



## PRODUCTION OF BIOGAS FROM MICROBIAL LABORATORY CULTURE MEDIA WASTE, ANALYSIS OF METHANOGENS AND DIGESTED SLURRY UTILISATION AS BIOFERTILIZER

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### ABSTRACT:

This study explores using Microbial Laboratory Culture Media Waste to produce biogas in an anaerobic digester, offering a cheap and eco-friendly waste management method and protect the environment from pathogenic microorganisms. Biogas production was measured at 148 mL over 35 days. Bacterial isolation identified Methanobacterium genus. The bio-slurry residue can be used as fertilizer, providing readily available nutrients for crop and aquaculture production.

**Keywords:** - Biogas, Methanogens, Microbial Laboratory Waste, Bioenergy, Biofertilizer.

### INTRODUCTION :

The pace of energy consumption is increasing at unforeseen rates with each passing day on this rapidly developing planet. Biogas is a renewable fuel that can be created when organic matter is broken down by microorganisms in the absence of oxygen. Whatever power we use, we use it as energy; it either originates from the earth as coal or petroleum, but all these things are such that sooner or later they will end. Waste is such a thing that is of no use to anyone but we will use it to create bioenergy. This biogas plant will run on such waste which can later be used as a biofertilizer, so many of our purposes will be solved.

Organic materials such as animal manure, plants and kitchen waste, but no one pays attention to microbial laboratory culture media waste. "Any biomass rich in cellulose, hemicellulose, proteins, lipids, and carbohydrates can be used to produce biogas (Weiland, 2010). Academic, research, pharmaceutical, and food industries routinely discard microbial culture media waste, burdening waste treatment plants and wasting

energy potential. Yet, repurposing this waste through pretreatment and anaerobic digestion could mitigate environmental harm and harness energy generation. Successful biogas trials in India significantly address the energy crisis, particularly in pastoral regions, with Maharashtra leading at over 931k plants, followed by Karnataka at 513k.

India boasts over 5 million biogas plants, providing sustainable energy without landscape degradation. Biogas is primarily comprised of methane (50-70%), carbon dioxide (20-40%), nitrogen (0-10%), hydrogen sulphide (0-3%), and trace moisture, (P. A. Sangeetha et al., 2013).

Biogas production technology offers numerous benefits: waste recycling, reduced greenhouse gas emissions, renewable energy, environmental protection, and high-quality fertilizer, Microbial formulations enhance nutrient availability for plants by boosting specific microbial activities. (Leo et. Al., 2002). However, reliance on synthetic and chemical fertilizers remains widespread despite their environmental impact (Sepril S. 2012).

### Anaerobic Digestion

Biogas is created through a complex process called anaerobic digestion, involving four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

In hydrolysis, organic matter is transformed into complex organic material. In acidogenesis, the formation of organic acids, H<sub>2</sub> and CO<sub>2</sub>, In acetogenesis, acetogenic bacteria convert alcohol into acetate, and in methanogenesis, methanogens convert acetogenesis byproducts, primarily acetic acid, into methane, with carbon dioxide also contributing to methane and water production (Iftikhar A. Raja et al., 2017).

Methanogens, belonging to Archaeobacteria, thrive in oxygen-depleted environments like mud, animal guts, and deep marine sediments. As strict anaerobes, they are autotrophs obtaining energy and carbon from decomposition products, where they convert formic acid and carbon dioxide into methane (Patel, A. H. 2012, Industrial Microbiology).

### MATERIAL AND METHODS :

#### Sample collection

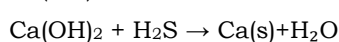
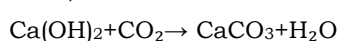
4kg of microbial lab culture media waste from Sardar Patel Mahavidyalaya, Chandrapur, Maharashtra, was mixed with water (6:4 w/v) to form a semi-solid substrate.

#### Experimental set up for biogas production

A 15-liter plastic oil can was converted into a laboratory-scale batch anaerobic digester. It features an opening on top for gas collection, a tire tube for biogas collection, and a saline wire and burner for detection. A thermometer is attached for the observation of temperature, and a purification system is also connected.

