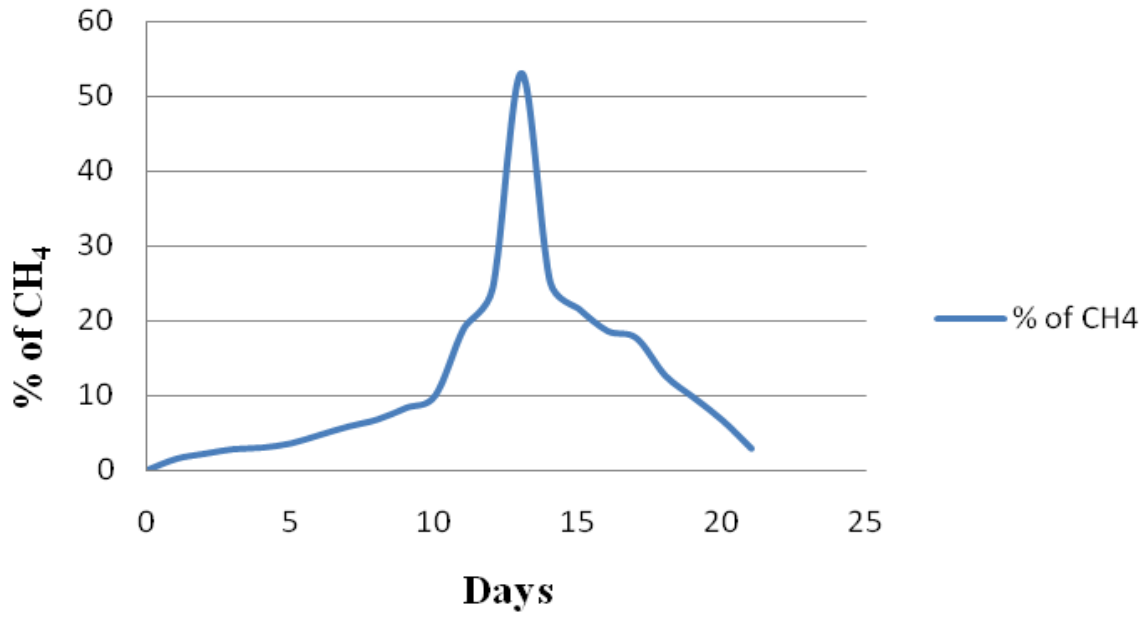


Fig.6. a) Variation of Methane (%) with time for gobar gas slurry

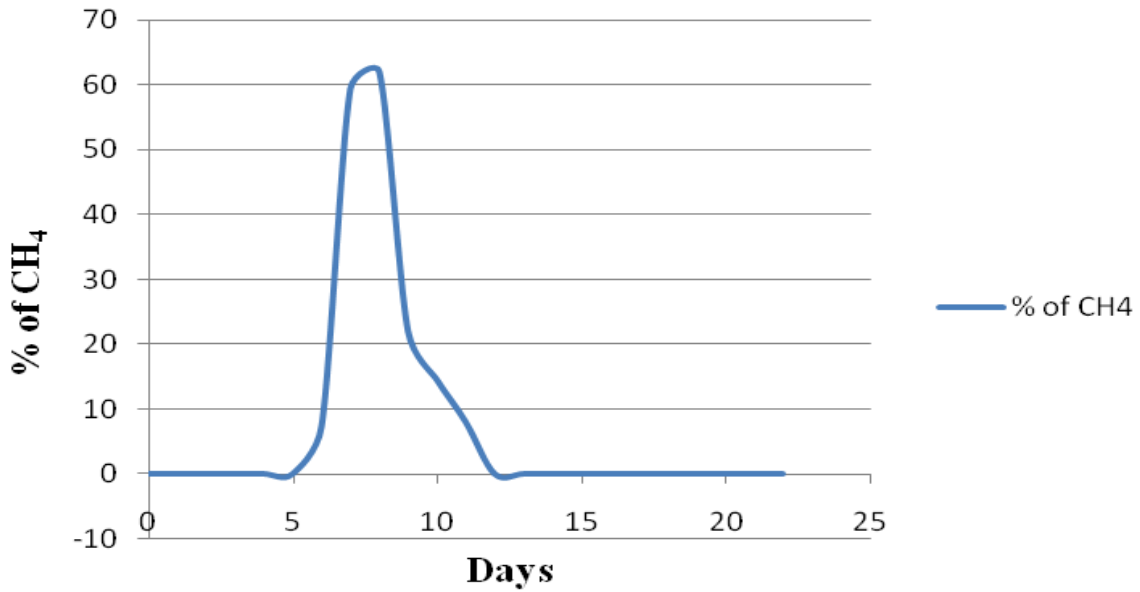


Fig.6. b) Variation of Methane (%) with time for sewage sludge

The production of methane (CH₄) was monitored daily by Methane gas analyzer. The highest amount of methane (62%) was obtained on 9th day of the experiment because when biogas production is there the reaction takes place and methanogens bacteria becomes strong and active. It is also observed from Fig. 6a & Fig. 6b that after 9th day methane gas production was reduced. This is due to the decay of the bacterial growth and at the end of the experiment there has been no appreciable biogas generation when the bacterial action has practically ceased. The results are in conformity with the amount of bio gas produced in other studies[26].

4. CONCLUSION:

Anaerobic digestion of *Parthenium hysterophorous* with gobar (Cow dung) gas sludge and sewage sludge was conducted under semi-batch reactor to investigate the dependence of methane yield using parthenium substrate under varied condition. In this process microbiological population is vital and boosts the methane generation rate. Along with this anaerobic conditions must be maintained properly otherwise aerobic conditions will inhibit the whole process. The highest amount of methane (62%) was obtained on 9th day of the experiment using sewage sludge as inoculum with parthenium. So, commercialization of the whole process needs to be done, as significant yield in methane gas is found from this experiment. This would not only help to mitigate the daily energy crisis easily at a cheaper cost with available infrastructure mainly in rural India, but also control the parthenium growth which is a menace for the society.

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HIGHLIGHT

- A promising alternative raw material for the accomplishment of Biomethanation is utilization of obnoxious weed. *Parthenium hysterophorus* L.
- *Parthenium hysterophorus* was utilised to optimize the yield of methane gas with cow urine used as a catalyst.
- Different parametric range like pH 6.5-7.5, temperature 35-40 °C and total solid loading (TS) 7.5 - 8.1% was utilized.
- The volatile fatty acid (VFA) yield was 128-942 mg/L and the maximum amount of methane produced was 62%.

Figure

Fig.1. Experimental set up using *parthenium hysterophorus*

Fig.2.a) pH variation with time for gobar gas slurry

Fig.2.b) pH variation with time for sewage sludge

Fig.3.a) VFA conc. Variation with time for gobar gas slurry

Fig.3.b) VFA conc. Variation with time for sewage sludge

Fig.4. Variation of total solid (% TS) of Influent and Effluent slurry

Fig 5. Variation of COD of Influent and Effluent slurry

Fig.6. a) Variation of Methane (%) with time for gobar gas slurry

Fig.6. b) Variation of Methane (%) with time for sewage sludge