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METHANOGENESIS OF PROSOPIS JULIFLORA

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Abstract - *This project involves the experimental analysis of biogas produced by using Prosopis juliflora (an invasive species in South India) as biomass and waste water sludge as substrate. Fabrication of bio-gas sampling device is done and the anaerobic condition is created to produce bio-gas. The conventional biogas sampling method is employed to analyse the biogas produced. The characteristics of biomass (plant specimen) and substrate (waste water sludge) are studied and the methane potential test is conducted to analyse the biogas production efficiency for a given substrate to biomass ratio. The conclusions are made based upon the efficiency of the bio-gas.*

Key Words: Methanogenesis, Prosopis juliflora, Invasive species, methane, biogas, Seema karuvelam, Anaerobic.

1. INTRODUCTION

The Prosopis juliflora commonly known as *Seema karuvelam* in Tamil is a species native to West Africa. It was brought to Tamil Nadu in 1960's for eliminating fuelwood scarcity, these seeds then started drifting into dams and rivers, causing some serious problems.

Now, the process of uprooting these trees is speed up by the Tamil Nadu government to prevent groundwater loss. The uprooted tree is generally dumped at a place and it is either burnt or left to decay, but the seeds in the uprooted trees won't die and will germinate in the nearby areas. Even if the tree is burnt the seeds will thrive to spread.

These piled up mass of organic waste can be used as a source for biogas production through anaerobic digestion. The biogas production plant uses all types of organic waste from the municipal solid waste like vegetable waste, agricultural waste and paper waste. The uprooted tree can be shredded using a tree shredder and can also be used for methane gas production. This ideology will create a purpose for this uprooted invasive plant.

This paper gives the detail experimental analysis of biogas production through anaerobic digestion of shredded Prosopis juliflora. The efficiency of the biogas production is analysed and compared with the conventional sources of biogas production.

1.1 Prosopis juliflora: in India

The Prosopis juliflora was first imported by the colonial British to afforest the wastelands of western India. In Tamil Nadu the fuel fire wood is the main fuel supply for the cooking purpose in the rural and drought-prone area especially in Ramanathapuram, Virudhunagar and Thirunelveli districts. During 1960's to face the fuelwood shortage and deforestation, the Tamil Nadu government sowed the seeds of this tree from helicopters on the desert wastelands. But the introduction of this species resulted in a major ecological disaster.

1.2 Prosopis juliflora: Characteristics

Prosopis juliflora is a tree that can grow up to 12m (40ft) and has a trunk diameter of up to 1.2m (4ft). It is a drought as well as fire resistant crop having root system able to grow to a depth of above 50m (164ft) in search of water. The tree has thrones in pairs at the nodes making it not suitable for feeding cattle. The gum of the tree has cerotic acid which is poisonous for cattle.

1.3 Objectives

1. To prepare the plant sample and to study its property.
2. To collect the substrate and study its property.
3. To perform methane potential test to analyze the biogas collected from bio-reactor setup.
4. To check the composition of biogas for maximum efficiency.

1.4 Scope

The ground water crisis is a major factor that the government has to deal with in Tamil Nadu. The Prosopis juliflora once sowed in the desert lands as a savior of life now turned into a vicious threat by depleting ground water resource and invading native plant species.

This ideology of using the uprooted tree in anaerobic digestion will result in evading further germination of this tree and production of fuel. The large pile of this organic mass will produce huge amount of biogas if treated properly which is practically possible.

2. EXPERIMENTAL STUDY ON SLUDGE AND PLANT SAMPLE

The substrate used for anaerobic digestion of the shredded plant (Prosopis juliflora) specimen is anaerobic digester sludge collected from the Perungudi sewage treatment plant. The main reason for selecting this sludge as the substrate is that it contains enough bacterial content and pH necessary for the anaerobic digestion to take place.

The Chemical Oxygen Demand (COD) and Total solids tests were done and the substrate to inoculum ratio is fixed.

The substrate to inoculum ratio depends on the Volatile suspended solids present in the sludge. The pH of the sludge is the main factor to be considered and it should be within permissible value in the range of 7.0 to 7.8. pH value below 7 will result in the acidic environment which is not suitable for bacterial growth. If the pH is acidic then $NaHCO_3$ is added to maintain the pH within permissible limits. Also another factor to be considered is temperature. The sludge must be kept in place where the temperature does not exceed 35°C in order to favor the growth of micro-organisms.

In general, the sludge will have more Oxygen demand than the biomass hence the degradation of the biomass takes place rapidly. The anaerobic process can be done in two types: - Single stage or two stage anaerobic digestion.

Here, the process is carried out in single stage in a bio-reactor, where the sludge is mixed with biomass in calculated amount and left in anaerobic condition for gas production by maintaining the temperature and pressure.

2.1 Anaerobic sludge and plant sample collection

The anaerobic sludge which is to be used as a substrate in this single stage anaerobic digestion is collected from the Perungudi Sewage treatment plant. The permission for 35L of sludge collection was got and collected on 18/2/19. The main reason for choosing this sludge as a substrate is that it contains large amount of methanogens, less acidity and high solid content which are suitable for attaining maximum methane yield.

