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Trichodermaatroviride As Microbial Strain In Biogas Production From Lignocellulosic Wastes.

B Senthil Rathi*, Vinod Kumar S, Sivaranjanee R, N Deepa, and G Sampath Kumar[2].

1 Department of Chemical Engineering, St. Joseph's College of Engineering, Chennai -600119, India
2 Department of Chemical Engineering, TheKavery Polytechnic College, Salem – 636453, India

ABSTRACT

Lignocellulosic waste is abundantly available raw material on the Earth which is classified as different waste streams from various industries, forestry, agriculture and municipalities. Hydrolysis of these materials is the first step for either digestion to biogas (methane) or fermentation to ethanol. In this work the co-digestion of biogas by *Trichodermaatroviride* 9641, from the lignocellulosic waste materials such as orange peel waste, litter leaf, paper waste, and dairy waste was studied in an anaerobic reactor. It was studied by measuring the biogas concentration at regular intervals. The maximum biogas (470 ml) was obtained on the 10th day of incubation. Production of biogas by *Trichodermaatroviride*9641 on all the four substrates was enhanced by optimizing the pretreated substrate, pH, substrate concentration; inoculum concentration of the culture medium.The experimental data (pretreated substrate) showed maximum gas output (482 ml)of gas production when compared without pretreated substrate. Under the optimized conditions, the Orange peel waste showed maximum production at pH 5.5 (445 ml), 15g/ml of substrate concentration (480 ml) and 6 ml of inoculum concentration (465 ml).Hence, the orange peel can produce the maximum amount of biogas using *Trichodermaatroviride*9641 compared with other Lignocellulosic waste.

Key words: *Trichodermaatroviride 9641,* lignocellulosicwastes, biogas, anaerobic reactor

Corresponding author

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INTRODUCTION

Biomass is a renewable energy source, biological material from living, or recently living organisms. As an energy source, biomass can either be used directly, or converted into other energy products such as biofuel. Biomass is carbon, hydrogen and oxygen based. Biomass energy is derived from five distinct energy sources: garbage, wood, waste, landfill gases, and alcohol fuels. [1, 2]. Lignocellulose composes more than 60% of plant biomass produced on earth [3]. Fruit/vegetable waste includes refused fruits and vegetables from the municipal terminal market which is about 20 - 50% of municipal solid waste cities. Annually, there were 100 million tons of fruit/ vegetable generated [4]. Wood energy is derived by using lignocellulosic biomass (second generation biofuels) as fuel. Biogas is typically referred to as a gas produced by the breakdown of organic matter in the absence of oxygen. The process is known as anaerobic digestion and performed through the biological activity of microorganisms. This phenomenon naturally occurs at the bottom of ponds and marshes, which results in production of methane [5]. Nowadays it is produced from sugar-and starch-based materials such as sugarcane and corn. However, the second generation production of ethanol derived from lignocellulosic materials is now being tested in pilot plants [6, 7]. Recently, the considerable attention of research activity on fermentative hydrogen-production has been focused on the conversion of biomass reproducible resources to hydrogen by mixed cultures [8 -9]. Biogas production from jatrophadeoiled cake and orange peel waste was established here to be feasible at room temperature. [11]. The methane yields from the mango peels of some of the varieties, orange wastes, pomegranate rotten seeds and lemon pressings were significantly higher than the cellulose [12]. The performance of biohydrogen production using the raw wheat straw and HCl pretreated wheat straw was then compared in batch fermentation tests [13].

The optimum conditions for biogas production where the initial concentration of mixed xylose/Arabin ose 5g/l each, initial cultivation pH 5.5 & temperature 55^{0}C. Under the optimum conditions, a maximum biogas yield of 2.49 mol-gas/mol-sugar consumed was obtained [14].With hydrothermal heating, organic dissolving into liquid phase increased the COD, VFA and TOC concentration. That improves the biodegradation of biomass waste. [15]. The highest methane yield was obtained by pretreatment of the substrate at 20°C for 10 min with orange peel waste and hexane ratio of 1 : 12 which results in three times higher methane yield compared to the untreated wastes [16, 17].

