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BIOGAS PRODUCTION FROM CO-DIGESTION OF TEXTILE DYE AND RICE HUSK USING *AGARICUS HETEROCYSTIS*

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ABSTRACT: The waste generated from the textile industry is used for the production of biogas under the anaerobic digestion method. This study investigated the effect of various parameters such as Temperature 27 °C – 31 °C, pH 8-10, Inoculum concentration 35-55 ml on the textile dye with rice husk by the *Agaricus heterocystis* for biogas production using Response surface methodology. Maximum biogas yield 93 ml was obtained under the optimized condition of pH 9, Temperature 29 °C, and Inoculum volume 45 ml. The modified Gompertz model was used for maximum biogas production (1140 ml) from reactor 1 of textile dye (100 ml), rice husk (20 g), and inoculum volume (45 ml). The maximum biogas production (350 ml) was obtained under the optimized condition of Incubation time 144 h; Dye 100 ml/L and Rice husk 20 mg/L under the classical method. Produced biogas was confirmed and analyzed the composition: Methane - 78% and Carbon dioxide - 13% by Gas Chromatography (GC) method. The present study provided an efficient method for textile effluent co-digestion with rice husk for biogas production and also to overcome the textile dye disposal issue faced by leading textile industries.

INTRODUCTION: Energy is the most important factor to global prosperity. Naturally occurring gas that is generated by anaerobic process and it is used in energy production. Biogas differs from natural gas. It is a renewable energy source produced by anaerobic digestion^{1, 2}. Anaerobic digestion is the process in which microorganisms break down biodegradable wastes in the absence of oxygen. Anaerobic digestion can be used to treat various organic wastes and recover bio-energy in the form of biogas, which contains mainly CH₄ and CO₂³⁻⁵.

Methane could be a source of renewable energy producing electricity in combined heat and power plants. Biogas can also be produced in anaerobic digesters from plant or animal waste. The use of biogas is a green technology with environmental benefits. Biogas combustion results in a net reduction in greenhouse gas emissions.

Biogas production on the farm can reduce the odors, insects, and the pathogens associated with traditional manure stock piles. Due to increase in the price of oil biogas can be used as an alternative source. Biogas is the process of conversion of biodegradable waste into biogas under anaerobic process. Biogas comprised of methane gas (55%-75%), carbon monoxide, and trace amounts of nitrogen and hydrogen⁶⁻⁸. Co-digestion is the simultaneous digestion of a homogenous mixture of

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two or more substrates. The most common situation is when a major amount of a main basic substrate (textile dye) is mixed and digested together with minor amounts of a single, or a variety of additional substrate. The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates⁹⁻¹¹. Co-digestion was used by researchers such as to improve biogas yield by controlling the carbon to nitrogen ratio. Co-digestion can provide a better nutrient balance and, therefore, better digester performance and higher biogas yields¹².

The utilization of substrate in the form of textile dye was co-digested with rice husk in order to obtain optimum biogas production rate kinetics at ambient temperature^{13, 14}. Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques for modeling analysis. A response of interest is influenced by several variables. The objective is to optimize this response^{15, 16}. The objective of this study was to treat the waste and produce methane gas from the textile dye with rice husk as adsorbent using *Agaricus heterocystis* and to investigate the effect of temperature, pH, and inoculum concentration for an anaerobic digestion process to produce biogas. Optimization was carried out for biogas production using Response Surface Methodology.

MATERIALS AND METHODS:

Collection of Textile Dye: The textile dye was obtained from a textile processing industry in Tiruppur, Tamil Nadu. The textile dye was light blue in color with a foul smell due to the mixture of several dyes like disperse dyes, direct dyes, cationic dyes, sulfur dyes, anionic dyes, and azoic dyes. The dyes were collected from the exit discharge of the industry. The effluent was stored in plastic bottles in a dark place under normal room temperature.

TABLE 1: CHARACTERIZATION OF TEXTILE DYE (BEFORE)

S. no.	Parameters	Units	Conc.	Limits
1	BOD at 20°C at 5 Days	mg/l	224	<30
2	Cod (Open Reflux Method)	mg/l	2250	<250

The dye was analyzed between the ranges 200 to 800 nm spectrum in the (Shimadzu-1800) UV-Vis spectrophotometer, and λ_{\max} of the effluent was found to be 578.8 nm. The characterization of a textile dye of parameters has shown in **Table 1**.

