

# Kinetic Studies on Biogas Production Using Orange Peel Waste

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## Abstract

The production of biogas by *Trichoderma atroviride* 9641 from lignocellulosic waste such as orange peel, litter leaf, paper, and dairy waste was studied in an anaerobic reactor. The biogas production of waste using *Trichoderma atroviride* 9641 was studied by measuring the biogas collected at regular intervals. A maximum biogas volume of 470 ml was obtained on the 10th day of incubation. The production of biogas from *Trichoderma atroviride* 9641 on orange peel waste was enhanced by optimizing the pretreated substrate, pH, and substrate concentration. The pretreated substrate showed maximum biogas production of 482 ml when compared to the substrate without pretreatment. Under the optimized conditions, the orange peel waste showed maximum production at a pH value of 5.5 and a substrate concentration of 475 ml. Peptone showed the best result for biogas production 480 ml. when compared to other nitrogen sources potassium nitrate and ammonium nitrate. When orange peel was used as substrates for biogas production observe in 457 ml were among the trace elements. Maximum biogas production in the orange peel waste 460 ml was observed when n-hexane was used as a solvent for the extraction of D-Limonene content when compared with other solvents.

**Keywords:** *Trichoderma Atroviride 9641, Lignocellulosic Wastes, Biogas, Anaerobic Reactor, Kinetic Studies.*

## 1. INTRODUCTION

Biogas can be generated from waste such as sewage, distillery waste, food waste, feed stock and pome the produced biogas contains 99% of the biogas after passing through a KOH solution to remove CO<sub>2</sub>. Cirne et al. (2007) indicated that the feasibility of biogas generation utilizing dairy manures as feedstock by anaerobic fermentation was demonstrated in this study.

The major challenges limiting its commercialization relate to its low production rate, yields, and the economic viability of the processes involved. Biogas production can be achieved by dark fermentation as well as photofermentation, with a higher rate of production reported Arunsri, F., Alissara, R. (2011). Tengerdy, R.P and Szakacs, G. (2003).investigated the effect of an initial pH between 5 and 10 on biogas production from crude cheese whey (87.5% by butyl aceticum. Biogas was produced over the pH range studied. The biogas production rate and yield peaked at an initial pH of 6 and then steadily decreased as the pH increased. VanGinkel, S (2001) et al. indicated that the optimal pH and starch had a maximum biogas yield of 186 ml of starch. The main problem with this formula is that biodegradable and non-

biodegradable matter are both taken into account for the calculation, while only the biodegradable matter is metabolized into methane.

Fan, YT. et al. (2007) indicated that the optimum conditions for biogas production were the initial concentration of mixed xylose and arabinose (5 g/l), initial cultivation pH 5.5, and temperature 550 °C. Under the optimum conditions, a maximum biogas yield of 2.49 mol of gas per mol of sugar consumed was obtained. Elaiyaraju, P., Partha, N. (2011) investigated the effect of pH and intermediate product formation on biogas production using *Enterobacter cloacae*. Initial pH was found to have an effect on gas production potential, while regulating pH 6.5 throughout the fermentation was found to increase the cumulative gas production rate and yield significantly.

Martin et al. (2010) indicated that the optimal operating conditions for biogas production occurred when the pH was 7 and the temperature was 550 °C, with the highest gas production of 7.8 mmol. The optimal recovery time for biogas was 25 hours in the batch experiments. Biogas is estimated to have the potential to replace around 17% of vehicle fuel. Biogas is a renewable fuel, so it qualifies for renewable energy subsidies in some parts of the world. Biogas can also be cleaned and upgraded to natural gas standards when it becomes biomethane, as indicated by Munnings, C. et al. (2014).

In our study, the percentages of total solids decreased from 10% to 5.2%, and the percentages of volatile solids decreased with time from 70% to 21.2%. The presence of biogas in orange peel waste was confirmed by gas chromatography analysis. The application of the modified Gompertz equation was able to predict the pattern of biogas production with time. It was observed that the maximum biogas production was obtained from various flask 1 compared to flasks 2 and 3. Hence, we conclude that the orange peel waste resulted in maximum biogas production. Out of the four lignocellulosic wastes used in this study, orange peel waste holds the greatest promise for low-cost biogas production and biogas Production using Modified Gompertz Equation.