The plant sample was made by cutting the branches, stem and root of the tree and shredding it into smaller size for improving the digestion rate.

There are certain preliminary processes involved in anaerobic digestion which includes adding chemicals to maintain pH, creating favorable conditions for the growth of methanogens and monitoring COD values. This is done by adding $NaHCO_3$ of 0.1mM (10ml for each 1L mix) so as to maintain growth of methanogens and using both NaOH and HCl to maintain pH.

Fig -1: Shredded plant sample

2.2 Anaerobic sludge and plant sample characteristics

The plant sample and sludge are tested for COD, pH and Total solids. The plant sample must be shredded and made into pulp by adding distilled water before testing.

The test results are given in the table:

Table -1: Sample test results

Tests/sample	Plant	Sludge
COD	0.9 g/L	1.3 g/l
Total solids	894 g/L	257 g/l
Volatile suspended solids (VSS)	10 g/L	1.2 g/l
pH	6.9	7.56

3. FABRICATION OF BIO-REACTOR

The sludge to biomass ratio (S_0/X_0) is an important factor in the net methane yield. The gas sampling is done for various sludge to biomass ratio so as to determine the efficient one. The volume of sludge and the biomass needed is calculated based on this ratio only. Initially the COD of the biomass and the Volatile solids of the sludge must be known in order to find the sludge and biomass volume required. This is done by using the formula:

$$S_0/X_0 = \frac{V_{Substrate}\,(L) * \text{substrate TCOD (g/l)}}{V_{Sludge}\,(L) * \text{sludge VSS (g/l)}}$$

The sludge available to prepare three samples is only 35L. Hence, the volume of the container must be designed in such a way that the available sludge is utilized up to great extent. Hence the total volume of the container must be a sum of volume of sludge, volume of biomass and volume required for the bio-gas to get filled.

Table -2: Volumetric calculation

Sludge to biomass ratio	0.5	1	1.5
Volume of sludge ($V_{Substrate}$) (L)	12	8.56	6.67
Volume of plant specimen ($V_{Substrate}$) (L)	8	11.44	13.33
Mass of plant specimen (Kg)	7.2	10.3	12

Thus, the total volume of the bio-reactor to be fabricated must accommodate the sample of volume 20L and also the space for bio-gas produced as a result of anaerobic digestion.

3.1 Materials used for fabrication

It is mandatory that the container to be fabricated must have calculated volume and must withstand the vacuum pressure created while creating anaerobic condition in order to avoid damage to the container.

The material employed for fabricating the container is Hot rolled mild steel (MS) sheet of thickness 1.6mm which often used for all engineering purposes. The sheet of 8ft * 4ft was used to fabricate three containers having capacity each of 37.5L.

¼ inch air hose is connected to the container by using MS connector by welding it. The opening for the container is provided by 2inch GI Coupling with inner thread for lid.

The three bio-reactor contains must be checked for any possible leakages in order to make it completely air tight.

Fig -2: Bio-reactor

4. BIO-GAS COLLECTION

The substrate and biomass are mixed in calculated ratio and poured into the containers. The container is sealed using M-seal and vacuum pressure of 400mmHg is created in it using gear oil vacuum pump. $NaHCO_3$ of 200ml (0.1mM – 10ml for each 1L mix) is added as buffer.

The container is kept under room temperature for 14 days. Container must be periodically shacked and checked for pressure drop.

Fig -3: Pouring substrate and biomass mix

4.1 Gas quality standards for testing

The gas to be analyzed in Gas chromatography must have certain characteristics in order to avoid blocking of the gas inlet and damaging the equipment. There is no problem to be dealt when the gas to be tested is given as input through online. But in case of gas collected from external sources there are some restrictions that must be considered. Some of them are:

i. The gas to be analyzed must not have any solid particles suspended in it.
ii. Gas should not contain moisture.
iii. Sulphur content should not be present in it.

The main reason for these restrictions is that the gas inlet of the analyzer should not get blocked by the solid particles or by moisture. Because, the inlet tube of the analyzer consists of ¼ inch tube connected with a 1/8inch tube which in turn is fitted to a 1/16inch tube. So, there are possibilities for blockage of the inlet. Hence care should be taken to avoid this situation.

Blockage can possibly be avoided by treating the gas with NaOH solution to remove the Sulphur particles and the moisture can be avoided by collecting the gas in room temperature. For safety purpose the gas can be checked with naked eye for presence of moisture and solid content after collecting it in injector.

The gas collection process is provided in the following flow chart:

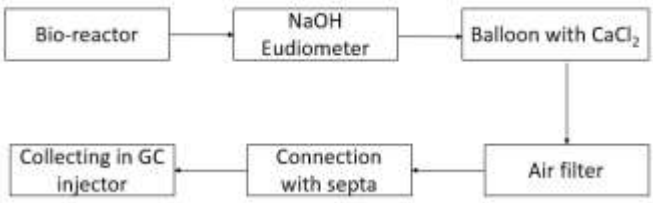

Fig -4: Gas collection process

4.2 Gas purification

As the gas to be analyzed is a product from the anaerobic digestion of sludge there is a possibility of Sulphur presence which should be avoided. Hence the generated biogas must be treated before collecting for analysis. This is done with the help of Eudiometer set up.