Collection of Fungi: Fungi sample was ordered in MTCC (*Agaricus heterocystis*) - 4124. *Agaricus heterocystis* is the poisonous species which is the new species of fungi. Fungi are used for degrading the dye that has been used to degrade the textile dye.

Preparation of Potato Dextrose Broth: The potato dextrose broth was prepared at the concentration of 100 ml for each setup of the methanogenic activity test. The broth consists of potato infusion and dextrose for the preparation of the broth. The fungi sample was inoculated in broth and observed after 7 days.

Growth Characteristics of Fungi: The growth curve of the fungi sample (*Agaricus heterocystis*) was studied every 3 h. Absorbance was taken at 540 nm. **Fig. 1** illustrates the growth pattern of fungi.

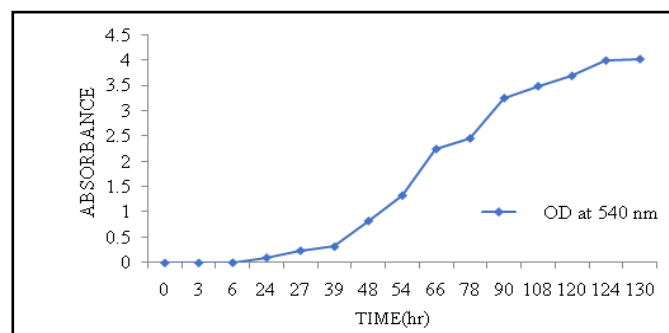


FIG. 1: GROWTH PATTERN OF AGARICUS HETEROCYSTIS

Methanogenic Activity Test for Textile Dye: In this test, activity was not determined directly as the substrate utilization rate; rather, the methane production rate was noted. A textile dye was placed in a serum flask of 500 ml (100 ml of textile dye + 235 ml of water). Water was added to a level of 3 cm from the top of the flask, and then 5 ml of the stock solution of acetic acid are added. The rubber stopper was placed, and the flask was then connected to the liquid displacement system. The serum flask containing textile dye, water, rice husk, fungi (*Agaricus heterocystis*), and addition of acetic acid were connected under a liquid

displacement system. The volume of the NaOH solution in the liquid displacement system of the blank could be comparable to the volume of the liquid displacement system that was connected with the serum flask that contains the sample shown in **Fig. 2**. The first reading of gas production was performed after one day (overnight incubation). This reading is the first reading.

The volume of displaced NaOH is not only the result of gas production but also of 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 methanogenic activity. After the zero reading, reading should be executed three times a day, and before every reading, the dye flask has to be mixed thoroughly. The liquid displaced by the blank should be measured for every reading^{17, 18}.

Methane production sludge = Displaced liquid by sample - Displaced liquid by a blank

After every reading, the methane production was calculated. The total production of methane was increased above 400 ml. The experiments were completed after the production of gas on 400 ml approximately, and 400 ml to 500 ml of methane gas was produced.

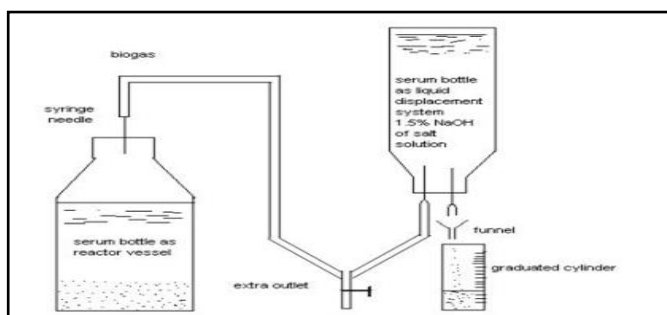


FIG. 2: SCHEMATIC DIAGRAM FOR METHANOGENIC ACTIVITY TEST AND REACTOR SETUP

Experimental Design: The experimental design for the anaerobic digestion of rice husk and fungi (*Agaricus heterocystis*) was carried out of different temperatures of 27 to 32 °C in four batch reactors.

