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Aerobic Biosensor-A Novel Approach of Microbial Fuel Cell with A Halotolerant *Priestia Megaterium*-BorS17B13

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ABSTRACT

Discovering the unknown crannies of mangrove associated microbes constitutes the secret to revealing the unexplored realm of industrial prospective and maintaining vital products such as protease. A halo-tolerant microbe, Priestia megaterium strain BorS17B13 was isolated from the mangrove swamps of Monari Creek, Borivali, Maharashtra, India. The organism was aerobic, Gram +Ve and abundantly produced protease enzymes while uptaking as crude source of bothnitrogen and carbon (Gram flour). At 72 hr, the optimum protease activity was 423 U/ml. Furthermore, their bioenergy generation potential was evaluated in a double chamber Oxy Anodic Microbial Fuel Cell (OA-MFC) with an agitator for equivalent setups, such as a bioreactor. They were also investigated with an external resistor or load of 47 ohm and 100 ohm. The maximum power generated was 259 mW, while the volumetric power density was 0.34 mW/m^3 (100 ohm). By incorporating MFC technologies into aerobic biosensors, the unique concept of OA-MFCs will showcase the possibilities for emerging technologies and will move aerobic microbiota to aerobic MFC-based sensors. Mangrove-rich halotolerant microorganisms are rarely investigated for their OA-MFC potential, making our research fascinating.

Keywords- *Halo-tolerant, OA-MFC, Mangroves, Protease, Crude sources, Gram flour.*

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INTRODUCTION

Mangrove ecosystems have evidence of highly diverse and rare microbiota. Research in mangrove-associated land has always drawn the attention of many diverse fields of scientists. Isolates obtained from them and their by-products have fascinated many research groups. Extremophile organisms are rare bugs with fantastic behavioural mechanisms, and their products are in demand in many industries. Grand View Research predicts a +$9.2 billion increase in the global enzymes market till 2027 (Report ID: 978-1-68038-844-2). Bacterial-origin proteases have been the subject of extensive study since the inception of enzymology. Microbial proteases are extensively applied in biotechnological fields due to their outstanding productivity, low timing and space requirements, potential for genome modification, and cost-effectiveness. The structural configuration of microbe proteolytic enzymes was characterised by a relatively smaller size and a dense form [1]. Various agro-industrial waste materials, namely wheat bran, chickpea (Gram flour), red gram, green gram, and black gram, were tested for producing protease by *Bacillus sp*. The results indicated that the highest enzyme production was recordedwith green gram, while the lowest protease production was observed with red gram [2]. The scientific community worldwide has shown significant interest in the potential of Microbial Fuel Cells (MFC) as devices for processing waste into energy. The application of MFC has demonstrated its capability to serve a dual function of generating electrical energy concomitantly with the treatment of waste. The phenomenon of conductivity has been found to exhibit a direct correlation with the ionic strength of the electrolyte in microbial fuel cells (MFCs). This correlation, in turn, leads to a drop in the internal resistance of the system, thereby resulting in an increase in the power produced by the MFC. The production of power in a microbial fuel cell (MFC) was contingent upon various factors, including the distance between electrodes, the ionic strength of the electrolyte, and the ambient temperature. The presence of salinity in waste has been observed to facilitate power production in microbial fuel cells (MFCs), thereby conferring an advantageous effect. This effect was reliant upon the availability of an appropriate consortium of productive bacteria that are capable of degrading organic matter from waste water [3]. The scientific investigation of applying microbes as the

catalyst in fuel cells, known as Microbial Fuel Cells (MFC), dates back to the 1970s [4]. The initial demonstration of microbial fuel cells for the treatment of domestic wastewater occurred in the year 1991. MFC via improved outputs of electricity have been developed only recently, presenting potential prospects for real-world use. The biologically produced convertible substrate undergoes transformation via MFC into electrical power. This phenomenon occurs as a result of bacterial biological switching from a naturally occurring electron acceptor, such as oxygen or nitrate, to an insoluble acceptor, such as an MFC-anode [5-8]. The current investigation highlights the application of a crude source, *Cicer arietinum L.* (Gram flour) as the only source of nitrogen as well as carbon for the production of an industrially important protease from the *Priestia megaterium* strain BorS17B13 in the mangrove-ecosystem of Monari Creek, Borivali, Maharashtra, India. Isolate was subjected to further analysis to determine electricity production using the novel idea of an aerobic anode chamber MFC, whichwas also referred to as Oxi Anodic Microbial Fuel Cell (OA-MFC). A newly developed technology, OA-MFC, expands the possible possibilities for MFCs beyond the confines of anaerobic microbes. The application of this method in the future could contribute to the discovery of a new horizon for "Aerated anodic biosensors".

