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# Screening and Characterization of Micro Organisms for the bio-production of Electricity from Rice Straw using Microbial FUEL CELL

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**ABSTRACT:** The demand for the fossil fuels such as petrol, diesel, kerosene and other petroleum derivatives is increasing day to day due to the enormous increase in the vehicles and population. These are nonrenewable energy sources which will be depleting soon due to their continuous usage. Therefore the presently available renewable energy sources must be properly utilized. There is an immediate need for the search of alternate energy sources. The rice straw which is abundantly available all over the can be used as an alternate energy source. Electricity can be produced from rice straw by using Microbial fuel cell (MFC) technology, which uses microorganisms to transform chemical energy of organic compounds of the rice straw into electricity. In this article, electricity generation from rice straw in two-chambered microbial fuel cells (MFC) using rice straw and microorganisms is described. The MFC produced the maximum Voltage of 3.1 and corresponding to the maximum current of 3.9 amperes which results in power output of 12.09 W. The results of this study can contribute for the improvement, understanding and optimizing electricity generation from rice straw in microbial fuel cells.

**KEYWORDS:** Rice straw, Microbial fuel cell, Electricity, microorganisms, Alternate energy source.

### I. INTRODUCTION

In recent years the demand for the energy supply has become one of the major issues worldwide. Every day the energy requirement is an ever increasing entity throughout the world due to the enormous increase in the vehicles and population. Continuous use of fossil fuels, especially petroleum oil and natural gas, has led to the environmental pollution. In this regard, renewable energy source have been viewed as a possible alternative to fossil fuels, which have potential to generate energy and also environmental friendly energy sources. Use of lignocellulosic biomass offers the enormous potential for bioenergy production of the second generation of biofuels. Depending upon the end use applications, a variety of biofuels, such as bioethanol, methane, hydrogen and bio oils, can be extracted from lignocellulosic biomass. Rice straw is considered to be one of the waste materials suitable for renewable energy resource for electricity production using microbial fuel cell. Rice straw contains bio-masses such as cellulose, hemicellulose, and some lignin [1]. The hemicellulose undergoes degradation to its constituent sugars by acidic and/or enzymatic hydrolysis. The sugars produced from hydrolysis can be further used as substrates to for the production of ethanol or organic acids [2]-[3]. Microbial fuel cell is a system in which the micro organisms converts the chemical energy produced by the oxidation of organic or inorganic compounds into ATP by sequential reactions in which

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electrons are transferred to a terminal electron acceptor to generate an electrical current[4]-[6]. The microorganisms play important role in the conversion of organic contaminants into energy production [7].

The electrochemically active bacteria attached to the electrode converts the energy available in biodegradable substrates such as glucose, sucrose, starch, acetate, oxalate, fumarate and amino acids directly into electricity by catalytic activity. These bacteria can oxidize organic compounds to carbon dioxide and transfer electrons to the anode. Later the electrons pass through an external circuit to the cathode to produce current. Protons migrate to the cathode and combine with  $O_2$  and with electrons which released from anode to form water. Electron transfer occurs through agar salt bridge. The microorganisms that have been used in Microbial fuel cell are cellulose degrading bacteria.

## II. MATERIALS AND METHODS

### Collection of rice straw

Rice straw was obtained from the agricultural fields of Gubbi, Tumakuru, Karnataka, India (Fig.1) and then cut into lengths of 3 cm with scissors and washed thoroughly with tap water until the washes were clean. The pieces were dried in a hot air oven at  $60^\circ C$  for 3 h and then powdered in a blender (Fig. 2).