## 2. MATERIALS AND METHODS

### 2.1 Microbial Strain

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

**Table 1: Growth Medium**

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

### 2.2 Preparation of Raw Waste

The waste is collected from industries and in the college canteen. The organic wastes (orange peel waste and litter leaf) are collected, dried, and powdered in a crusher. The industrial effluent (paper waste and dairy waste) collected from the industries is getting diluted. The diluted waste is added to the serum bottles. The medium composition is given in Table 2. The medium was autoclaved for 20 minutes at 121 °C. After cooling, a fresh fungus was inoculated into the autoclaved flask containing medium. Then the serum bottles were kept at room temperature under static conditions for a period of 10 days.

**Table 2: Composition of Biogas Production Medium**

Components	Concentration (g/l)
NH <sub>4</sub> HCO <sub>3</sub>	5.24
NaHCO <sub>3</sub>	6.72
TRACE ELEMENTS	
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.005
CoCl <sub>2</sub> .5H <sub>2</sub> O	0.000125
MgCl <sub>2</sub> .6H <sub>2</sub> O	0.1
MnSO <sub>4</sub> .6H <sub>2</sub> O	0.015
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.025
Na <sub>2</sub> S.H <sub>2</sub> O	0.25

### 3. EXPERIMENTAL SETUP AND PROCEDURE

#### 3.1 Reactor Setup

A 500-ml serum flask 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 500-ml serum flask with the NaOH solution, is plugged by a rubber stop perforated by two hollow needles and placed upside down. Biogas produced by the serum flask will accumulate in the liquid displacement system, displacing the NaOH solution. CO<sub>2</sub> will be absorbed in the NaOH solution. The displaced liquid is therefore considered to have the same volume as that 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 are covered with a funnel and placed below the second hollow needle of the NaOH flask. The displaced liquid is gathered in this flask or cylinder. The blank and sample measure gas production due to changes in temperature and pressure, which should be subtracted from the values obtained in the experiment.

#### 3.2 Experimental Procedure

Figure 1 for a waste sample is placed in a 500-ml serum flask (5 ml of waste +1 ml of inoculum). The production media are weighed, and then, up to 500 ml, they are added to the serum flask. The rubber stop is placed, and the flask is connected to the liquid displacement system. The serum flask for the blank (containing only water in the same volume of liquid as 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. The first reading of gas production is performed after one day of overnight incubation. This reading is the zero reading. The volume of displaced NaOH is not only the result of gas production but also the realization of equilibrium between the liquid displacement system and ambient pressure. Therefore, the amount of liquid produced in the zero reading is not included in the calculation of the biogas activity. After the zero reading, reading was executed three times a day. Before every reading, the flask was thoroughly mixed. The liquid displaced by the blank was measured for every reading. After every reading, the accumulated biogas production was calculated. Approximately 500–600 ml of biogas was produced. A graph is to be prepared with on the X-axis the time and on the Y-axis the gas production.