MATERIALS AND METHODS

Collection of Raw Materials

Four raw materials were chosen for this work. (i) Paper Waste, (ii) Dairy Waste, (iii) Orange Waste, (iv) Litter Leaf. All these materials were collected from St. Josephs college of Engineering, Chennai

Microbial Strain

Trichodermaatroviride(9641), obtained from MTCC (Chandigarh), was used for the production of biogas. Stock cultures were maintained in malt extract medium (Table 1) and sub cultured at monthly intervals.

Table 1: Growth Medium

Components	Concentration (g/l)
Malt extract	40
Agar	20

Pretreatment of Lignocellulosic Waste

The wastes were collected from industries and college canteen. The organic wastes (Orange peel waste + Litter leaf) were collected, dried and powdered in a crusher. The industrial effluents (Paper waste + Dairy waste) collected from the industries were diluted. The diluted wastes are then added into the serum bottles. The medium composition is given in the Table 2. The medium was autoclaved for 20min at 121°C. After cooling, a fresh fungus was inoculated to the autoclaved flask containing medium. Then the serum bottles were maintained at room temperature under static conditions for a period of about 10 days.

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Table 2: Composition of Biogas Production Medium

Components	Concentration (g/l)
NH_4HCO_3	5.24
$NaHCO_3$	6.72
Trace Elements	
$CuSO_4.5H_2O$	0.005
$CoCl_2.5H_2O$	0.000125
$MgCl_2.6H_2O$	0.1
$MnSO_4.6H_2O$	0.015
$FeSO_4.7H_2O$	0.025
$Na_2S.H_2O$	0.25

Experimental Setup

The experimental set up for the CO-digestion of Biogas were carried out at ambient temperature ranging from 27°C to 32°C in four batch reactors Labeled 1 to 4 which is shown in Fig. 1.A serum flask of 500ml contains the sludge sample. The flask is plugged with a rubber stop perforated with a hollow needle. A liquid displacement system (Mariotte flask), consisting of a 500ml serum flask with the NaOH-solution and plugged by a rubber stop perforated by two hollow needles and placed upside down. Biogas produced by the in the serum flask will be accumulated in the liquid displacement system, displacing the NaOH solution.CO_2 will be absorbed in the NaOH solution. The displaced liquidis therefore considered to have the same volume as the produced by the gas. The sludge flask and the NaOH-flask are interconnected with a tube that is connected at both ends to syringes. Conical flasks or graduated cylinders is covered with funnel and is placed below the second hollow needle of the NaOH-flask. The displaced liquid is gathered in this flask or cylinder.