Fig.1 Rice straw

Fig.2 Blended rice straw

### Determination of cellulose, Hemicelluloses and Lignin content in the Rice straw

2g of rice straw was boiled in ethanol for 15min and washed thoroughly with distilled water later kept in hot air oven to dry at  $40^\circ C$  overnight. The dried rice straw was divided into two parts in which one part was considered as A fraction and the second part is considered as B fraction. The second fraction (B) of residue was treated with 24% KOH for 4hrs at  $25^\circ C$ , then it was washed thoroughly with distilled water at  $80^\circ C$  overnight and its dry weight was noted. The same sample again treated with 72%  $H_2SO_4$  for 3hrs to hydrolyze the cellulose and the refluxed with 5%  $H_2SO_4$  for 2hrs.  $H_2SO_4$  was removed completely by washing with distilled water. Then it was dried at  $80^\circ C$  in hot air oven overnight, the dry weight of the fraction was taken as C fraction.

$$\text{Cellulose} = B - C$$

$$\text{Hemicellulose} = A - B$$

$$\text{Lignin} = C$$

### Determination of moisture content

2g of rice straw was cut into small pieces and dried in hot air oven for 1hr at  $60^\circ C$ . Then its weight was measured and moisture content was determined.

### Screening and Characterization of Cellulose Degrading Bacteria

#### Culture inoculation and MFC operation

A mixed culture of cellulose degrading bacteria was cultured on a modified Dubos's salt medium (Table. 1) amended with carboxy methyl cellulose (CMC) as the sole carbon source. The cellulose degrading bacteria were isolated by transferring of the 1g of soil solution to the media. The bacteria culture was incubated at  $30^\circ C$  for one week.

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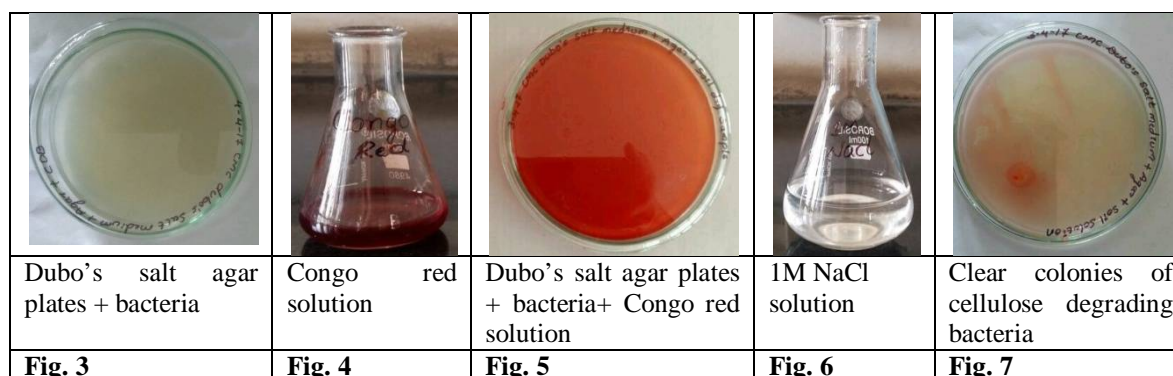
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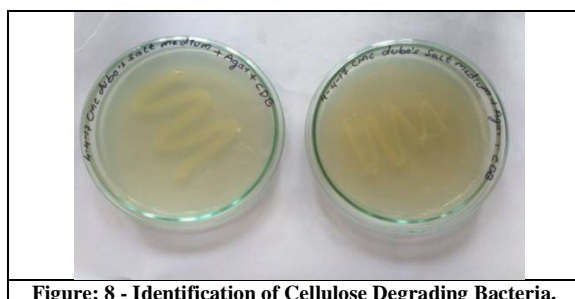
Table. 1 Dubos's salt medium

| Components                  | Grams per litre |
|-----------------------------|-----------------|
| Carboxy methyl cellulose    | 10              |
| Sodium nitrate              | 0.5             |
| Dipotassium hydro phosphate | 1               |
| Magnesium sulphate          | 0.5             |
| Potassium chloride          | 0.5             |
| Ferrous sulphate            | 0.001           |

The incubated bacteria was inoculated on carboxymethyl cellulose amended Dubo's salt agar plates (Fig. 3). After one week Congo red solution (Fig. 4) is poured on to the cultured plates (Fig. 5). The cultured plates were washed with 1M NaCl (Fig. 6) to check the clear colonies of cellulose degrading bacteria (Fig.7). The cellulose degrading bacteria were screened by streaking the pure colony of cellulose degrading bacteria obtained from the culture on the carboxy methyl cellulose amended Dubos's salt agar medium (Fig. 8).



