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A Study of Biomass Activity That Leads To Electricity Generation from Mud Soil

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----------ABSTRACT----------

Metabolic activity within a microbial fuel cell (MFC) is critical for generating power for use as a renewable energy source. The study's goal is to track both exogenous and endogenous metabolic activity for various biomass-substrates combinations used to generate energy from mud soil MFCs. Oxygen consumption rate and methane production rate were used as an indicator for microbial activity in this study. A total of six identical cells were made for six different criteria, three of which have a low concentration of bio-consortium and another three have a high concentration of bio-consortium. Two different substrates were used too. A total of six experiments were conducted to observe the metabolic rates and power output of microbes present in a special bio-consortium of both exogenous and endogenous states utilizing cohesive soil. 300ml of the prepared sample with Zinc and Carbon felt as electrodes were used. The highest recorded methane value and oxygen consumption percentage were detected at 4847 µg/mol and 14%. Power output was also considered in this study. Though power output was irregular, however after around 48 hours of observation, the peak power output was 16.56 watt/m2 and the highest total electricity production was 1556998.848 J/m2. Further study is needed to have a better understanding of the metabolic activities of microbes inside MFC and to keep the MFC output constant at the maximum achievable power.

Keywords**–***Bio-consortium, Endogenous, Exogenous, Metabolic activities, Methane generation.*

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I. INTRODUCTION

Renewable energy will play a significant role in global energy production and consumption in near future [1]. In recent decades, there has been a positive trend in the use of energy throughout the world. As oil reserves dwindle, the global energy issue has prompted researchers to look into other energy sources. Microbial fuel cells (MFCs) are gaining popularity due to their low operating cost and use of a variety of biodegradable substrates as fuel. This MFC produces bioelectricity by using an active microorganism as a biocatalyst in an anaerobic anode compartment [2]. Almost all MFCs are made up of anode and cathode chambers that are physically separated by a proton exchange membrane (PEM). The anode's active biocatalyst oxidizes the organic substrates, producing electrons and protons. The PEM transports the protons to the cathode chamber, whereas the external circuit transports the electrons. MFCs can be used for power production, battery replacement, and wireless sensor networks. These are used to charge cell phones, low-energy gadgets, house lamps, and other domestic electronic components. Despite the fact that the power levels in all of these systems were rather modest, MFC is a remarkable technology that can combine a broad variety of substrates, materials, and system structures with bacteria to achieve bioenergy production [3]. It is long-lasting and can be recommended for long-term use due to improved safety and health concerns. Bettin demonstrated that an MFC capable of delivering 25mW of power might be used for cardiac stimulation; nevertheless, the required surface area is relatively huge [4]. Besides that, low energy recovery and high costs have been reported as limitations in the field of MFC technology [5]–[7]. Although many researchers have worked on improving energy recovery in MFC systems using low-cost materials, further study is needed to improve the electric power density in MFCs [8]–[10].

Initially, in MFC, the anaerobic exoelectrogenic bacteria in the anodic chamber begin to oxidize the supplied substrates and release electrons (e^-) as well as protons (H^+) towards the anode. As a result of oxidation, carbon dioxide (CO_2) is created. The electrons generated in the process are transported from the anode to the cathode via an external circuit, creating electricity in the process. The protons enter the cathode, where they reduce oxygen (O_2) and generate water (H_2O) [11]. When bacteria consume a substrate such as sugar in aerobic circumstances, carbon dioxide and water are produced. They produce carbon dioxide, hydrons (hydrogen ions), and electrons when oxygen is not present. The reactions at the anode and cathode electrode in a typical MFC using acetate (CH_3COO^-) as a model substrate model [11] are presented below:

Anodic reaction: $CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

Cathodic reaction: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

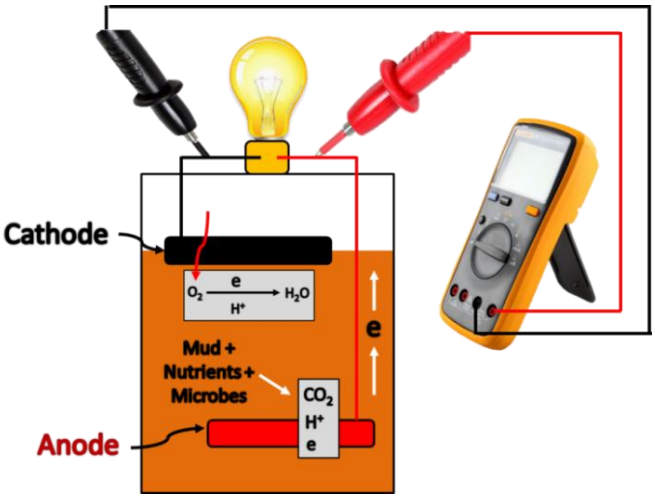

Fig -1: Mechanism of electricity generation using mud soil based MFC

The anaerobic digestion process, which takes place in MFC's anodic chamber, includes four main steps. To begin with, the hydrolysis phase breaks down big organic substrates into smaller molecules. Second, acidogenesis converts the first reaction's outcome into short-chain compounds like volatile fatty acids, ketone, and alcohol. The products of acidogenesis are then converted into hydrogen, carbon dioxide, and acetic acid by acetogenic bacteria in Acetogenesis. Methanogenesis is the final stage, in which methanogens convert these compounds to methane and carbon dioxide. The overall process can be summarized by the chemical reaction below, in which organic material such as glucose is biochemically degraded by anaerobic microorganisms into carbon dioxide (CO_2) and methane (CH_4):

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$$

So theoretically in the single cell MFC as time goes on, oxygen content would reduce resulting in the depletion of electron acceptors and the content of methane would increase with time until it reaches its full potential.