MATERIAL AND METHODS

Enrichment and Isolation

Samples were collected from mangrove forests and Monari Creek, Borivali (19.2179° N, 72.8087° E), Maharashtra. The sampling type was "Random sampling". Soil (1g w/v), water (1 ml v/v), and root (1 g w/v) samples were collected and processed by directly inoculating samples in enrichment media. All the samples were incubated for 24hr on a rotatry shaker (180 rpm) at 37˚C. One loop of culture from enrichment broth was streaked on the Complete Medium Plate (glucose, 1%; K_2HPO_4, 0.5% ; yeast extract, 0.5%; peptone, 0.5% and Agar-agar 3 % (w/v). Enrichment broth was prepared with two NaCl concentrations (10% and 20%) for each sample, 7 pH of the medium was adjusted by separately autoclaved Na_2Co_3 (20%, w/v).

All the isolates were screened for industrially important enzymes like; protease, amylase, cellulase [9]. From the isolates, optimum protease was further identified ***on the basis of 16s rRNA gene sequencing.***

Growth and Enzyme kinetics (submerged flask fermentation) with gram flour

Isolate, BorS17B13 was grown in medium contains gram flour as sole source of nitrogen and carbon with minimal salt (KH_2PO_4, 0.5% w/v, K_2HPO_4, 0.5% w/v and NaCl, 0.25% w/v) and separately autoclaved Na_2Co_3 to adjusting pH (pH 7). Active culture of 9% was inoculated in the broth and incubated on rotator shaker (180rpm) at 37˚C. At predetermined time intervals, growth (600nm) and protease enzyme activity (280nm) was performed. Enzyme activity was carried by Anson-Hagihara's method (casein as substrate). Protease enzyme activity was calculated using tyrosine as standard, where one unit of enzyme activity was equal to amount of enzyme releasing 1µg of std. tyrosine per minute under assay condition [10-11].

Media optimization

Optimization of was performed by One Variable At a Time (OVAT) method. Primary sources (Carbon, Nitrogen, Cations) required for microbial growth were optimized. Other important parameters like; Salt concentration, Inoculum size, pH was also optimized. Optimization was carried by submerged fermentation in flask and growth (UV-spectrometer at 600nm) and enzyme activity (UV-spectrometer at 280nm) was carried for the parameters. Crude enzyme was used for scrutinizing protease activity [12].

Oxygenated Anode MFC (OA-MFC) Setup

Oxy Anodic-MFC was designed specifically for applications incorporating aerobic microbes into MFCs. The MFC configuration was based on the double chamber model of MFCs (Figures 1 and 2), in which the anode compartment comprised bacterial broth and the cathode compartment contained the electrolytes potassium ferricyanide; 16.46 g/l, and Di-hydrogen potassium phosphate; 2.71 g/l and water (v/v) as per the experimental requirement. One of the electrodes was an anodic other was a cathodic electrode. In both instances, the electrode material consisted of aluminium-mesh. Crocodile pins with wires were attached to each of the electrodes in a closed-circuit setup. To make it easier to measure current and voltage, electrons were collected on a strip of aluminium (1 ohm resistance). Proton Exchange Membrane (PEM) was used inthe dual-chamber OA-MFC, and PEM was made up of ceramic clay material [13]

In a circuit, electrons and protons migrate from the anode to the cathode via wire and PEM, respectively, releasing a water molecule and producing a current surge. As the microorganisms used were aerobic in nature, oxygen was provided to the anode chamber using OA-MFC (Oxy Anodic-MFC). The cathode in this configuration received oxygen in a complete closed circuit. The impeller, which was regulated from the top of the setup, served the dual purpose of homogenizing and dissolving oxygen in anodic chamber for simple availability of oxygen to aerobic microbes in the OA-MFC, and mixing medium broth so that bacteria get

sufficient motion to utilise medium. There was a pipe for sampling, a impeller (100 rpm) and an aerating pipe that included a bacterial filter to evacuate the pressure built up in the device during the growth of bacteria. The voltage was measured in an Open Circuit Voltage (OCV) condition. OCV is when no external resistance was supplied to the circuit and a direct connection was monitored to measure voltage. In the experiment, Short Circuit Current (SCC) was measured when a very low external resistor, in this example, a 10 ohm resistor, was put in series with the circuit. The maximum output of the circuit was calculated with respect to time. External resistors (47 ohm and 100 ohm) were used to measure current and voltage in close circuit in series and parallel, respectively.