Screening and identification of Cellulose Degrading Bacteria



## Preparation of media for MFC.

Nutrient mineral buffer media was taken in both anode and cathode compartments of the microbial fuel cell. It consisted of 0.31 g/L  $\text{NH}_4\text{Cl}$ , 3.13 g/L  $\text{NaHCO}_3$ , 0.75 g/L  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 2.75 g/L  $\text{Na}_2\text{HPO}_4$ , 4.22 g/L  $\text{NaH}_2\text{PO}_4$ , 0.13 g/L KCL and trace metals and vitamin solutions, then the media was sterilized using autoclave for 20 minutes[8].

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## Construction and operation of Microbial fuel cell

H- Type Microbial fuel cell was constructed by using two chambers as anode and cathode (Fig. 9). The anode chamber was anaerobic and cathode chamber was aerobic. The two chambers were constructed from plastic boxes of diameters suitable to hold the agar salt bridge which was clamped by means of valves fitted with rubbers. On each side of agar salt bridge two non-wet proof copper electrodes as anode and cathode were inserted inside the chambers.

The microbial fuel cell tests were operated at a fixed external circuit resistance. The two chambers were filled with 1.5 L nutrient mineral buffer solution. The anode compartment was inoculated with cellulose degrading bacteria and 6 g / L rice straw was added as the electron donor during the set-up of the MFC. The anode chamber was flushed with N<sub>2</sub> gas for 10 min and then sealed with a rubber cap to maintain anaerobic condition. 50 mM K<sub>3</sub>Fe (CN)<sub>6</sub> was used as the terminal electron acceptor. Once the microbial fuel cell produces a repeatable cycle of power generation, the anode compartment was refilled with a fresh nutrient mineral buffer solution and rice straw.



Fig. 9 MICROBIAL FUEL CELL

## Calculations of power generation.

The MFC tests were performed at a fixed external resistance of 1000 Ohm. The MFC system was monitored using a precision multi meter for recording the voltage and current. The voltage readings were recorded for every 30 min. The maximum voltage and current obtained in MFC was 3.1V and 3.9 amperes respectively. The product of voltage and current gives the total power, which was found to be 12.09.

$$P = VI$$

$$P = 3.1 \times 3.9$$

$$P = 12.09 \text{ W.}$$

## III. RESULTS AND DISCUSSION

### Determination of cellulose, Hemicelluloses and Lignin content in the Rice straw.

The cellulose, Hemicellulose, lignin and moisture content were determined (table.2).

Table 2: Composition of Rice straw in terms of percentage

| Components       | In percentage |
|------------------|---------------|
| Cellulose        | 36            |
| Hemicellulose    | 17            |
| Lignin           | 12            |
| Moisture content | 13            |

### Biochemical characterization of Cellulose degrading bacteria

The cellulose degrading bacteria were characterized by biochemical tests such as IMVIC, Starch Hydrolysis, Carbohydrate Fermentation, Catalase, Urease, Hydrogen Sulphide, Nitrate Reduction, Gram positive, and Gram negative tests using standard procedures.

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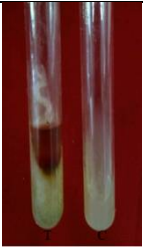