Reactor 1: 100 ml of textile dye + 235 ml of water and 20 g of husk + 40 ml of culture.

Reactor 2: 100 ml of textile dye + 235 ml of water and 20 g of husk.

Reactor 3: 100 ml of textile dye + 235 ml water and 40 ml of fungi (broth).

Reactor 4: 100 ml of textile dye + 235 ml of water and 30 g husk + 30 ml of culture.

The reactors were set up, and biogas measurement was carried out under liquid displacement method. 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 ambient temperature measured was determined with a mercury bulb thermometer. In our study, the effect of mixing ratios of textile dye and rice husk to methane production in the anaerobic digester was analyzed^{19, 20}.

Carbon Dioxide and Methane Content: In the liquid displacement system, 1.5% NaOH was filled in the serum bottle during the production of gas which allows only methane, while other gases are absorbed by sodium hydroxide solution. For the analysis of biogas, the gas was collected in the rubber balloon, and it was estimated by gas chromatography analysis.

Gas Chromatography Analysis: Biogas was produced from the anaerobic digestion of mixed textile dye with rice husk. It was quantified in liquid displacement system. The volume of biogas was measured by the volume of water displaced in a graduated measuring jar. JEOL GC mate instrument was used for the analysis of biogas. JEOL GC mate instrument parameters were injection temperature 220 °C, temperature range 40 to 100 °C, flow rate of temperature 2 °C / min and helium gas was used as a carrier gas. The column of JEOL GC mate HP5 (Hewlett Packard) was used. The biogas analysis was done at Tamil Nadu Agricultural University (TNAU) in Coimbatore.

Response Surface Methodology: Response surface methodology (RSM) is a collection of mathematical and statistical techniques through which we can determine the optimum concentration or conditions using central composite designs. RSM is a second-order polynomial equation that works on relationships between diverse explanatory variables and response variables. The system was explained by the second-order polynomial equation:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3$$

Where, Y is the response measured (dye degradation %) X₁, X₂, X₃ are the parameters. The optimum value is obtained by the regression equation, by analyzing the plots.

In the present work three significant parameters (Temperature, pH, Inoculum concentration) were chosen according to central composite design were fixed constant to determine the most significant factor for adsorption process. The three-parameter ranges are temperature (27-31 °C), pH (8-10), and substrate concentration (35-55 ml). **Table 2** summarizes the Response surface methodology (CCD) was developed for 3 process parameters over 20 trials using the Minitab19 commercial software.

The optimization experiments were performed as batch adsorption studies in 250 ml Erlenmeyer flasks containing 100 ml of diluted dye effluent. The dye was diluted appropriately to respective concentrations using distilled water. The factor levels were maintained during the experiment. The adsorption studies were performed in the incubator for static conditions and in the orbital shaker for agitated conditions.

TABLE 2: PROCESS PARAMETERS FOR RESPONSE SURFACE METHODOLOGY (CCD)

Parameters	Low Value	High Value
pH	8	10
Temperature	27 °C	31 °C
Inoculums concentration	35 ml	55 ml

On reaching equilibrium conditions, the effluent was centrifuged at 2500 rpm (Remi R-8C Laboratory Centrifuge) for 5 minutes to remove the biomass debris, and the supernatant was preserved.

TABLE 3: RSM DESIGN FOR OPTIMIZING SIGNIFICANT VARIABLES FOR DYE DEGRADATION

Run Order	pH	Temperature (C)	Inoculum (ml)	Cumulative Biogas Prod (ml)
1	10.000	31.000	35.00	82.37
2	8.000	27.000	35.00	84.36
3	9.000	29.000	45.00	93.12
4	9.000	29.000	45.00	93.08
5	8.000	31.000	55.00	81.49
6	10.000	27.000	55.00	79.25
7	9.000	25.734	45.00	82.61
8	10.633	29.000	45.00	80.86
9	9.000	32.266	45.00	85.29
10	9.000	29.000	61.33	84.76
11	9.000	29.000	28.67	88.63
12	9.000	29.000	45.00	93.12
13	9.000	29.000	45.00	93.07