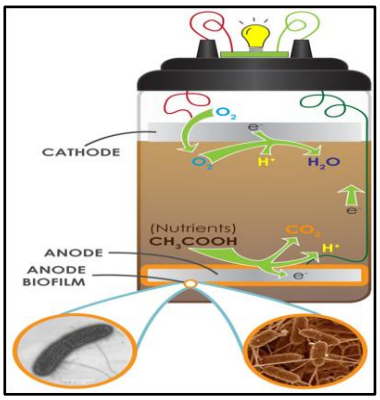

Fig -2: Traditional soil based microbial fuel cell

In the last decade, the role of electrogenic bacteria in biofilm development has grown dramatically, as has the interest in Microbial Fuel Cells (MFCs) as an efficient energy-producing technology [12]–[15]. The primary aspect of metabolic activity is cellular respiration, and oxygen consumption can be used as a reliable diagnostic of bacterial aerobic respiration, even in facultative anaerobic bacteria [16]. A number of researchers have suggested that the oxygen consumption of developing bacteria cultures could be used as a growth criterion [17], [18]. The rate of oxygen uptake was compared to the pace of bacteria growth by Clifton and Martin [18], [19]. Martin demonstrated that the oxygen consumption per cell and the surface area per cell both reached their maximum levels at the same time.

Methane fermentation is carried out by bacteria that get energy for development by catabolizing anaerobically degradable organic materials to COs and CH_4 Reference as end products [20]. Because it promotes the anaerobic breakdown of complex organic materials to relatively clean and easily purified gaseous products, CO_2 and CH_4, with a very low growth yield of bacteria, methane fermentation is particularly significant in the carbon and other elemental cycles in nature [21], [22].

This research aims to investigate the metabolic activity of various microbe-substrates mixes in the production of electricity from mud soil MFCs. The novelty of this study lies in tracking out the endogenous and exogenous metabolic activity for different microbe-substrates combinations for electricity generation in MFC. A total of six identical cells were made, three of which have a low concentration of bio-consortium and another three have a high concentration of bio-consortium for both the endogenous and exogenous case. In the exogenous case, glucose and sucrose were used separately as the substrates. Cohesive soil was used, and carbon felt as a cathode and zinc as an anode was used in this study. This study examines and describes the power output and restrictions. Improvements to the MFC are also recommended.

II. MATERIALS & METHOD

2.1 Sample Preparation

Cohesive soil used for commercial pottery works, passing through 50 no. sieve was prepared with distilled water and converted to well-mixed mud. 1200g of the mud soil mixed with 300 ml solution of bio-consortium (1 or 5 capsules) for each cell was used. Glucose and sucrose solution had been added according to each experimental configuration as the substrate to feed the microbes. The overall test samples used in the study can be summarized below (Table -1).

Table -1: Test samples with the ingredients used in the study

Test Sample	**Microbes**		**Substrate**		**Target test**
	Type	Amount	Type	Amount	
1	Bio-consortium	1 capsule (4 billion)	None	-	Endogenous
2	Bio-consortium	5 capsules (20 billion)	None	-	Endogenous
3	Bio-consortium	1 capsule (4 billion)	Glucose	10g	Exogenous
4	Bio-consortium	1 capsule (4 billion)	Sucrose	10g	Exogenous
5	Bio-consortium	5 capsules (20 billion)	Glucose	10g	Exogenous
6	Bio-consortium	5 capsules (20 billion)	Sucrose	10g	Exogenous

Table -2 and 3 presents some important physical properties of the soil sample and the composition of the bio-consortium capsule used in the experiment. An X-ray diffractometry test was used to determine the chemical characteristics of the mud soil (data is not shown here). This test was performed by Lafarge Surma Cement Ltd. The Chemical Composition of the bio-consortium was provided by the provider.

Table -2: Physical properties of the tested mud soil

Name of the physical properties	Values
Specific gravity	2.67
pH value	6.9

Table -3: Composition of bio-consortium

Components	Amount
Lactobacillus acidophilus	2 billion
Bifidobacterium bifidum	1 billion
Lactobacillus bulgaricus	1 billion
Fructo-oligosaccharides	100mg

Fig -1: Prepared samples for the experiments

a. Operation

i. Design and Fabrication of Microbial Fuel Cell

The whole experimental setup was planned to fabricate in a cost-effective way. Locally available materials along with some commercial parts were used to build the setup. Each cell was composed of 300ml of samples, with a zinc plate as the anode (6cm*2.5cm) and carbon felt as the cathode (Thickness 5mm, 7cm*3.5cm). The anode was placed in the soil and the cathode was placed at the top of the soil so that it could make a good contraction area with both the soil and the air as it will be reducing oxygen from the air. The distance between anode and cathode was 3cm. During the investigation, the room temperature was considered a thermal background for microbial metabolism. It varied from 27℃ to 29℃.