RESULTS AND DISCUSSION

Isolation

The samples, comprising soil, water, and root samples, were procured from the vicinity of Borivali, Monari Creek, Maharashtra, located in the western region of India, which was known for its abundant mangrove vegetation. A total of 62 isolates were collected from 30 samples that were isolated through the enrichment method. The isolates were screened for the presence of enzymes that have industrial significance, such as protease, amylase, and cellulase. Protease enzyme stays stable for longer time in crude enzyme form so was selected for further research. From the isolates, optimum protease producer, BorS17B13 was selected for further research [14, 15, 16. Selected isolate, BorS17B13 was screened on the basis of 16s rRNA gene sequencing and was found to be closely related to *Priestia aryabhattai* strain. This isolate's 16s rRNA gene sequence was submitted at NCBI (Accession No. OM743775, *Priestia megaterium* B21). The isolate, BorS17B13 a halotolerant, gram positive and protease producer was assessed for MFC potential (Electricity production/Power generation).

Growth and Enzyme kinetics using gram flour (Crude source)

Growth and enzyme kinetics for *Priestia megaterium* strain BorS17B13 using gram flour (Figure 1) as a crude source. Growth and protease activity were increasing with time. Protease activity increased to 375 U/ml at 24 hr, 396 U/ml at 48 hr, and at the end of 72 hr, enzyme activity was 423 U/ml. Prakasham et. al., established that production of alkaline protease by a *Bacillus sp*. was impacted by the physiological and chemical properties of the gram husk [15].

This production was associated with the growth of the bacteria strain. The investigation revealed that the optimal enzyme yields was attained by controlling the size of the particles of the solid matrix [15]. An alkaline protease by *Bacillus horikoshii* using soyabean as crude source [18]. A research supporting our study, found that the growth of *Bacillus mojavensis* A21, protease production wasincreased using gram flour/chick pea flour [17]

Patel et al., reported 183 U/ml protease activity while growing the organism on wheat flour and molasses [13]. In comparison to these data, our isolate produced 423 U/ml protease activity with the crude source ***Cicer arietinum L.*** (gram flour) that was significantly high and also proposed the enzyme having great commercially viability.

OA-MFC; Novel biosensor approach

The application of Oxi-Anodic Microbial Fuel Cell (OA-MFC) represents a novel and modern methodology for expanding the scope of Microbial Fuel Cell (MFC) applications, transitioning from anaerobic to aerobic bacterial environments. A significant number of aerobic bacteria have been utilised for various applications pertaining to human welfare. The theoretical framework of OA-MFCs was established through the prospective application of aerobic microorganisms as biosensors through the implementation of MFC methodologies. The term "Oxi" denotes the presence of oxygen, a vital element for the survival of aerobic microbes. A proton exchange membrane (PEM) was employed in conjunction with a two-chamber MFC as depicted in figures (Figure 2 and Figure 3). Similar to our study, in a research by Gajda et al., they claim to use low cost ceramic based MFC, they worked on electro osmotic drag where catholyte received as by product of power/electricity production [2]. The anodic chamber contained a culture of bacteria, while the cathodic chamber was filled with electrolyte solution. Electrons were observed to undergo a transfer from the anode to the cathode, thereby achieving a closed circuit. Hydrogen ions in theform of protons (H+) were observed to exit the inner anodic chamber through the proton exchange membrane. External resistors, denoted as R_{ex}, were employed to measure the current in a closed circuit; alternatively, load can be used to complete the close circuit.

Numerous investigations have been conducted in this particular domain, encompassing the impact of heightened salinity/conductivity on the power generation capabilities of microbial fuel cells (MFCs). In the majority of these investigations, freshwater bacteria were utilised and a positive correlation was discovered between power output and conductivity [24].

Correlation of OCV verse SCC with respect to time

OCV and SCC both parameters were frequently used for measuring the potential of current and voltage from the MFC setup. At the end of the experiment (9 hr) of the OA-MFC (Figure 4) growth was 0.7 O.D. andOptimum OCV and SCC during the experiment were 678 mV and 2.77 Am^{-3} respectively. Similar to our experiment in a research six different type of MFC were used from them one group (three MFCs) were operated in OCV mode as in our experiment. The OCV group together obtained about 750mV [2]. These results were close to our readings but in contrast to that we obtained these with single MFC unit.