The filtrate after each trial was analyzed using (Shimadzu UV-1800) UV-Vis spectrophotometer at 578.8 nm with sterile distilled water as blank and the readings were tabulated. Effluent without adding biomass was used as control. The decolorization percentage was calculated using the following equation^{21,22}.

$$Y = (\text{Initial absorbance} - \text{Final absorbance} / \text{Initial absorbance}) * 100$$

Where, Y denotes the decolonization percentage

Analysis of Data: Minitab19 was used for the statistical and regression analysis of data. The is chosen since it provides a very comprehensive and easy interpretation of results

RESULTS AND DISCUSSION:

Optimization Studies on Response Surface

Methodology: Response surface methodology (CCD) is a statistical and experimental design to determine the significant parameter effective for the degradation process. The effect of parameters on dye degradation efficiency is investigated using a central composite experimental design²³. The result was obtained by central composite design to identify the significant factor among the time, pH and temperature.

ANOVA: The analysis of variance (ANOVA) for the degradation of oleander biomass is given in the table. A *P-value* less than 0.05 indicates that the models are significant. Based on the results, the relationship between the degradation percentage of dye and the parameters is expressed by the second-order polynomial equation. The R-square value is 98.68 % for the removal of dye from using fungi with rice husk.

14	7.367	29.000	45.00	86.72
15	8.000	31.000	35.00	78.45
16	10.000	27.000	35.00	82.34
17	9.000	29.000	45.00	93.08
18	9.000	29.000	45.00	93.12
19	8.000	27.000	55.00	85.38
20	10.000	31.000	55.00	81.23

The result shows a great variation in the parameters for degradation process for the production of biogas.

TABLE 4: ANALYSIS OF VARIANCE FOR DEGRADATION USING CENTRAL COMPOSITE DESIGN

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	11	502.206	45.655	16.21	0.000
Blocks	2	7.825	3.912	1.39	0.304
Linear	3	18.858	6.286	2.23	0.162
pH	1	14.825	14.825	5.26	0.051
Temperature (C)	1	0.874	0.874	0.31	0.043
Inoculum (ml)	1	3.159	3.159	1.12	0.021
Square	3	447.529	149.176	52.95	0.000
pH*pH	1	204.286	204.286	72.52	0.000
Temperature (C)*Temperature (C)	1	198.101	198.101	70.32	0.000
Inoculum (ml)*Inoculum (ml)	1	106.792	106.792	37.91	0.000
pH*Temperature (C)	1	17.435	17.435	6.19	0.038
pH*Inoculum (ml)	1	8.591	8.591	3.05	0.011
Temperature (C)*Inoculum (ml)	1	1.970	1.970	0.70	0.027
Error	8	22.537	2.817	-	-
Lack-of-Fit	5	22.534	4.507	4744.03	0.000
Total	19	524.743	-	-	-

Surface Plot of Cumulative Biogas Production:

The surface plot shows the uptake of the dye. The plot gives the interaction between the inoculum and pH. From the plot, we can observe that the maximum degradation was found in both the

parameters, such as pH and time. The response obtained from the interaction also shows the high biogas yield^{24, 25}. Similarly, the surface plot for other parameter combinations was given in Fig. 4 and 5.

