Anaerobic Microbial Hydrolysis of Agriculture Waste for Biogas Production

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ABSTRACT
Agriculture waste is an organic material and can be used to produce biogas through anaerobic digestion, thus providing an alternative for Agriculture waste use and mitigating the pollution. Agriculture waste is mainly composed of three groups of polymers, namely cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes. Hydrolysis is the process that converts complex organic matter(cellulose and hemicellulose) to soluble compounds such a simple sugar that can be used by bacteria performing fermentation and compound such as ethanol, biogas organic acids and others are produced. Lignin is a very complex molecule and is closely bound to cellulose and hemicellulose. It provide rigidity and cohesion to the material cell wall, and thus form a barrier against microbial attack and prevents the accessibility of enzymes to their substrate, hence making hydrolysis a rate limiting factor in anaerobic digestion of agriculture waste. The main objective of this study is to accelerate the biogas generation from agriculture waste by enhancing the hydrolysis process of agriculture waste using anaerobic hydrolytic microorganism isolated from cattle dung and by providing pre-treatment to agriculture waste prior to hydrolysis. Screening for cellulase and xylanase activity by clear zone formed around colonies in carboxymethyl cellulose (CMC) and Xylan based agar media and to determine the effect of isolated hydrolytic microorganism on hydrolysis rate of agriculture waste and biogas generation potential.

General Terms
Cellulose, Xylanase, COD, Thioglycolate.

Keywords
Anaerobic, Agriculture waste, Biogas, Hydrolysis.

1. INTRODUCTION
Lignocellulose in the form of forestry and agriculture wastes is accumulated in large quantities every year. It is used as animal feed, fuel for cooking, house heating and papermaking. A significant amount of agriculture waste remains unused and burned in open fields, causing serious environmental and safety problems, such as air pollution. On the other hand agriculture waste is an organic material and can be used to produce biogas through anaerobic digestion, thus providing an alternative for Agriculture waste use and mitigating the pollution.
Agriculture wastes are mainly composed of three groups of polymers, namely cellulose, hemicellulose, and lignin. The amounts of carbohydrate polymers and lignin vary from one plant species to another. However, cellulose is usually the dominant structural polysaccharide of plant cell walls (35–50%), followed by hemicellulose (20–35%) and lignin (10–25%). Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes. Hydrolysis is the process that converts complex organic matter(cellulose and hemicellulose) to soluble compounds such a simple sugar that can be used by bacteria performing fermentation or other microbes and compound such as ethanol, biogas organic acids and others are produced. The main drawback with anaerobic digestion of agriculture waste is the presence of lignin, Lignin is a very complex molecule constructed of phenylpropane units linked in a large three-dimensional structure. Three phenyl propionic alcohols exist as monomers of lignin: p-coumaryl alcohol, coniferyl alcohol and sinapylalcohol. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion to the material cell wall, to prevent water permeability to xylem vessels, and to form a physic–chemical barrier against microbial attack. Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation. Thus its form a barrier preventing the accessibility of hydrolytic enzymes to their substrate making hydrolysis a rate limiting factor in anaerobic digestion.Various factors affect biogas productivity these include pre-treatment of the feed stock, Microbial biomass activity, the C: N ratio, pH, volatile fatty acids, Substrates.
The present study have focused on to accelerate the biogas generation by enhancing the hydrolysis process of agriculture waste using anaerobic hydrolytic microorganism isolated from cattle dung and by providing pre-treatment to agriculture waste prior to hydrolysis. To determine the effect of isolated hydrolytic microorganism on the rate of hydrolysis and biogas generation potential and to develop the laboratory scale model for biogas generation.

2. MATERIALS AND METHODS
2.1. Materials:
Agriculture waste particularly rice straw was collected from a field in Ahmedabad, Gujarat. It was chopped in to 1-2 cm length. The chopped waste was dried in sunlight for 1 day and was stored at room temperature for further use.
Fresh cow dung was collected from Ahmedabad, Gujarat and was used immediately.

2.2. Enrichment of anaerobes:
100gms of agriculture waste previously chopped were wetted with 1 ppm formalin and 1 lifter of water and was kept for 24hr incubation. Water was drain from the straw after 24hr, Upon draining water from straw 80 % moisture is retained by rice straw. 10gm of this rice straw was mixed 20ml thioglycolate broth supplemented with 1gm of cow dung in a sterile test tube equipped with cotton plug and was kept in air tight container for two weeks, at ambient temperature (25±5^{o}C).
2.3. Isolation and screening of saccharolytic strains:
The isolation of hydrolytic bacteria was carried out by serial dilutions. Ten-fold serial dilutions of enriched sample were prepared in sterilized distilled water and 0.5 mL diluted