#### Quantitative and Quantitative Estimation

The research measured biogas production using water displacement and purified it with limewater and activated charcoal (Mel, M. et al., 2014).



### Microbial (Methanogenic bacterial) Analysis:

The digested slurry sample was collected, serially diluted, and plated on anaerobic agar. After incubation for 72 hours at 37°C, bacterial isolates were identified based on cultural, morphological, and biochemical characteristics (Bello et al., 2019).

### Effects of the biogas slurry fertilization on chickpea plant

Two pots were labeled as control (no fertilizer) and bio-slurry biofertilizer. Chickpea seeds were planted in each pot with soil samples. 50 ml of bio-slurry fertilizer was applied weekly. After 45 days, soil fertility analysis measured pH, nitrogen, phosphorus, and potassium.

### RESULTS AND DISCUSSION :

This chapter presents three key sections: 1. Biogas production from microbial culture waste. 2. Isolation and identification of methanogens from digested slurry. 3. Impact of biogas slurry fertilization on chickpea growth and soil analysis.

### Microbial Laboratory Culture Media Waste as Fermentable Material

The substrate performances are listed in Table 1, with an average digester temperature of 33.5°C. Table 2 indicates a temperature rise during biogas production, with a hydraulic retention time of 35 days.

#### Quantifying Biogas Production

The biogas volume, measured by water displacement, was 148 ml after 35 days of fermentation, using 420 ml of water initially.

### Methanogenic Bacteria Isolation From Digested Slurry

Methanogenic bacteria were isolated using anaerobic agar and incubated in an anaerobic chamber at 37°C for 72 hours. Identification relied on morphological traits: motile, bacilli/short rod-shaped colonies with raised surfaces, flat edges, greenish-white color, and small diameter. Gram staining revealed both Gram-negative and Gram-positive colonies.

### **Methanogenic Bacteria Identification**

Bacterial identification was conducted via biochemical tests including IMViC, Urease, TSI, Catalase, starch and casein hydrolysis, and carbohydrates fermentation (Table No. 3).

In the microbiological analysis, isolates were identified as Methanobacteria, belonging to the family Methanobacteriaceae.

### **Comparative Study of Plant Growth**

Chickpea plants were weekly treated with biofertilizer for 7 weeks, with plant height measured and recorded in centimeters.

### **Soil Fertility Analysis**

Chickpea plants treated with digested slurry fertilizer for two months showed higher levels of nitrogen, phosphorus, and potassium in the soil compared to the control, indicating an increase in NPK content (Table 4).

### **CONCLUSION :**

This study shows that flammable biogas can be produced from microbial culture media waste via anaerobic digestion, addressing environmental and health concerns. Culturing on a methanogenic medium maximizes inoculum for methanogenic bacteria growth, crucial for biomethanation. The study demonstrates that using biofertilizer from anaerobic digester significantly enhances chickpea plant growth compared to control, showcasing the potential of biogas as a sustainable renewable energy source, thus reducing reliance on fossil fuels and offering economic benefits

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**Table 1: Performances of Substrate for Biogas Production**

Parameters	Test Substrate: Microbial Laboratory Culture Media Waste
Initial pH	7.5
Day production started	3 <sup>rd</sup>
Day flammability started	9 <sup>th</sup>
Total gas (ml)	148
Final pH	6.5

**Table 2: Temperature's impact on biogas production in anaerobic digesters.**

Days	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week
Temperature	26°C	28°C	33°C	38°C	38°C

**Table 3: Biochemical test results of methanogenic bacteria colony**

Sr. No.	Biochemical Test	Biochemical Test Results
1	Indol Test	+
2	MR Test	+
3	VP Test	-
4	Citrate Test	+
5	Urease Test	+
6	TSI Test	+
7	Catalase Test	-
8	Starch Hydrolysis Test	+
9	Casein Hydrolysis	+
10	Carbohydrates Fermentation Test	+