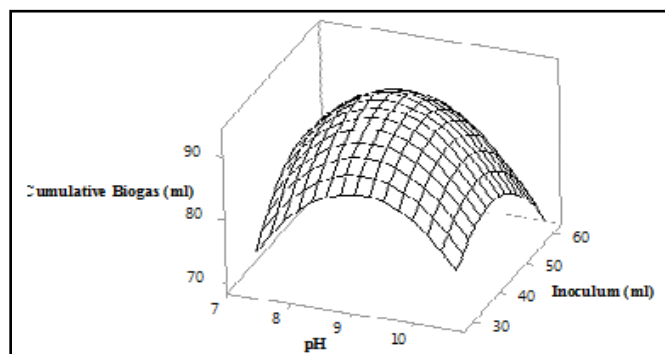


FIG. 3: SURFACE PLOT REPRESENTING THE INTERACTION BETWEEN INOCULUM (ML) AND pH

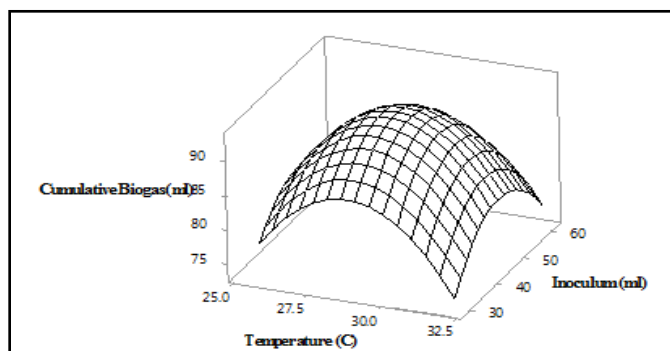


FIG. 4: SURFACE PLOT REPRESENTING THE INTERACTION BETWEEN INOCULUM AND TEMPERATURE

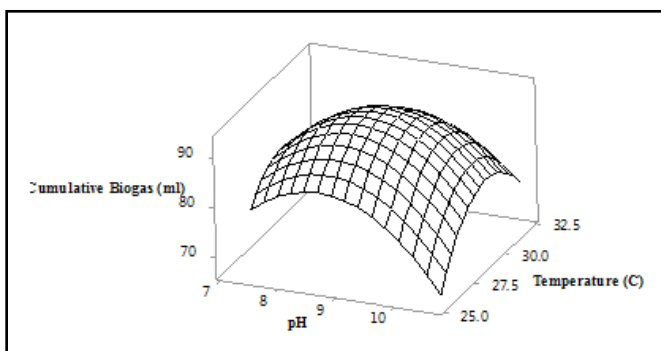


FIG. 5: SURFACE PLOT REPRESENTING THE INTERACTION BETWEEN pH AND TEMPERATURE

Contour Plot Representing the Interactions Between Given Parameters: The counterplots are the graphical representation which is in three dimensions. The plots shown in **Fig. 6-8** explained the interaction between the given parameters such

as inoculum, temperature, pH, and the optimum condition for degradation. The counterplot of the second-order polynomial equation is done by the interaction of two variables within the experimental ranges.