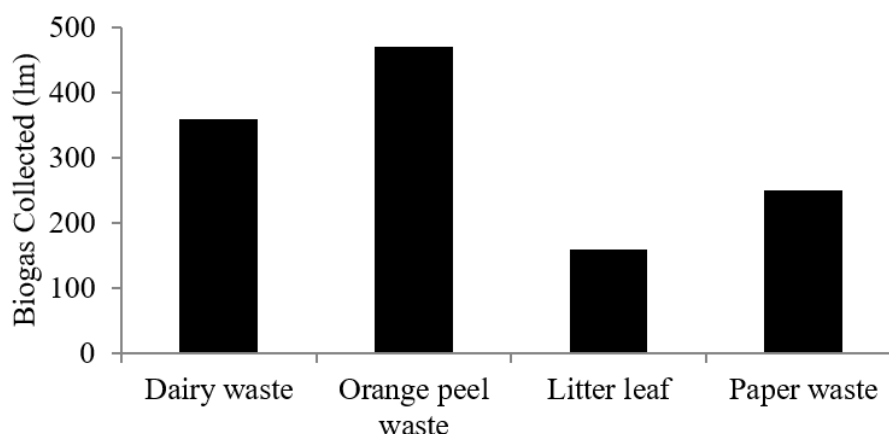


**Figure 1: Experimental Setup for Biogas Production**

## **4. RESULTS AND DISCUSSION**

### **4.1 Effect of Different Wastes on Biogas Production**

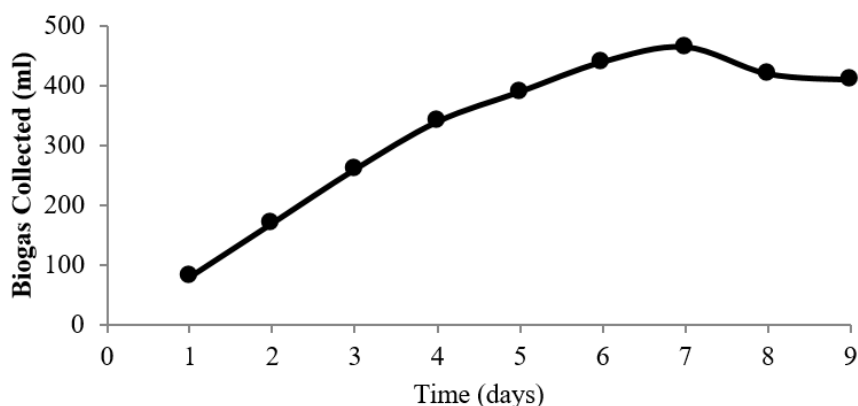
It is inferred from the results that the maximum production of biogas occurs in a medium containing orange peel waste as the carbon source Gopi Krishna Kafle, Lide chen (2016) and Csiszar, E., Szakacs, G., Koczka, B. (2007). It was found to be 470 ml. Maximum biogas production was observed in orange peel waste, as this contains more hexose molecules compared to other waste used as a substrate, as shown in figure 2.



**Figure 2: Effect of Different Wastes on Biogas Production**

### **4.2. Effect of Time**

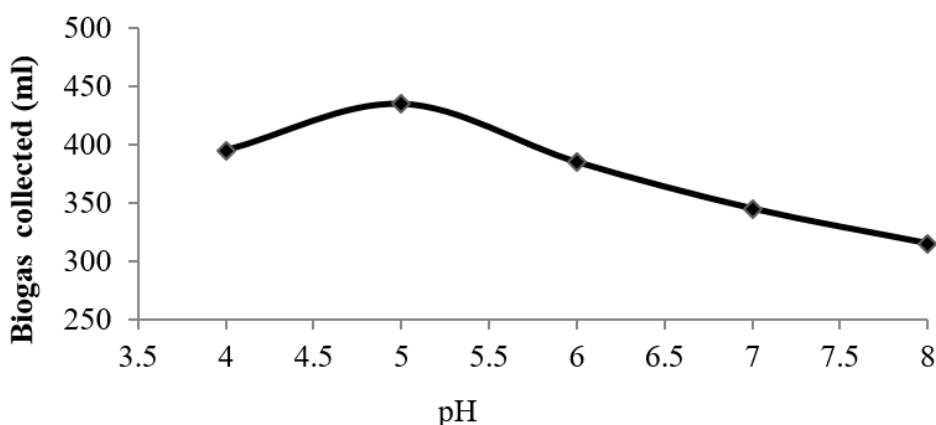
It is inferred from the results that the orange peel waste was found to have potential for biogas production of 465 ml on the seventh day of biogas production due to the presence of a high hexose content present in it, which will result in an increase in biogas production as shown in figure 3.



**Figure 3: Effect of Time**

#### 4.3 Effect of pH

The effect of pH was studied by adjusting the media (with 1.0 M NaOH or HCl) to different pH values from 4 to 8. The media were autoclaved, cooled, and inoculated with an overnight culture of *Trichoderma atroviride* 9641. The effect of pH on biogas production was investigated at five different pH values (from 4 to 8). As seen from the results summarized in Fig. 4, biogas production occurred within the pH range investigated. However, in batches with a pH above 5, the yield decreased drastically. This finding contradicts that of Elaiyaraju, P and Partha, N. (2011), who reported the optimal initial pH of 5 for a closely related organism, *Trichoderma atroviride* 9641, utilizing glucose as a substrate, where pH 5 was found optimal for biogas production. 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 cases of pH 7.0 and 7.5, respectively. Biogas production followed a similar trend to glucose consumption; however, no significant change in biogas production was observed when increasing the pH beyond 5.