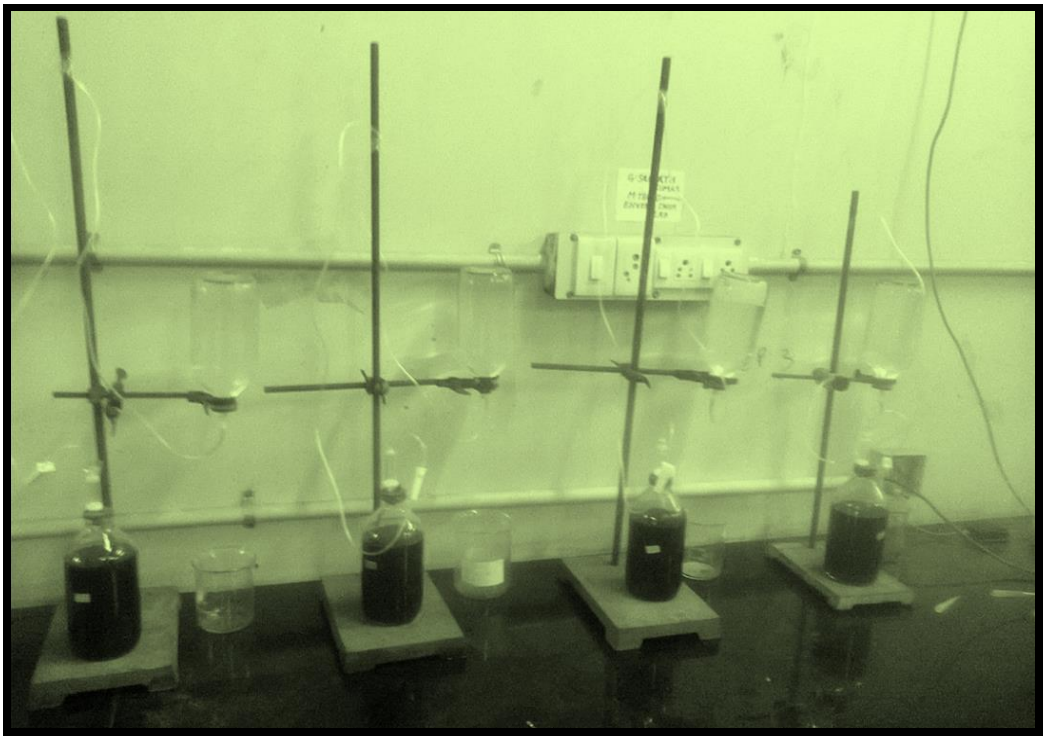

Figure 1: Experimental Setup for Biogas Production

Experimental Procedure

Waste sample is placed in a serum flask of 500 ml (5 ml of waste +1 ml of inoculum). The production media were weighed and then it is made up to 500 ml and it is then added into the serum flask. The rubber stop is placed and the flask is connected to the liquid displacement system. The serum flask for the blank

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(containing only water, in the same volume of the liquid in the serum flask containing the sludge sample) is also connected to a liquid displacement system. The volume of the NaOH solution in the liquid displacement system of the blank should be comparable to the volume of the liquid displacement system that is connected with the serum flask that contains the sample .Approximately 500-600 ml of biogas was produced.

RESULTS AND DISCUSSION

Effect of different wastes on Biogas Production

The maximum production of biogas occurs in a medium containing Orange peel (Fig. 2) which has carbon source and contains more amount of hexose molecule when compared to other waste used as a substrate it was found to be 470 ml at 10th day of Biogas Production shown in Fig. 3.