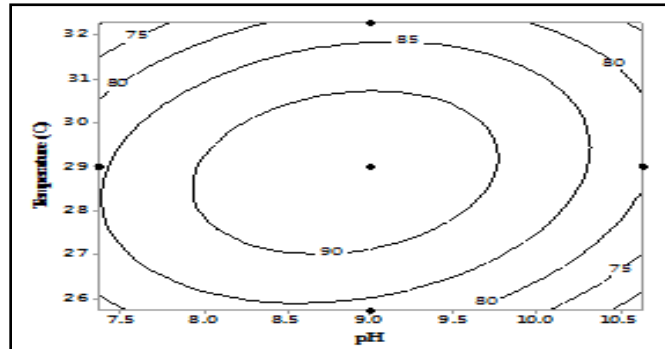


FIG. 6: CONTOUR PLOT OF CUMULATIVE BIOGAS PRODUCTION VS PH, TEMPERATURE

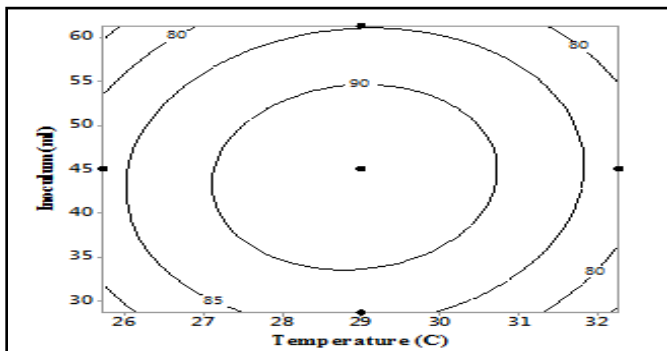


FIG. 7: CONTOUR PLOT OF CUMULATIVE BIOGAS PRODUCTION vs. INOCULUMS (mL), TEMPERATURE

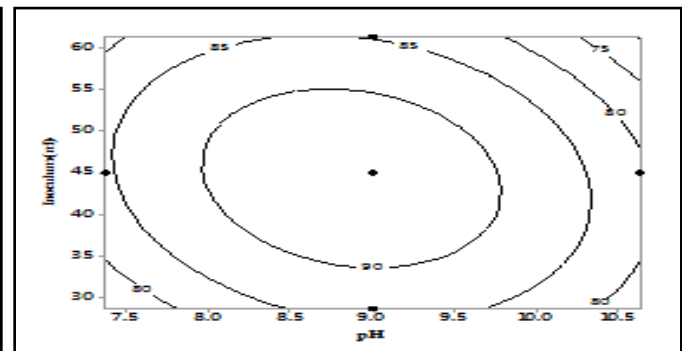


FIG. 8: CONTOUR PLOT OF CUMULATIVE BIOGAS PRODUCTION vs. pH, INOCULUMS (mL)

Effect of Various Parameters on Cumulative Biogas Production: The biogas was produced from the batch reactor of serum bottle 500 ml was measured by liquid displacement method on a daily basis over a period of 7 days as shown in **Fig. 9**. The graph shows the maximum production of gas produced under the liquid displacement method of 350 ml in 168 h. **Fig. 10** shows the maximum production of gas produced under the liquid displacement method of 350 ml in 168 h.

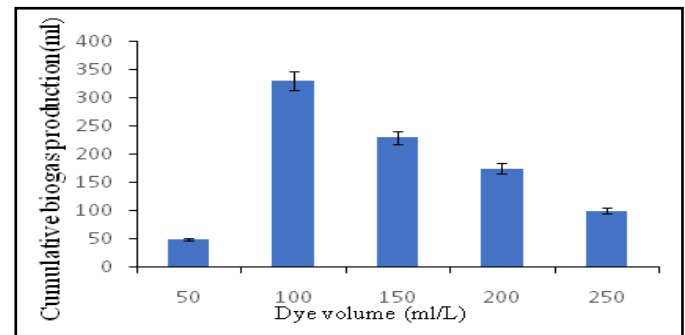


FIG. 10: METHANE PRODUCTION DURING DYE CONCENTRATION

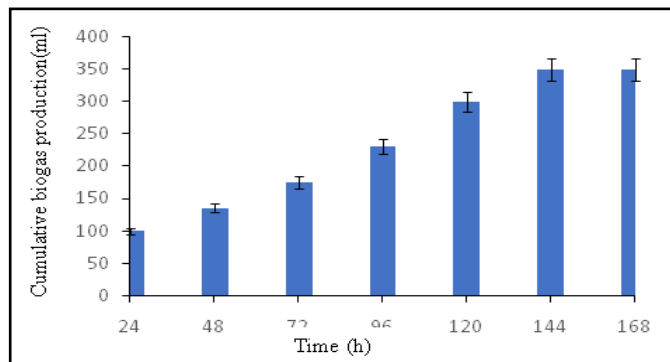


FIG. 9: CUMULATIVE BIOGAS PRODUCTION DURING METHANOGENIC ACTIVITY

The biogas produced in dye concentration ^{26, 27}. The textile dye concentration gives maximum production of biogas in the range of 100 ml, as shown in the figure. The biogas was produced from the batch reactor of serum bottle 500 ml was measured by liquid displacement method on a daily basis over a period of 7 days showed in **Fig. 11**. The amount of substrate (Rice husk) of 20 mg/L gives maximum production of biogas, as shown in **Fig.11**.