Fig -3: Design of MFC, Zinc, and Carbon felt electrodes

b. Measurement

The experiments were conducted for 48 hours for each sample. The reduction of oxygen was measured over time using a Lutron YK22DO DO meter. As a container, a glass bottle was employed. This meter provides a direct readout of the percentage of oxygen in the air. Methane production was measured using a gas detector. The detector returned a result in µg/mol. Under each bottle, there was also a facility for stirring.

Fig -3: Experimental setup

i. Measurement of Oxygen Reduction

After stabilization, the DO meter was calibrated to a standard air oxygen percentage, and the bottle was adjusted with the DO meter. The DO meter's internal air volume was 400ml, and the reading was obtained for around 48 hours.

ii. Measurement of Methane Production

Methane production was measured using a gas detector. Under each bottle, there was also a facility for stirring. After adjusting the container bottle, the gas detector was set to zero reading. The reading from the gas detector was in µg/mol. The bottle's internal air volume was 400ml. The data was collected for around 48 hours.

iii. Measurement of Power Generation

A digital multimeter was used to measure the voltage output for open-circuit condition. After a regular interval, the voltage and current of the MFC were measured. This measurement lasted about 48 hours.

c. Calculations

2.4.1Calculation of Oxygen Reduction and Methane Generation

The DO meter was used to monitor the decline in oxygen percentage in the air. This meter provides a direct readout of the percentage of oxygen in the air. The gas detector gave a direct result in µg/mol for methane generation.

2.4.2 Calculation of Power Generation

Average power output was calculated via,

$P = VI/A$

In which, P = Power density (W/m^2 Per growth area),

V = Voltage (V),

A = Area of working electrodes(m^2),

I = Current (Ampere).

Power output and Current densities are all normalized to the working electrode surface area, which is equal to the 2*area of the anode. Calculations for total electricity production were performed with,

Total electricity production (J/m^2) = $\sum P \times t$

In which, P = average power density in W/m^2 during 48hours and

t = 48 hours (172800 s).

III. RESULTS

The concentration and types of substrates, the concentration of the bio-consortium, and the temperature inside the MFC all have a role in the study's effectiveness. The mud soil was tested using several types of substrates and varying concentrations of bacteria to determine the microorganisms' activity. The generation of oxygen and methane versus time, as well as the accompanying power generation, illustrates the activity of microorganisms under various situations.

3.1 MFC Using One Capsule of Bio-Consortium (4 billion) (Endogenous Phase)

This experiment was carried out with one capsule of bio-consortium (4 billion) to observe metabolism in the endogenous phase for 48 hours. The graph of O_2 consumption and CH_4 production over time is shown in Chart-1, Chart-2 and Chart-3 depict the relationship between O_2 consumption and CH_4 production and electricity generation. During the first 6.5 hours, the present oxygen percentage increased, indicating that oxygen was not utilized initially, but after that, the oxygen percentage gradually increased. Oxygen consumption goes from 0 to 2.5 percent after 48 hours. The CH_4 production graph shows that the generation was continuous for the first 24 hours and reached 724 gm/mol, after which it declined and stopped at 268 gm/mol. Power generation rose for the first 20 hours, then decreased for the next 20 hours, then progressively grew for the next 25 hours and lasted until 45 hours 11.19 watt/m^2 was the maximum power output.

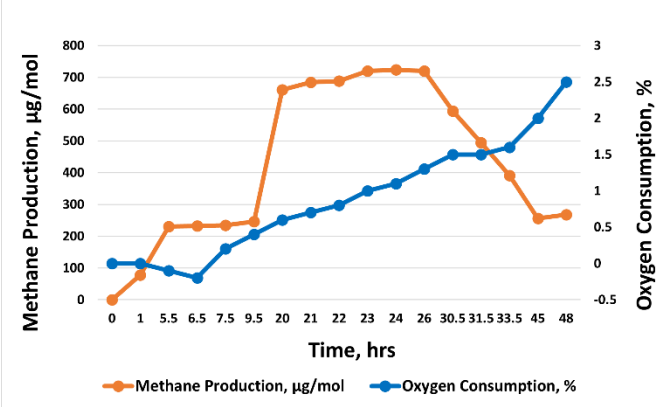

Chart -1: Time vs CH4 production and O2 consumption curve (using one capsule of bio-consortium)

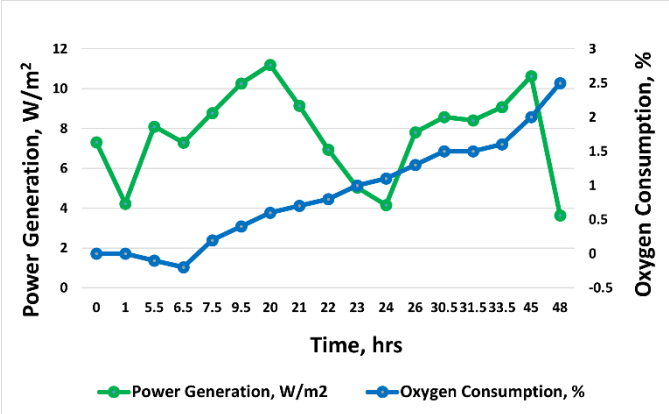

Chart -2: Time vs power generation and O2 consumption and curve (using one capsule of bio-consortium)