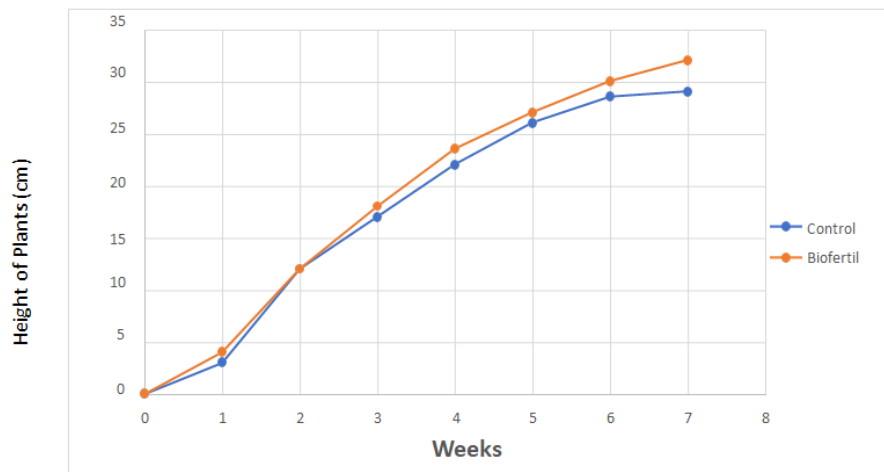
The symbol (+) represent Positive Test and the symbol (-) Negative Test

**Table No. 4: Height of chickpea plant treated with biofertilizer**

Treatment	Control	Digested slurry fertilizer
1 <sup>st</sup> week	3 cm	4 cm
2 <sup>nd</sup> week	12 cm	12 cm
3 <sup>rd</sup> week	17 cm	18 cm
4 <sup>th</sup> week	22 cm	23.5 cm
5 <sup>th</sup> week	26 cm	27 cm
6 <sup>th</sup> week	28.5 cm	30 cm
7 <sup>th</sup> week	29 cm	32 cm

**Table No. 5: Study of pH, EC, OC, and NPK of soil after applying Biofertilizer**

Sr. No.	Test Parameters	Units	Normal range	Initial	Control	Digested slurry fertilizer
1	pH	-	6.5 – 7.5	6.70	6.90	7.20
2	EC	-	0.1	0.54	0.64	0.55
3	OC	-	0.40-0.60	0.36	0.34	0.39
4	Available N	Kg/hect	280-420	107.88	125.44	188.16
5	Available P	Kg/hect	14-21	23.03	21.71	27.41
6	Available K	Kg/hect	150-200	192.22	184.83	348.53



**Fig. No. 1 : Height of chickpea plant treated with biofertilizer**



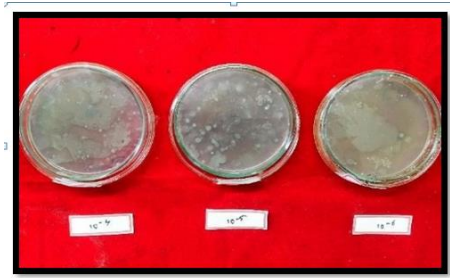
**Fig No.2: Microbial Laboratory Culture Media Waste**



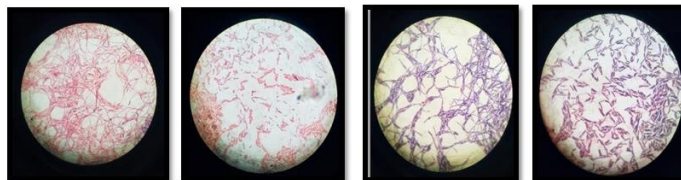
**Fig. No. 3: Anaerobic Digester**



**Fig No. 4: Biogas**



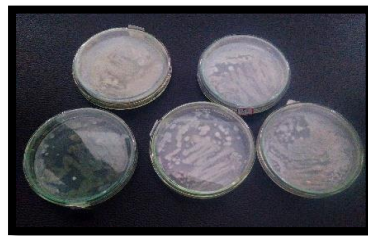
**Fig No. 5: Colonies on Anaerobic Agar**



**Fig. No. 6: Gram Staining of Bacterial Isolates**



**Fig. No.7:IMViC, Urease and TSI Test**



**Fig. No. 8: Casein Hydrolysis Test**



**Fig. No. 9: Starch Hydrolysis Test**



**Fig. No. 9: Pot Culturing of Chickpea plant after 3<sup>rd</sup> and 5<sup>th</sup> week**