The Eudiometer set up consist of an inverted jar for gas collection and a bath for NaOH solution. Gas is sucked out from the container by creating a negative pressure in the inverted jar, then the gate valve is opened. The collected gas should be kept in the inverted jar for a retention time of 2-3minutes so that the Sulphur particles will get precipitated. This procedure is similar to the Scrubber mechanism which is conventionally used for gas purification.

Then the gas is then transferred to a balloon containing $CaCl_2$ which is connected with a gate valve by inserting the tube connection from the valve into the gas filled area of the flask and opening the gate valve. Initially, the balloon should be emptied and the air in the tube must be sucked out so as to avoid the entry of the solution. The gate valve is connected to the septa via 12mm diameter tube stuffed with cotton that filter the solid particles from the gas.

Fig -5: Gas collection set-up

5. ANALYSIS OF BIO-GAS

There are various methods in the analyzing of the biogas for methane composition depending on the sample media, method of analysis, volume and pressure requirement. Some of them are

1. Thermal- desorption Gas-Chromatography mass spectrometry
2. Gas Chromatography-Atomic Emission Detector
3. Gas chromatography- Flame ionization detector (FID)/mass spectrometry
4. Microwave plasma atomic emission spectrometry
5. Gas chromatography- Inductively coupled plasma mass spectrometry

Here, the Gas chromatography- Flame ionization detector /mass spectrometry is used for analysis.

Now-a-days, there facilities available for automatic biogas analysis in which the solution is directly fitted to the analyzer and the gas collected is directly analyzed without the need for any transfer and collection. By using this analyzer, the rate of gas production can also be charted eventually.

5.1 Analysis of test results

The gas thus collected are tested using FID in the gas chromatography. The area percentage report is then used to interpret the composition of methane in the bio-gas.

The FID detect only the flammable gases present in the input. Here, Nitrogen used as a carrier gas. Therefore, the highest peak in the graph will represent presence of methane.

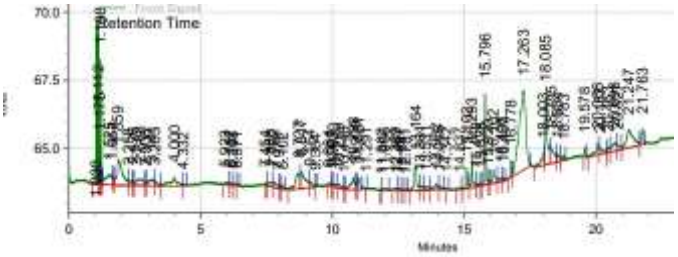

Fig -6: Gas chromatography result of gas sample 2

Fig -6. Represents the graph produced from the retention time and the volts generated from ignition of gas. Generally, the retention time of methane peak was 0.233 min at the column temperature of 150^0C. But in practice, the retention time may slightly differ.

Table -3: Methane peak data

Sample 2	
Retention time	1.03 minutes
Area	64380
Height	46328

Similarly, graphs and area percentage reports are obtained for other two samples.

5.2 Interpretation of results

The composition of methane in the biogas sample can be calculated based upon the retention time and the height of peak obtained for methane. The obtained peak data are compared with the standard biogas samples and the results are arrived.

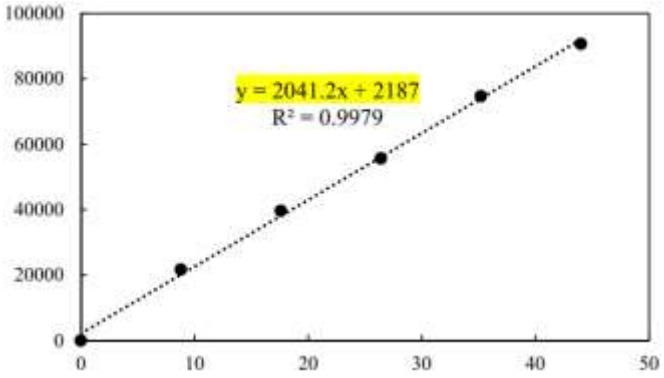

Fig -7: Standard methane peak graph of varying composition

The above graph is obtained by plotting the composition of methane on X-axis and area of methane peak on Y-axis for five standard bio-gas samples. The equation y = 2041.2x + 2187 gives the relationship between x and y axis which can be used for calculating the composition of methane in the unknown sample

Table -4: Methane composition

Substrate to biomass ratio	Methane %
0.5	29.5
1	31.5
1.5	26.7

From Table-4, we can interpret that the sample 2 produced maximum methane yield comparing with other two samples.

6. CONCLUSIONS

The bio-gas produced as a result of single stage anaerobic digestion of Prosopis juliflora was analyzed and composition of methane was found using Gas chromatography.

Therefore, the uprooted Prosopis juliflora tree can be used as a substrate in anaerobic digestion process for methane production rather than leaving to disperse in the nearby areas.

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