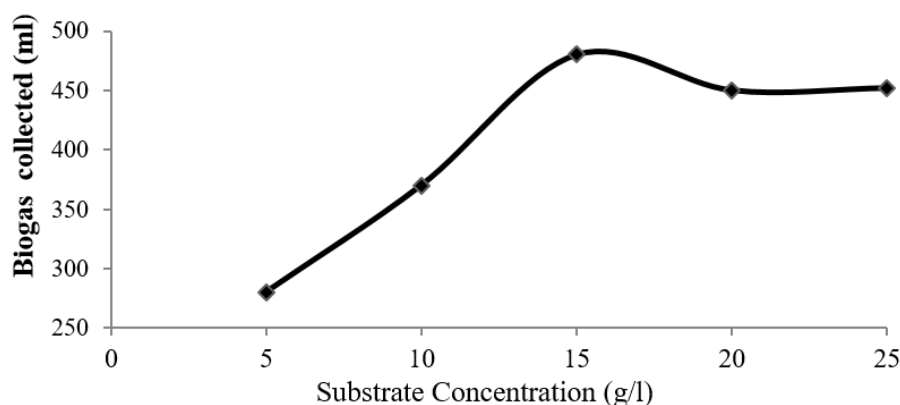


**Figure 4: Effect of pH**

#### 4.4 Effect of Substrate Concentration

The effects of substrate concentration on orange peel waste were tested at substrate concentrations ranging from 5 to 25 g/l of water. The incubation periods for all experiments were carried out for ten days. The production of biogas was determined on the tenth day of the process. Readings were noted at the end of the tenth day. The biogas production was found to

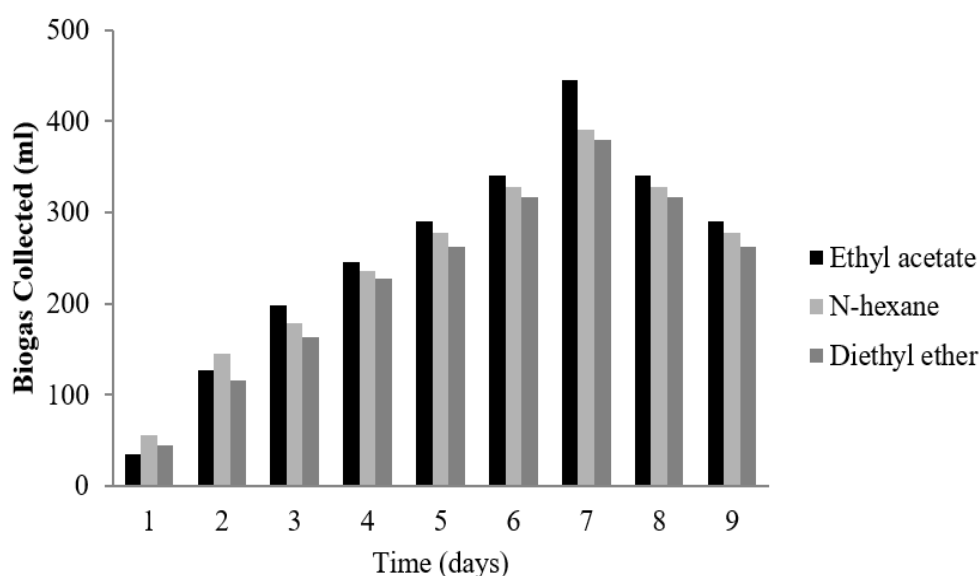
be dependent on the substrate concentration. In Figure 5, the total biogas quantity varied from 280 to 480 ml, and no further production was observed after 10 days. The peak biogas production of 480 ml was obtained at a substrate concentration of 15 g/l, and a further high concentration (20 g/l) was found to be inhibitory to gas production. The biogas accumulation gradually increased in the substrate concentration range of 5–15 g/l.