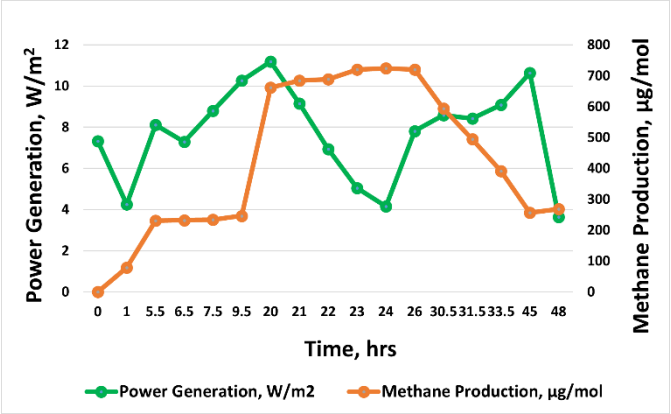

Chart -3: Time vs power generation and CH4 production curve (using one capsule of bio-consortium)

3.2 MFC Using Five Capsules of Bio-Consortium (20 billion) (Endogenous Phase)

This experiment was carried out with five bio-consortium (20 billion) capsules to investigate metabolism in the endogenous phase for 47.5 hours. The graph of O_2 consumption and CH_4 production over time is shown in Chart-4. The relationship between O_2 consumption and CH_4 production and power generation is depicted in Chart-5 and 6. During the first 6 hours, the current oxygen percentage increased and reached 21.3 percent, but after that, the oxygen consumption percentage gradually increased from 0.1 percent to 3.9 percent. The CH_4 production graph shows that the generation was continuous for the first 30 hours, reaching 1411 gm/mol; however, following that, the methane production declined and ended at 250 gm/mol. The first 25 hours of power generation were modest, but after that, there was a progressive increase in the generation that lasted until 43.5 hours. 8.96 watt/m^2 was the maximum power output.

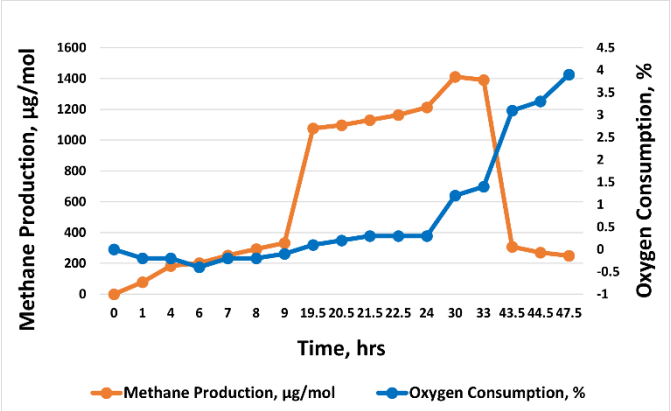

Chart -4: Time vs CH_4 production and O_2 consumption curve (using five capsules of bio-consortium)

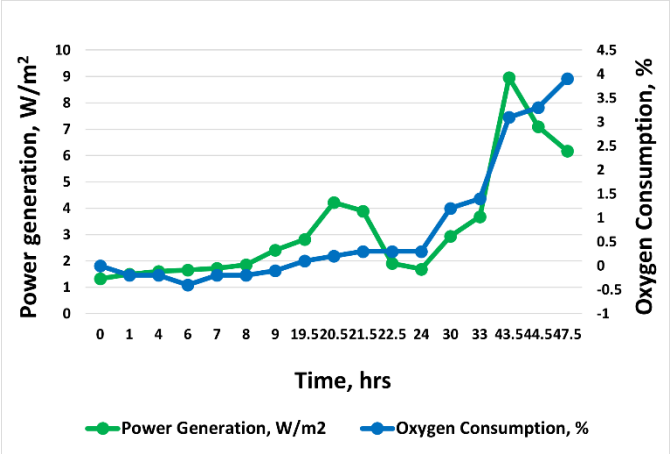

Chart -5: Time vs power generation and O_2 consumption and curve (using five capsules of bio-consortium)

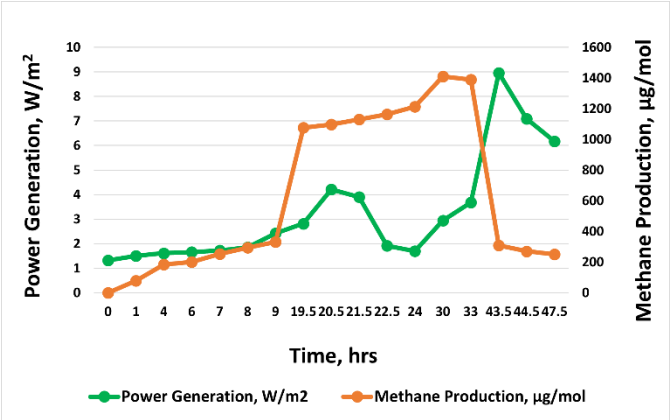

Chart -6: Time vs power generation and CH_4 production curve (using five capsules of bio-consortium)