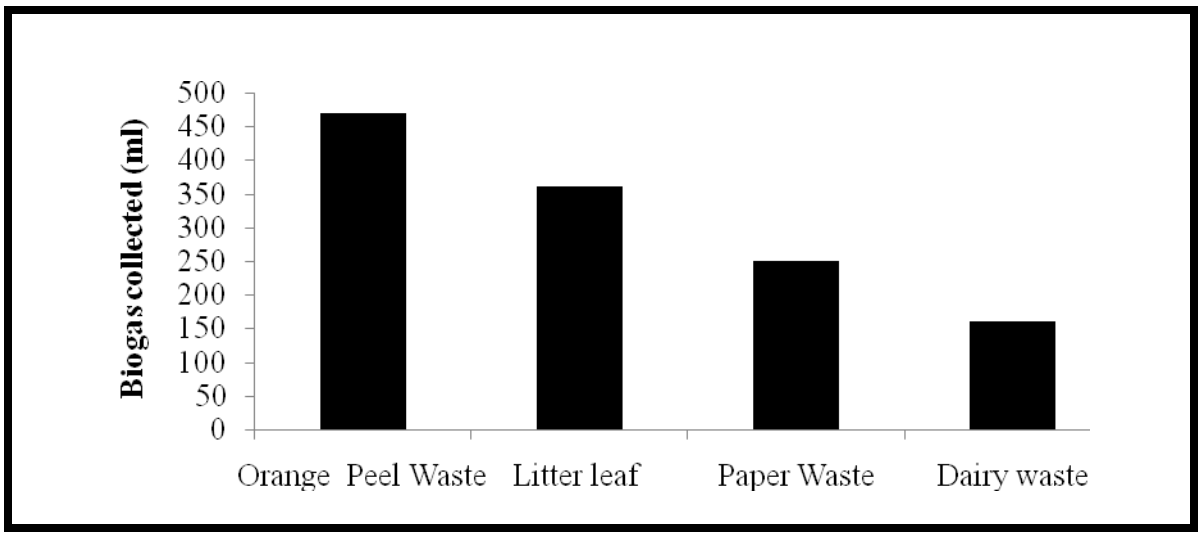

Figure 2: Effect of different wastes on Biogas Production

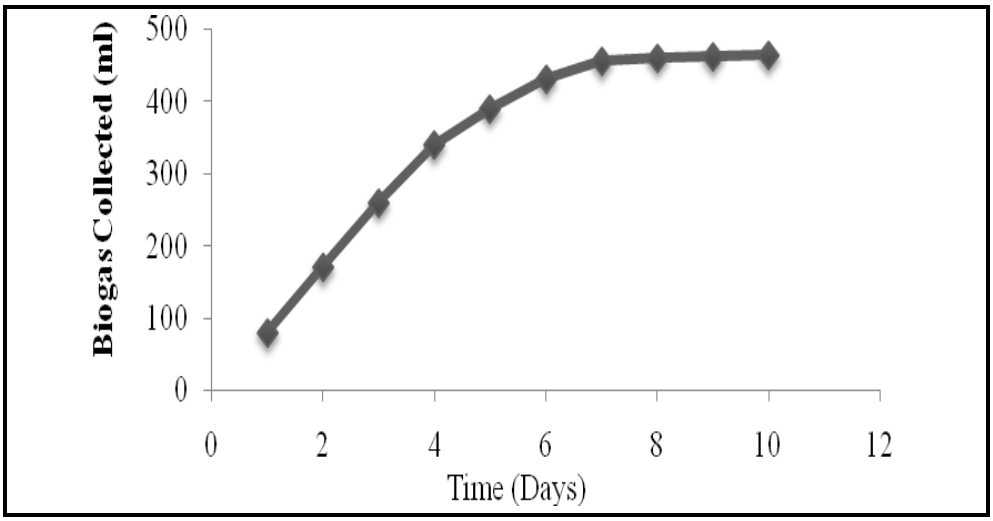

Figure 3: Biogas Production in Orange Peel Waste

Effect of Pretreatment

To make the components of lignocellulosics more accessible pretreatment was carried out. About 5g of finely ground above raw materials was mixed with 20ml of alkali (0.1N) solution and was soaked for a period of 12h. The conical flask containing these raw materials was filtered using muslin cloth. The residue was washed with water till a neutral pH was obtained. It was dried at 60°C over night and was used further. The

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cellulose content remained more or less constant till 10 days, however, further incubation led to decrease in cellulose content by 15.9%.(Fig. 4).However, further increase in the pretreatment period led to increase in hemicellulose content. The initial decrease in hemicellulose content for the first ten days might be the result of breakdown or hydrolysis of hemicellulose into fermentable sugars. This observation clearly indicates that the fungus has active hemicellulases during the 10 days of its growth cycle and acted clueless after the 10 day period.Thus *Trichoderma atroviride*(9641) can only convert the hexoses, such as glucose and mannose, and not the pointless, such as xylose and arabinose, that are found in the hemicellulose part of the substrate. Hence 10 days of incubation period were sufficient to increase the biogas production in the substrate.