sample was placed in the petri plate [2] and was covered by 20 ml CMC containing thioglycolate agar medium tempered at 50°C. The CMC-containing medium contained (g L-1): CMC 10, Casein 15, Yeast extract 5, Dextrose 5.50, Sodium Chloride 2.80, L - cystine 0.50, Sodium Thioglycolate 0.50, Resazurin sodium 0.001. After incubating at ambient temperature(25±5) for 4 days, Anaerobes were found growing in the deeper portion of agar medium and aerobics colonies were found growing on the surface of the medium. The individual anaerobic colony obtained was transferred to sterile distilled water with the help of a borer and spatula and was mixed well and was inoculated in several CMC containing thioglycolate agar slants in a sterile test tube equipped with a cotton plug, plug was ignited by passing it through bunsen burner and was pushed in to the tube by using glass rod until it nearly touches the slant, pyrogallic acid crystal was filled in the space between the cotton and the top of the slant tube, 2ml of 4% NaOH was added to the tube[4] and a rubber stopper was applied immediately to tighten each tubes and the tubes were kept invert in a decanter containing 20 ml 10% pyrogallic acid and 4ml 4% NaOH to maintain anaerobic condition inside the decanter and the decanter was sealed completely with the help of paraffin war[5] and was kept for incubation for 4 days at ambient temperature (25±5). The colonies grown on plates need to be sub cultured on thioglycolate agar plates and stored at 4°C for further studies and for bulk production the isolates need to culture in thioglycolate broth media containing powered rice straw as carbon source.

2.4. Screening of Cellulase Activity:

The purified colonies need to be further screened for identifying the presence cellulase activity. Pure individual cultures of bacterial colonies need to be inoculated on both CMC containing thioglycolate agar plates and on xylan containing thioglycolate agar plates, a disc of filter paper having same diameter as of petri dish is to be located on top of one half of the dish and a mixture of pyrogallic acid and sodium carbonate in dry powder form is to be spread on it, the inoculated plate is to be inverted over the filter paper and sealed tight with molten wax to produce anaerobic conditions and is to be kept in an air tight container[5]. After 4 days of incubation, the plates are to be flooded with 1% congo red and the plates should be allowed to stand for 20 minutes at room temperature. Then the plates should be thoroughly washed with 1M NaCl solution. A clear zone formed around the growing colonies of cellulase and xlyanase positive cultures against the dark red background is to be taken as the indication of cellulose and xylanase activity. The isolated bacterial colonies need to be further characterized for their morphological and gram reactions characters.

2.5. Pre-treatment of Agriculture Waste for biogas production:

A pre-treatment is required to break up the most recalcitrant component lignin of agriculture waste and to improve the accessibility of hydrolytic enzymes to their substrates. 200gm Agriculture waste for biogas production should be chopped in to 1-2 cm length and need to be wetted with 2ml formalin in 2ltr of water for 24hrs. Water should be drained from the straw after 24hr, and should be inoculated with white rot fungus [6] and need to be kept for 10 to 12 days at ambient temperature(25±5) for lignin degradation.

2.6. Determination of hydrolysis rate:

Two conical flasks are to be taken and both to be filled with slurry prepared of pre-treated straw: water ratio of 1:2. One flask containing slurry need to be inoculated only with cow dung, while second flask need to be inoculated with cow dung along with isolated microbes and is to be kept for 6 to 7 days incubation. Hydrolysis rate is to be determined by measuring the COD as per standard methods for both the samples before inoculation and after 7days incubation.

To determine the effect of pre-treatment on hydrolysis rate, Slurry prepared of untreated straw: water ratio of 1:2 is to be place in a conical flask inoculated with cow dung and is to be kept for 7 days incubation, COD to be measured before inoculation and after incubation. The COD measured for both pre-treated and untreated straw samples is to be compared.

2.7. Biogas generation potential:

Biphasic biodegradation of agriculture waste for biogas production is to be done; Hydrolysis and methanogenesis phase is to be separated in two different reactors. The hydrolytic cultures isolated from cow dung along with cow dung need to be inoculated in pre-treated agriculture waste for hydrolysis followed by methanogenesis for biogas production in separate reactor. Airtight 500 ml conical flask is to be taken as a laboratory scale reactor. U shaped glass pipe need to be connected to the mouth of the bottle. Another end of the pipe is to be opened in the inverted calibrated cylinder which is to be submerged in water. This cylinder will act as a biogas collection cylinder. The volume of gas produced in the reactor is to be measures by downward displacement of water in an inverted cylinder [8]. The experiment is to be carried out at ambient temperature. The schematic diagram is shown in figure 1.

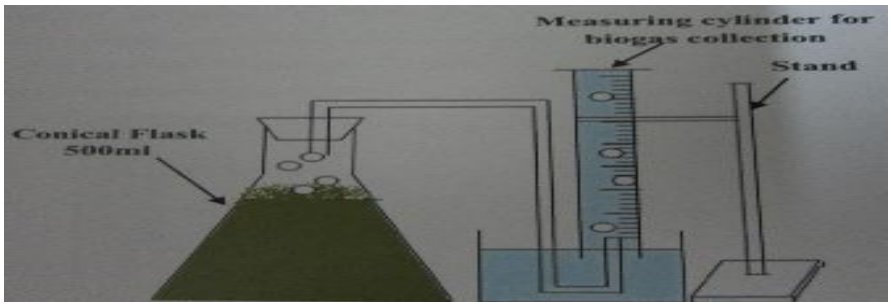

Figure No.1: Schematic diagram for laboratory scale mode

3. CONCLUSION

From the present study following results are anticipated

1. Biogas production will be enhanced by inoculating isolated hydrolytic microorganism to agriculture waste with decrease in detention time.

2. Pre-treatment of agriculture waste with white rot fungus is expected to maximize the accessibility of hydrolytic enzymes to its organic matter like hemicellulose and cellulose by lignin degradation.

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