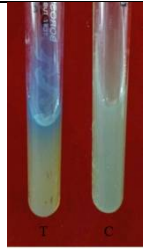

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**Table 3: Results of biochemical tests**

| Sl No. | Bio chemical test  | Result   |
|--------|--|----------|
| 01     | Iodine   | Positive |
| 02     | Methyl red   | Positive |
| 03     | Voges-proskauer  | Negative |
| 04     | Citrate utilization                                      | Positive |
| 05     | Starch hydrolysis  | Positive |
| 06     | Carbohydrate fermentation (Lactose, Glucose and Sucrose) | Positive |
| 07     | Catalase   | Positive |
| 08     | Urease   | Positive |
| 09     | Hydrogen sulphide  | Positive |
| 10     | Nitrate reduction  | Positive |

|   |   |   |   |   |   |
|---|---|---|---|---|---|
|  |  |  |  |  |  |
| Fig.6 Iodine test   | Fig.7 Methyl red test   | Fig. 8 Voges-proskauer test   | Fig.9 Citrateutilization test   | Fig. 10 Catalase test   | Fig. 11 Hydrogen sulphide test  |

|   |   |   |  |   |
|---|---|---|--|---|
|  |  |  |  |  |
| Fig. 12 Sucrose utilization test  | Fig. 13 Dextrose utilization test   | Fig. 14 Lactose utilization test  | Fig. 13- Starch hydrolysis test  | Fig. 14 -Nitrate reduction test   |
| Carbohydrate fermentation Test  |   |   |  |   |

## Start up and Enrichment of the MFCs

Initially MFC was operated using 1.5 g/L of rice straw as substrate. In order to form biofilm on the anode electrode. The biofilm was formed after 2-3 days of the MFC operation. After obtaining a stable voltage of the MFCs, the cathode and anode chambers were replaced by fresh medium. 1.5 g/L rice straw was again sent into the anode chamber. 5mM potassium ferricyanide solution was filled in the cathode chamber.



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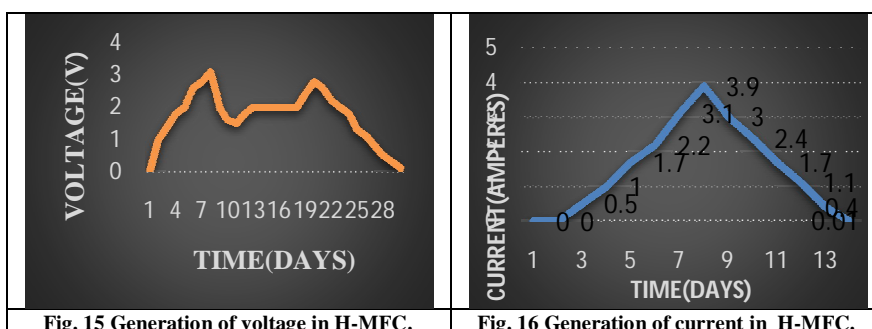
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## Electricity production in H-MFC

H- MFC was operated at room temperature by adding 20g of rice straw, 1.5L Nutrient mineral buffer (NMB) media, 25ml Cellulose degrading bacteria (CDB), and 5mM potassium ferricyanide catholyte. The voltage generated was noted at the intervals of every 24h and continued up to 30 days. A graph of voltage v/s time in days was plotted (Fig.15). The average voltage generated was 3.1V and stable voltage output of 12 days during the first period. In the second period between 13 to 21 days, the average voltage decreased to 2.8V because the inhibition of proton transfers produced by a lower water content, which was resulted from evapotranspiration. The performance of MFC was also investigated and current generated was plotted against the time in days and found that the average current was 3.1 V (Fig.16).



## IV. CONCLUSION

Rice straw is the main agricultural waste from rice plant, which is mainly used as feed for ruminants in all over the world. In the present study has shown the utilization of biomass for the production of electricity and to meet the countries energy demands. The present study demonstrated that stable power could be generated from rice straw. The MFC produced the maximum Voltage of 3.1 and corresponding to the maximum current of 3.9 amperes which results in power output of 12.09 W. In this experiment, crude rice straw was directly used as the substrate in the MFCs without any pre-treatment. Further studies are suggested in optimization of the media for getting maximum voltage, authentication of the bacteria and scaling up of the technology for the commercial utilization of the electricity produced from MFC.

## Competing interests

The authors declare that they have no competing interests.

## V. ACKNOWLEDGEMENT

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