3.3 MFC Using One Capsule of Bio-Consortium and 10g of Glucose (Exogenous Phase)

This experiment used one capsule of bio-consortium (4 billion) to observe metabolism in the exogenous phase for approximately 47.5 hours. As a substrate, 10 grams of glucose were employed. The graph of O_2 consumption and CH_4 production over time is shown in Chart-7. Chart-8 and 9 show the relationship between O_2 consumption and CH_4 production and electricity generation. The plot shows that oxygen consumption increased progressively over time, starting at 0% and ending at 8.3% after 47.5 hours. The CH_4 production curve shows that the generation was a little slower in the first 10 hours, followed by a quick spike in methane output. This generation was carried on for another 44.5 hours. The generation ended at 4642 gm/mol after 44.5 hours. For the first 22.5 hours, power generation increased, but then abruptly decreased. Power generation increased again after 32.5 hours 14.079 watt/m^2 was the greatest power output.

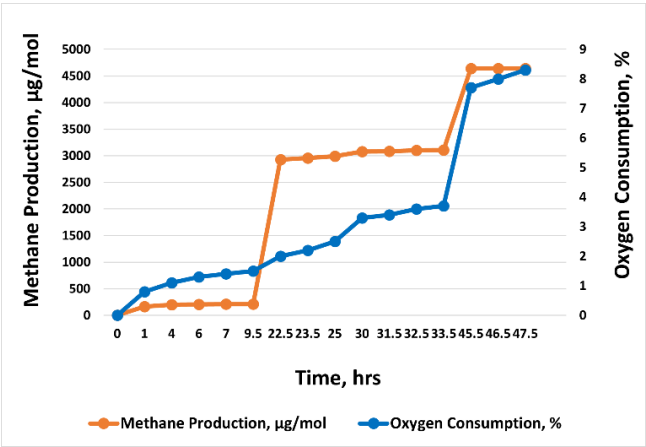

Chart -7: Time vs CH_4 production and O_2 consumption curve (using one capsule of bio-consortium and 10 gm of glucose)

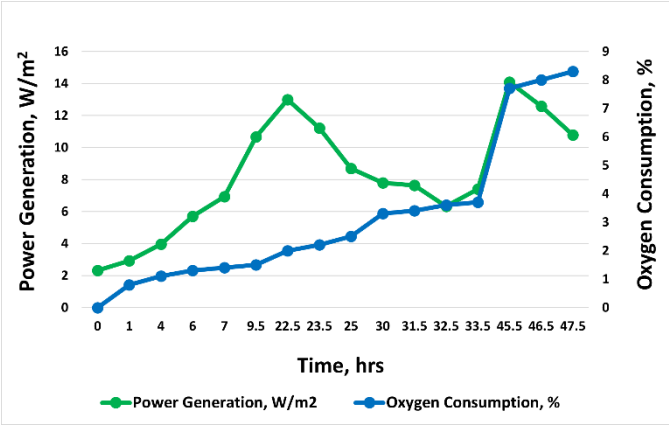

Chart -8: Time vs power generation and O_2 consumption and curve (using one capsule of bio-consortium and 10 gm of glucose)

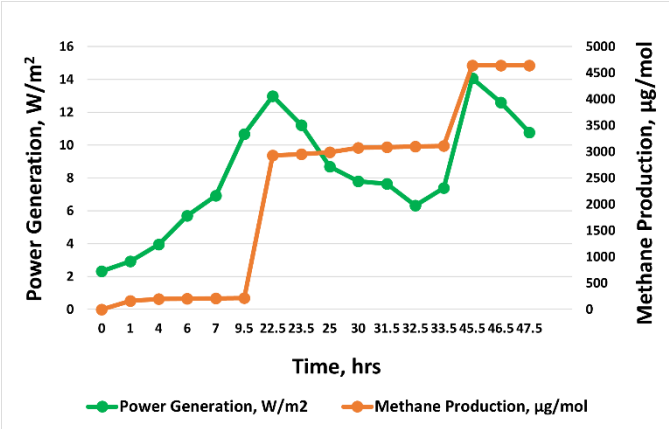

Chart -9: Time vs power generation and CH_4 production curve (using one capsule of bio-consortium and 10 gm of glucose)

3.4 MFC Using One Capsule of Bio-Consortium and 10g of Sucrose (Exogenous Phase)

This experiment used one capsule of bio-consortium (4 billion) to observe metabolism in the exogenous phase for approximately 47.5 hours. As a substrate, 10 grams of sucrose were used. The graph of O_2 consumption and CH_4 production over time is shown in Chart-10. Chart-11 and 12 depict the relationship between O_2 consumption and CH_4 production and the creation of electricity. It can be observed from the graph that oxygen consumption increased gradually throughout the journey, starting at 0% and ending at 6.6 percent after 47.5 hours. On the CH_4 production curve, it can be observed that the generation was a little lower in the first 10 hours, but then increased rapidly after that. This generation was carried on for 46.5 hours. The generation was terminated at 4655 gm/mol after 46.5 hours. For the first 22 hours, power generation increased, but after that, it suddenly decreased. There was a small rise in power generation after 31 hours 13.462 watt/m^2 was the maximum power output.