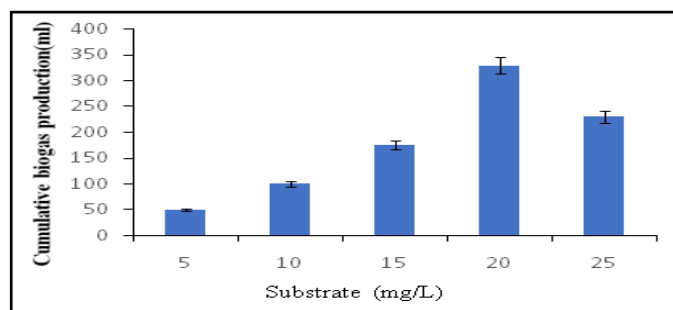


FIG. 11: METHANE PRODUCTION DURING SUBSTRATE VOLUME

Analysis of Gas Chromatography: In this experiment, the textile dye is used for biogas production at room conditions (such as ambient temperature without any physical treatment), and room temperature ranged between 27 to 31 °C. Biogas production was monitored and measured until biogas production reduced significantly using the modified Gompertz model. The study of biogas production from textile dye using rice husk was conducted in the reactor labeled 1 to 4. At the end of the 7-days period, it was observed that reactor 1 produced the highest cumulative biogas production potential (B) of 1159 ml at a maximum biogas production rate (R_b) of 6.678 ml/h, with a lag phase (λ) of -7.626 h. Reactor 3 had biogas production potential estimated to be 238.5 ml at a maximum biogas production rate of 1.433 ml/h, with a lag phase of -19.35 h. While in reactor 2, which comprised an equal amount of textile dye and rice husk, the biogas production potential was 490.4 ml at a maximum biogas production rate of 3.175 ml/h with a lag phase of 6.221 h. Finally, reactor 4 also

TABLE 5: BIOGAS PRODUCED IN ACCURATE REACTOR

Reactor	Biogas Produced (ml)	B (ml)	R_b (ml)	λ (h)	R^2
1	1140	1159	6.678	-19.35	0.9908
2	490	490.4	3.175	6.221	0.9970
3	244	238.5	1.433	-19.35	0.9701
4	447	464.6	3.349	7.96	0.9971

CONCLUSION: Biogas production from textile dye and rice husk using *Agaricus heterocystis* were studied in this present study at room temperature. The modified Gompertz equation is used for biogas production with time. It was observed that the maximum biogas production (1140 ml) was obtained from reactor 1 of textile dye (100 ml), rice husk (20 g), and inoculum volume (45 ml). The optimized values of pH 9; Temperature 29 °C, and Inoculum volume 45 ml were obtained using RSM in textile dye with a combination of rice husk for maximum production of biogas (93 ml). The

had biogas production potential estimated to be 464.6 ml at a maximum biogas production rate of 3.349 ml/h with a lag phase of 7.96 h. The modified Gompertz equation was observed to adequately describe biogas production with a goodness of fit (R^2) of 0.9908, 0.9970, 0.9701, and 0.9971 for reactors 1, 2, 3, and 4, respectively were displayed in Table 5. B is the biogas production potential (ml); R_b is the maximum biogas production rate (ml/day); λ is the lag phase (days), which is the minimum time taken to produce biogas or time taken for bacteria to acclimatize to the environment in days, R^2 is the goodness of fit^{28,29}.

Fig. 12 shows the analysis of produced biogas, and the composition of methane and carbon dioxide was confirmed as with standard values. Methane and CO₂ were analyzed by Gas Chromatography, and the corresponding values were found to be 78% and 13%, respectively, in produced biogas.

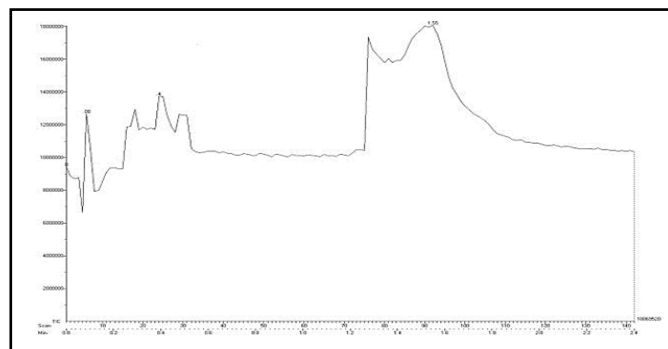


FIG. 12: GAS CHROMATOGRAPHY ANALYSIS FOR TEXTILE DYE WITH RICE HUSK

maximum biogas production (350 ml) was obtained under the optimized condition of Incubation time 144 h, Dye 100 ml/L, and Rice husk 20 mg/L. Produced biogas was confirmed and analyzed the composition: Methane - 78 % and Carbon dioxide - 13% by Gas Chromatography (GC) method. It can be concluded that an efficient method for biogas production using textile effluent co-digestion with rice husk under anaerobic conditions and also to overcome the textile dye disposal issue faced by leading textile industries.

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