**Figure 5: Effect of Substrate Concentration**

#### 4.5 Effect of D-Limonene Content in Orange Peel Waste

The effect of the D-limonene content in orange peel waste on biogas production was determined using three different solvents (ethyl acetate, diethyl ether, and n-hexane) to extract the D-limonene content in orange peel waste. Among these solvents, hexane (Figure 6) shows the maximum biogas production because it showed higher extraction efficiency toward D-limonene, and orange peel treated with n-hexane gave the highest biogas production than the peel treated with other solvents. The behavior of n-hexane can be due to either its high extraction efficiency or its being less toxic to microorganisms. Further experiments should be focused on pretreatment using n-hexane as a solvent and treating the peel with n-hexane for biogas production.



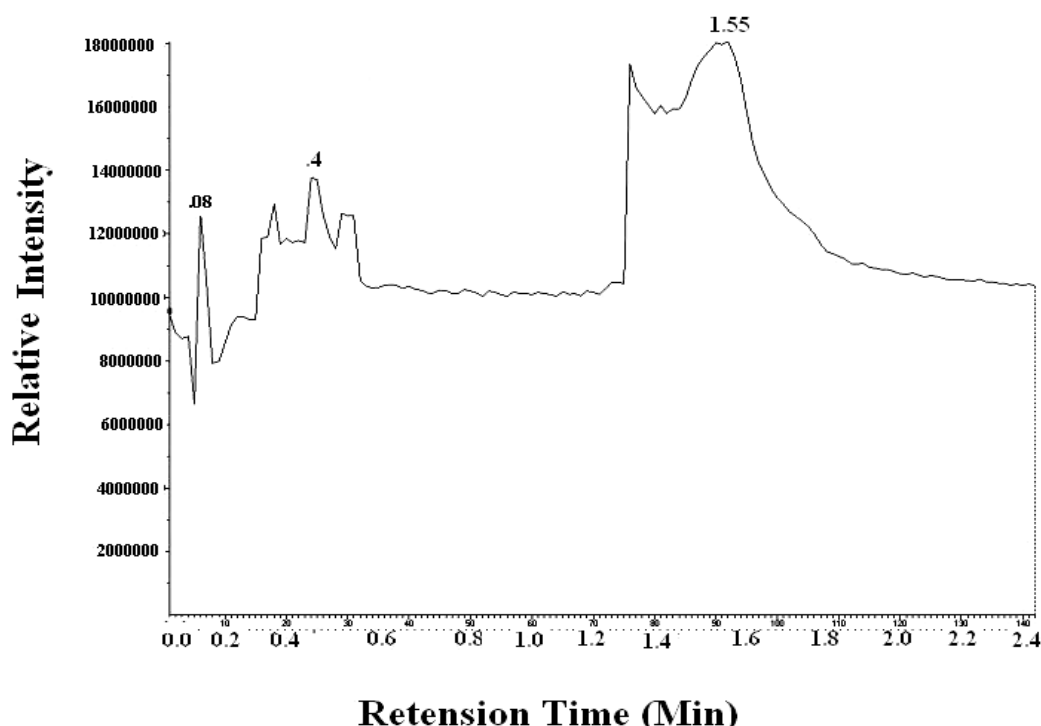
**Figure 6: Effect of Pretreatment of D-Limonene in Orange Peel**

#### 4.6 Gas Chromatography for Orange Peel Waste

Figure 7 shows the JEOL-supplied GC MATE II GC-MS with a data system at high resolution, and double-focusing gas chromatography was used for analyses of the biogas produced from the agricultural waste. The maximum resolution is 6000. Maximum calibrated mass: 1500 Daltons; source options: electron impact (EI); and chemical ionization (CI). Biogas produced from the anaerobic digestion of agricultural wastes was quantified in the liquid displacement system. The volume of biogas was measured by the volume of water displaced in a graduated measuring jar. JEOL GCmate II was used for the analysis of biogas. Parameters were used to inject a sample at a temperature of 220 °C, in the temperature range of 40 to 100 °C, with a with a flow rate of 2 °C/min, and helium gas was used as a carrier gas. The column of JEOL GCmate HP5 (Hewlett Packard) was used.