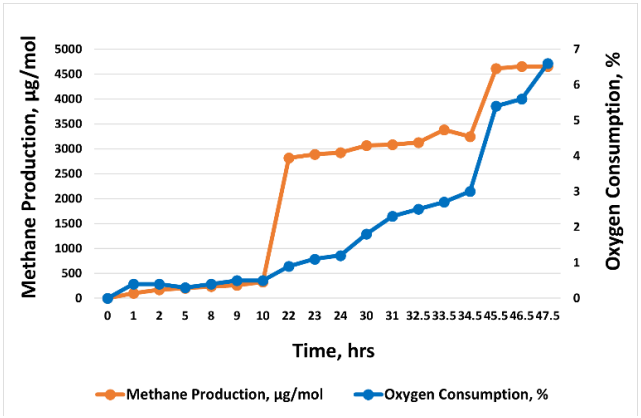

Chart -10: Time vs CH_4 production and O_2 consumption curve (using one capsule of bio-consortium and 10 gm of sucrose)

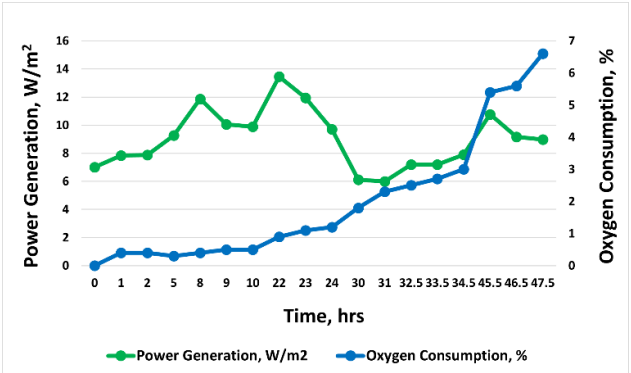

Chart -11: Time vs power generation and O_2 consumption and curve (using one capsule of bio-consortium and 10 gm of sucrose)

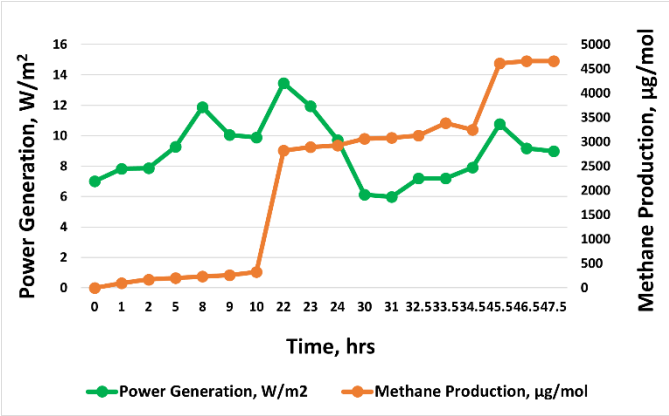

Chart -12: Time vs power generation and CH_4 production curve (using one capsule of bio-consortium and 10 gm of sucrose)

3.5 MFC Using Five Capsules of Bio-Consortium and 10g of Glucose (Exogenous Phase)

This experiment was carried out with five bio-consortium (20 billion) capsules to observe metabolism in the exogenous phase for roughly 45.5 hours. As a substrate, 10 grams of glucose were employed. The graph of O_2 consumption and CH_4 production over time is shown in Chart-13. Chart-14 and 15 show the relationship between O_2 consumption and CH_4 production and electricity generation. For the first ten hours, oxygen consumption was modest. After 10 hours, there was a progressive rapid increase in oxygen consumption, which began at 0% and finished at 14% after 45.5 hours. On the CH_4 production curve, it can be observed that the generation was a little lower in the first 5 hours, but then increased rapidly after that. This generation was carried on for another 44.5 hours. The generation ceased at 4847 gm/mol after 44.5 hours. For the first 26.5 hours, power generation was falling. The power output increased to 44.5 hours after 26.5 hours. There was a drop in power generation in our recent report. 16.56 watt/m^2 was the maximum power output.

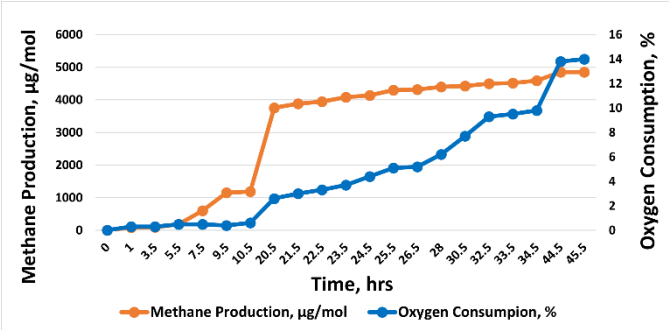

Chart -13: Time vs CH_4 production and O_2 consumption curve (using five capsules of bio-consortium and 10 gm of glucose)