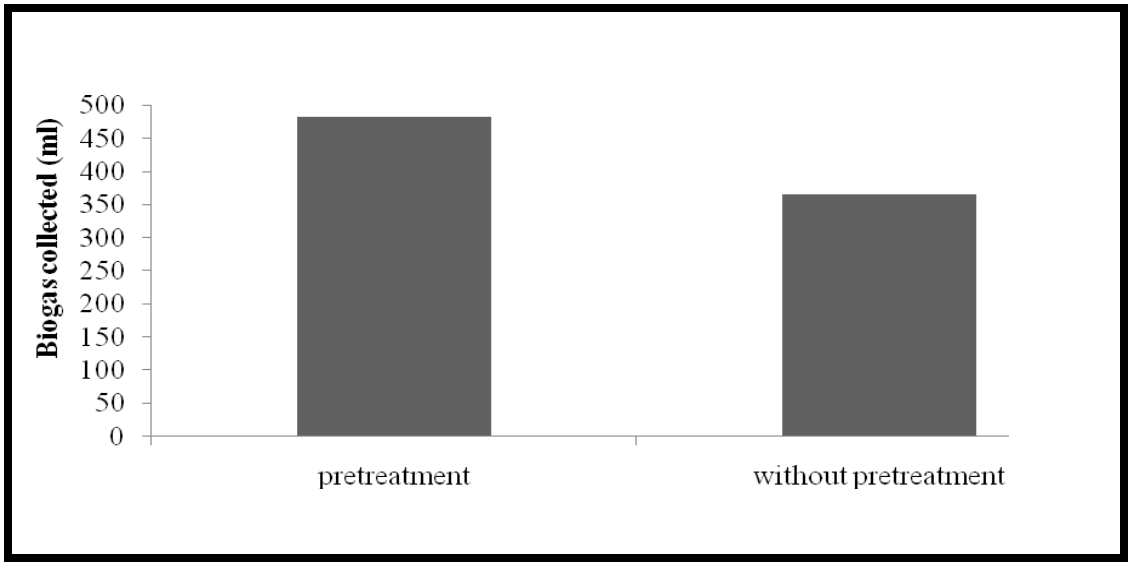

Figure 4: Effect of Pretreatment

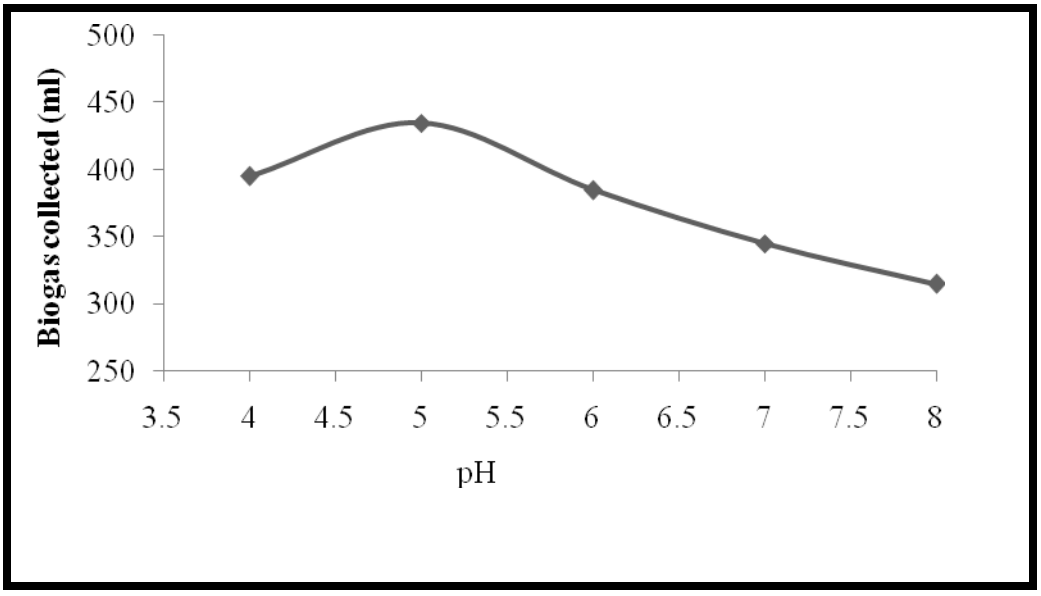

Figure 5: Effect of pH

Effect of pH

The effect of pH was studied by adjusting the media (with1.0 M of NaOH or HCl) to different pH values from 4 to 8. (Fig. 5). The media were autoclaved, cooled and inoculated with an overnight culture of *Trichodermaatroviride* (9641).*Trichoderma atroviride* (9641) utilizing glucose as a substrate where pH 5.5 was found optimal for biogas production [14].The sugar consumption varied from 66% to 83% between pH 5.0 and 6.0 suggesting the enhanced ability of the microorganism to consume sugar in this pH range. However, the

consumption decreased in case of pH 7.0 and 7.5 respectively. The biogas production followed a similar trend to glucose consumption, no significant change in biogas production was observed in increasing the pH beyond 5.5.

Effect of Substrate Concentration

An orange peel waste was tested at substrate concentrations from 5 to 25 g/l of water. The incubation period for all experiments was carried out for ten days. The biogas production was determined on the final day of the process. The total biogas quantity varied from 280 to 480 ml and no further production was observed after 10thday. The peak biogas production of 480 ml was obtained at a substrate concentration of 15 g/l and further high concentration (20 g/l) was found to be inhibitory to gas production (Fig. 6). The biogas accumulation gradually increased in the substrate concentration range of 5-15 g/l.