**Table 3: Composition of Biogas for the Orange Peel Waste as the Substrate**

S.No	Retention Time (min)	Molecule	Composition (%)
1	0.08	CH <sub>4</sub>	75
2	0.4	CO	9
3	1.55	CO <sub>2</sub>	16

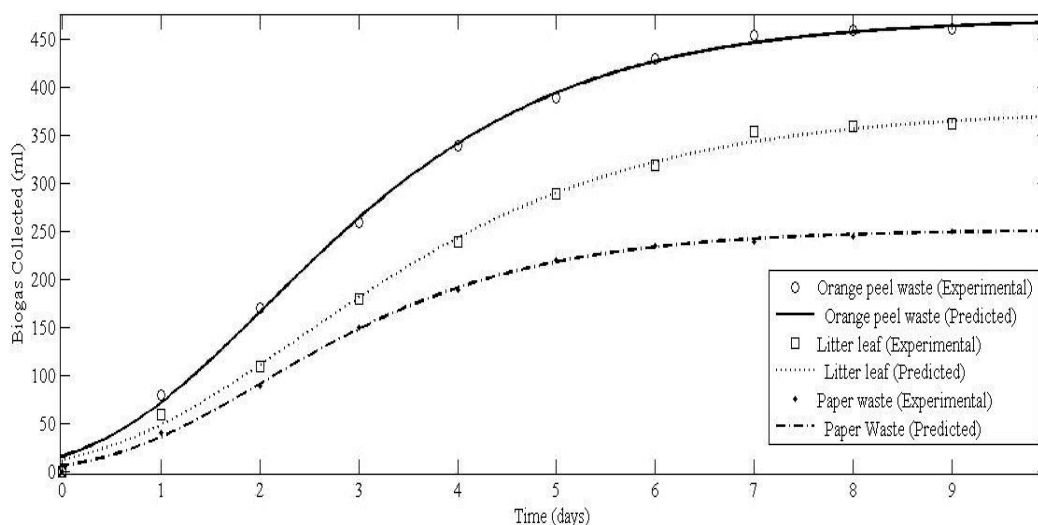


**Figure 7: Gas Chromatogram of Biogas Produced from the Orange Peel Waste as the Substrate**

#### 4.7 Kinetics Studies on Biogas Production using Modified Gompertz Equation

The modified Gompertz equation was used to fit the cumulative daily biogas production (Zhi-WuWang, YeboLi A. 2014), which was observed to adequately describe the biogas production from these substrates, as shown in figure 8.





**Figure 8: Kinetics Studies on Biogas Production using Modified Gompertz Equation**

The study of biogas production from three different wastes was conducted in the three serum flasks. The estimated kinetic constants using non-linear regression and other characteristics of the serum flasks 1, 2, and 3 are shown in Table 4.

**Table 4: Kinetics Studies on Biogas Production using Modified Gompertz Equation**

Time (Days)	Orange peel waste	Litter Leaf	Paper waste
0	0	0	0
1	80	60	40
2	170	110	90
3	260	180	150
4	340	240	190
5	390	290	220
6	430	320	235
7	465	365	260
8	430	320	235
9	390	290	220

At the end of the 9-day period, it was observed that flask 1 containing orange peel waste produced the highest cumulative biogas production potential (B) of 474.7 ml at a maximum biogas production rate (R<sub>b</sub>) of 164.3 ml/h, with a lag phase ( $\lambda$ ) of 0.5375 h. Flask 2 containing litter waste had a biogas production potential estimated to be 377.8 ml at a maximum biogas production rate of 84 ml/h, with a lag phase of 0.4802 h. Finally, flask 3 containing paper waste also had biogas production potential estimated to be 252.4 ml at a maximum biogas production rate of 71.24 ml/h with a lag phase of 0.4401 h. The modified Gompertz equation was observed to adequately describe biogas production with a goodness of fit (R<sup>2</sup>) of 0.9989, 0.9978, and 0.9966 for flasks 1, 2, and 3, respectively.

## CONCLUSION

It can be concluded that *Trichoderma atroviride* 9641 has the highest biogas production among the four wastes. Orange peel waste has the highest 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 7 days is enough for the maximum biogas production. It was also observed that pretreatment of substrate (5 g), pH (5), substrate concentration (15 g/l), and extraction of D-Limonene influenced the maximum biogas production. From the gas chromatography analysis, the produced biogas was confirmed to have gas present in it. From the kinetic studies, we confirmed that the pattern of biogas followed the modified Gompertz equation. Hence, we conclude that orange peel waste holds the greatest promise for the low cost of biogas production.

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