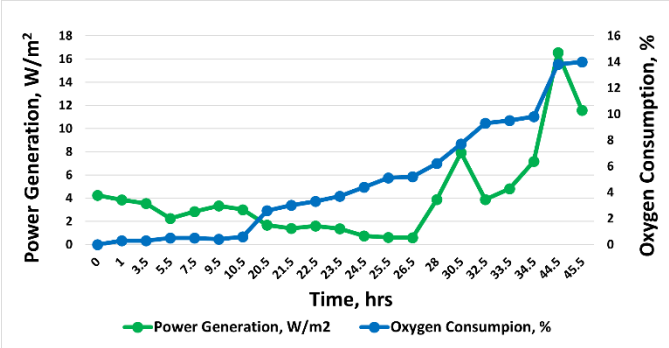

Chart -14: Time vs power generation and O_2 consumption and curve (using five capsules of bio-consortium and 10 gm of glucose)

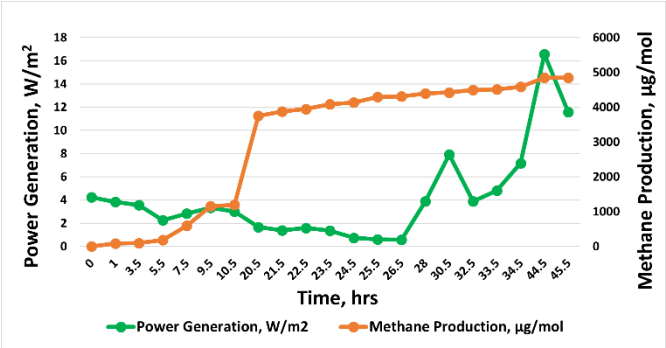

Chart -15: Time vs power generation and CH_4 production curve (using five capsules of bio-consortium and 10 gm of glucose)

3.6 MFC Using Five Capsules of Bio-Consortium and 10 gm of Sucrose (Exogenous Phase)

This experiment used five bio-consortium (20 billion) capsules to observe metabolism in the exogenous phase for 45.5 hours. As a substrate, 10-gram sucrose was used. The graph of O_2 consumption and CH_4 production over time is depicted in Chart-16. The relationship between O_2 consumption and CH_4 production and power generation is shown in Chart-17 and 18. The oxygen consumption in this segment increased progressively throughout the journey, starting at 0% and ending at 9.7% after 45.5 hours. The CH_4 production graph shows that the rate of methane production increased rapidly after the first 5 hours. This cycle was repeated for 43.5 hours. The generation reached 4650 gm/mol after 43.5 hours. For the first 5 hours, power generation increased; but, after 5 hours, power output abruptly decreased. There was another surge in power generation after 10 hours, and then the output gradually decreased until the end. 16.001 watt/m^2 was the highest power output.

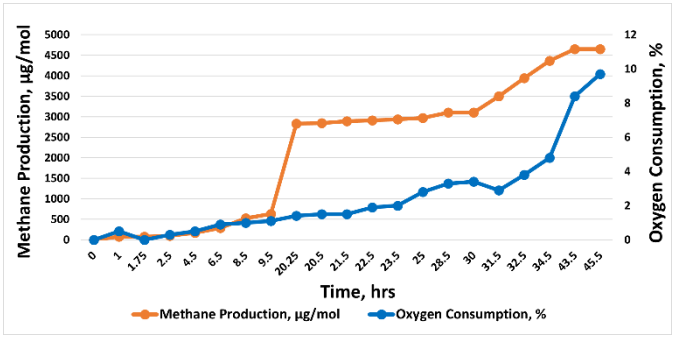

Chart -16: Time vs CH_4 production and O_2 consumption curve (using five capsules of bio-consortium and 10 gm of sucrose)

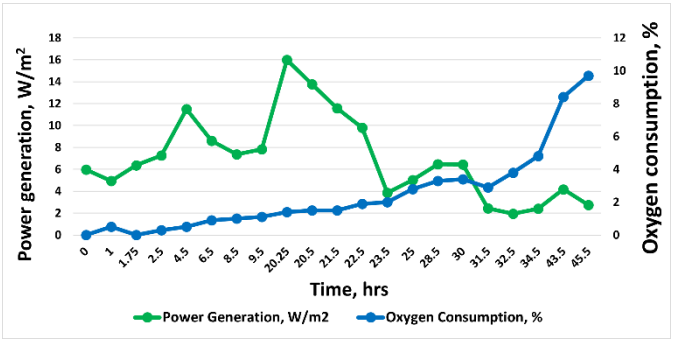

Chart -17: Time vs power generation and O_2 consumption and curve (using five capsules of bio-consortium and 10 gm of sucrose)

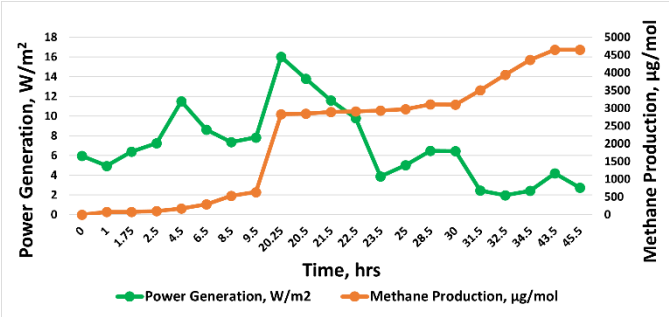

Chart -18: Time vs power generation and CH_4 production curve (using five capsules of bio-consortium and 10 gm of sucrose)