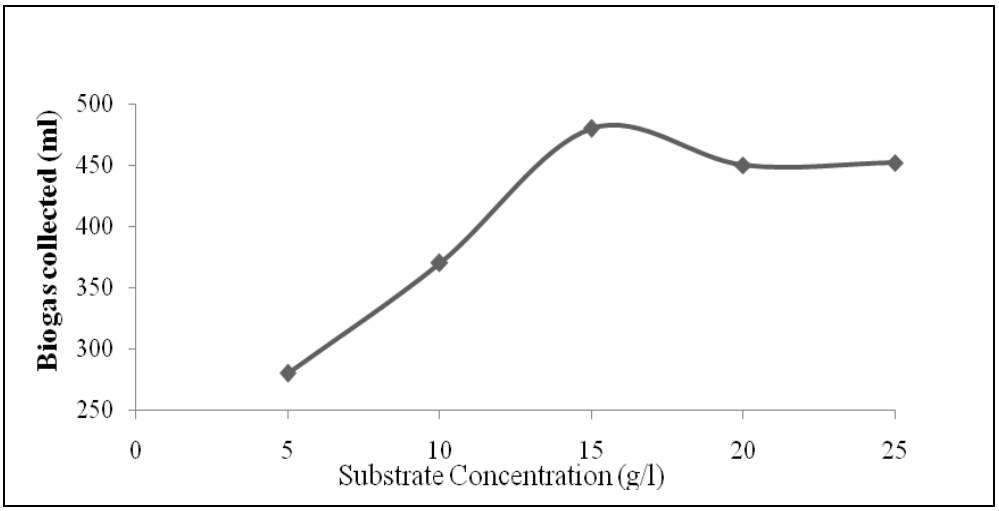

Figure 6: Effect of Substrate Concentration

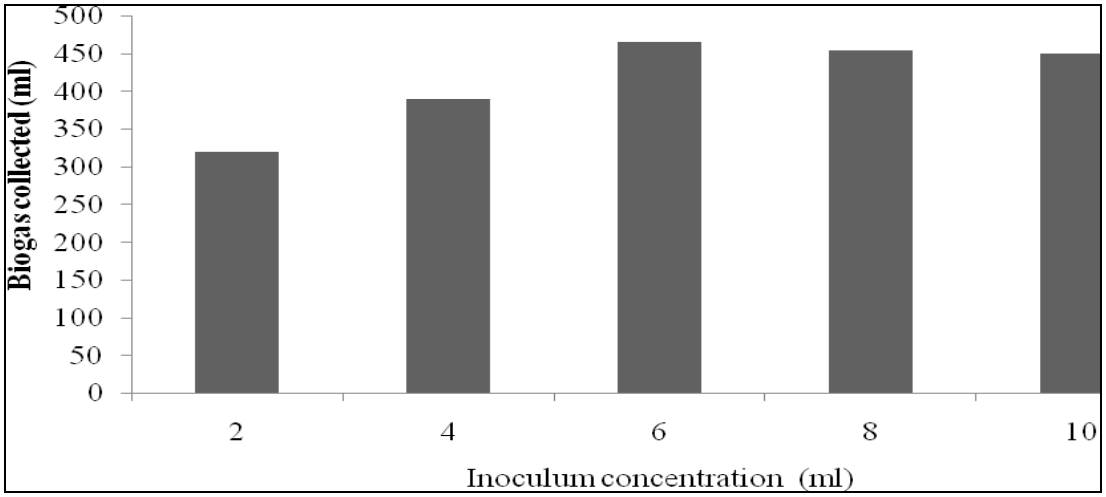

Figure 7: Effect of Inoculum Concentration

Effect of Inoculum Concentration

By adjusting the inoculum volume from 2 to 10 ml using an overnight culture of *Trichoderma atroviride* (9641). The production of biogas increased with increasing inoculum concentrations up to 6 ml and further increasing the concentration of inoculum the biogas production gets decreased due to the formation of biomass in the serum bottles. Hence maximum biogas production was observed at 6 ml of inoculum concentration.

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CONCLUSION

It can be concluded that biogas production by *Trichodermaatroviride*9641, among the four wastes, Orange peel waste has the maximum biogas production followed by litter leaf, paper waste and dairy waste. These waste materials constitute a renewable resource and can serve as an abundant and inexpensive carbon source. Moreover, the biogas production obtained indicates that a period of 10 days is enough for the maximum biogas production. It was also observed that pretreatment, pH, substrate concentration and inoculum concentration influenced the biogas production and these parameters should be maintained properly for the maximum production of biogas. As a result, the use of the above mentioned wastes for the production of biogas would decrease the cost of production in an environmentally sound manner.

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