3.7 Power Generation by Different Test Samples

This bar chart (Chart-19) depicts a comparison of the test samples where maximum power output is taken into account. The maximum power output was obtained from test sample 5 as shown in this graph. 16.56 watt/m^2 was the greatest power output. Five bio-consortium capsules and 10 grams of glucose made up test sample 5. Test sample 2, which was in the endogenous state and only consisted of 5 capsules of bio-consortium, produced the lowest power output. 8.96 watt/m^2 was the lowest power output.

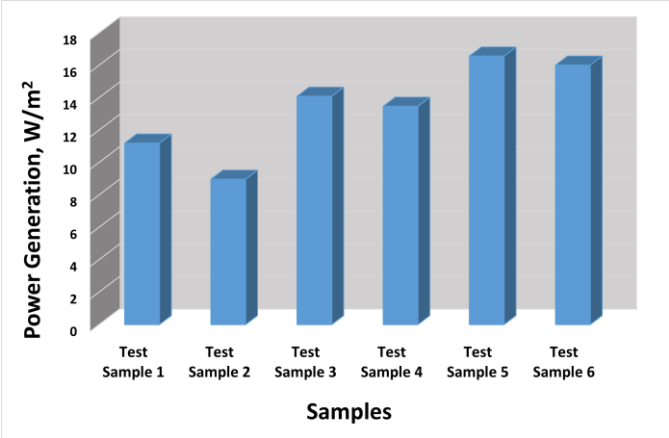

Chart -15: Power Generation (W/m^2) vs Test Samples

However, sample 4 gave the highest output during the experiment in terms of total electricity production. 1556998.848 J/m^2 was the highest total electricity production among the samples. The lowest one was given by sample 2. It was 563587.2 J/m^2.

IV. DISCUSSION

Microbial metabolic activity was observed for exogenous and endogenous conditions with different substrates as microbial feed. Percentage of oxygen consumption, methane production, and electricity generation were recorded for analysis. The activities in the exogenous condition were substantially higher than in the endogenous condition. When high concentrations of microorganisms were used, the activities were shown to be higher when glucose was employed as a substrate. Again, when sucrose was used as a substrate, the activities were not as high as when glucose was used.

The high-power generation for glucose as a substrate might be due to the fact that it is made up of simple monosaccharides that are easy for microorganisms to digest, whereas sucrose is made up of disaccharides and requires time to break down because it is made up of glucose and fructose. As a result, in the glucose substrate condition, activities were reported to be greater. The activities were substantially lower in endogenous conditions.The addition of a bio-consortium to the bio electrochemical system enhanced power generation. The increase in power can be explained by the oxidation of redox-active chemicals generated by bacteria, such as laccases [23]. Laccases may have a role to play once more. Laccase enzymes are found in fungi and bacteria and provide a variety of biological roles. They are also likely to be found in biofilms [24]. As a result, it's possible that bacterial laccases (either inside or outside the microorganisms) were involved in the process [25], [26]. After a certain period of time, the power generation started to decrease, a possible reason behind this might be substrate and microbes' limitations. Based on his experiments, Clifton hypothesized that the rate of bacterial metabolism per cell is maximum during the early stages of growth due to the larger size of

the cells. However, he stated that attempts to explain differences in the rate of metabolic activity at various stages of growth on the basis of changes in cell size and physiological activity of the cells had not been completely successful and that the decrease in metabolic rate as the culture aged was due to a depletion of available food and an increasingly unfavorable environment. Therefore, the creation of a multilayer bacterial biofilm, the accumulation of dead cells on the anode surface, and substrate depletion could be the cause of MFC performance degradation [27], [28].

Finally, it can be stated that the type and amount of inherent microorganisms as well as the substrate employed in the cell completely determine the effectiveness of MFCs. However, a large number of test experiments using various bio-consortium and substrates should be conducted for a better understanding. Finally, despite the fact that MFCs produce very little electricity, they might be considered a potential source of renewable energy. It can be improved by appropriately examining microorganisms' internal activity.

V. CONCLUSIONS

Various microorganisms and substrates have been used in the development of MFCs. However, the goal of this research was to learn more about the microorganisms' activity within MFCs. The actions of bacteria vary depending on the circumstances, as this study shows. When substrates are available, it is even greater. Again, activities are increased related to bio-consortium concentration as well as the specific substrate. The maximum power output was 16.56 watt/m^2 and the lowest was 8.96 watt/m^2. the activities decline due to a lack of substrates or the expiration of those that are available. As commercial soil was used in this research so due to the lack of availability of substrate for, a significant amount of inborn natural bacteria and microorganisms the MFC performance might be terminated after a certain period. Due to the limitation of time and other issues (Specially Covid-19), longer duration experiments and, experiments with different microbes-substrates combinations were not possible. These limitations could be potential research areas for further studies.

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