Voltage verse Current correlation with time for Rex 47 ohm and Rex 100 ohm

The current and voltage in the experimental MFC increased over time (Figure 5). To complete close circuit Rex of 47 ohm was connected. Initial hour (1hr) voltage was 101 mV and the current was 1.95 Am^{-3} and at the end of 9^{th} hr, the voltage output 67 mV, and the current was 1.13 Am^{-3}. Optimum voltage and current were obtained at 2^{nd} hr of the experiment, which were 107 mV and 2.07 Am^{-3} respectively. To complete close circuit Rex of 100 ohm was connected in a series and was parallel for current and voltage measurement (Figure 6). While initially voltage output was 162 mV and the current output, 1.48 Am^{-3}, by the end of 9^{th} hr, the current was 1.02 Am^{-3} and the voltage output was 102 mV. Optimum voltage was 169 mV and Optimum current was 1.86 Am^{-3} for the setup. In the MFC experiments many times close circuit system was used, were in different range of resistors are used according to the internal resistance of the circuit and selection Rex was also on the base of overcoming the losses for desired output [18-22]. Polarization study was carried in manyMFC experiments with varied Rex [22, 23]. In a research, author used 100 ohm external resistor as in our experiments. They have use Rex to electro-chemically characterise the electrodes half cell with help of linear sweep voltammeter [2].

Max. power production with Rex 47 ohm and Rex 100 ohm

Maximum power peak was calculated by plotting Power (mW) Vs Current Vs Time (Hr) (Figure 7). Maximum power peak (221 mW) was observed at 2^{nd} hr of the OA-MFC. From the figure (Figure 8), maximum power peak was calculated by plotting Power (mW) Vs Current Vs Time (Hr) graph. Maximum power peak (259 mW) was observed at 2^{nd} hr of the OA-MFC. These findings support our research, where we obtained power production using gram flour (Figure 4-8). In addition, our MFC unit also produced protease enzyme inside the anode chamber as the (Isolate- BorS17B13) could utilize Gram flour (Figure 1) medium for protease production. In the figure (Figure 9) correlation between volumetric power density and voltage, were shown for 100 ohm resistance. Volumetric power density was defined with respect to the volume of the anode compartment (bacterial broth), which was 750 millilitres in our case. As voltage increased, volumetric power density also increased, and both reached a peak at the 2^{nd} hr of the experiment then slowly dropped at end of 9 hrs. Optimum volumetric power density was 0.34 mW/m^3. MFC potential can be defined by different values like maxium power produced Power density (Size of electrode) and volumetric power density (Volume of anode). In a study by freguia et al., showed significant stable currentwas obtained by using non catalyzed cathode. Power produced was 21 W/m^3 [1].

Voltage drop with external resistor

The change in voltage with respect to time and external Load/Resistor (Rex) was studied (Figure 10). Difference between the OCV, R_{ex} 100 Ohm and R_{ex} 47 Ohm displayed a voltage drop after applied load. Voltage drop was greater in the 47 ohm close circuit then 100 ohm close circuit, but interestingly current (2.07 Am^{-3}) was greater with 47 ohm close circuit then 100 ohm (1.86 Am^{-3}) close circuit. A study supporting our results by show drops in current and voltage with change in resistance [25, 26].

The implementation of ceramic PEM in air-anode MFCs has been proposed as a means of enhancing their efficacy in practical applications such as energy recuperation. The results also support our MFC model, which utilises a ceramic proton exchange membrane. Additionally, this study serves as an illustration of the potential of MFCs in terms of measuring power and transforming it into electrical signal impulses for aerobic biosensor applications.

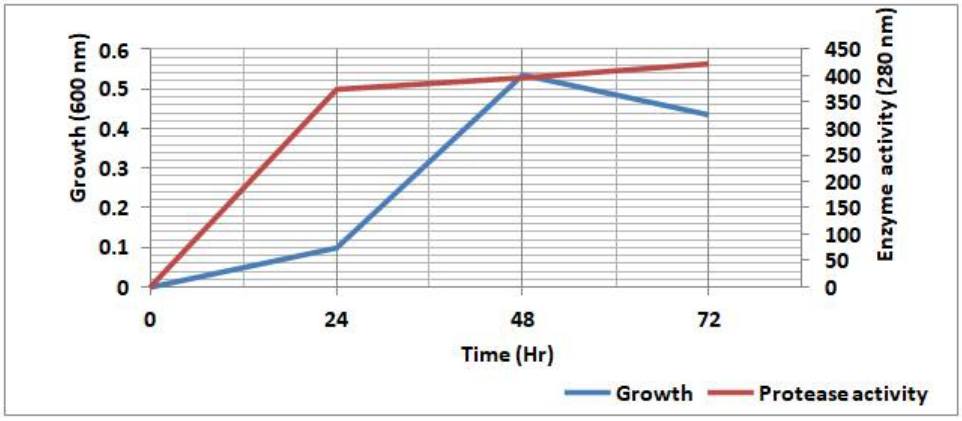

Figure 1. Growth and Enzyme kinetics of BorS17B13 using gram flour

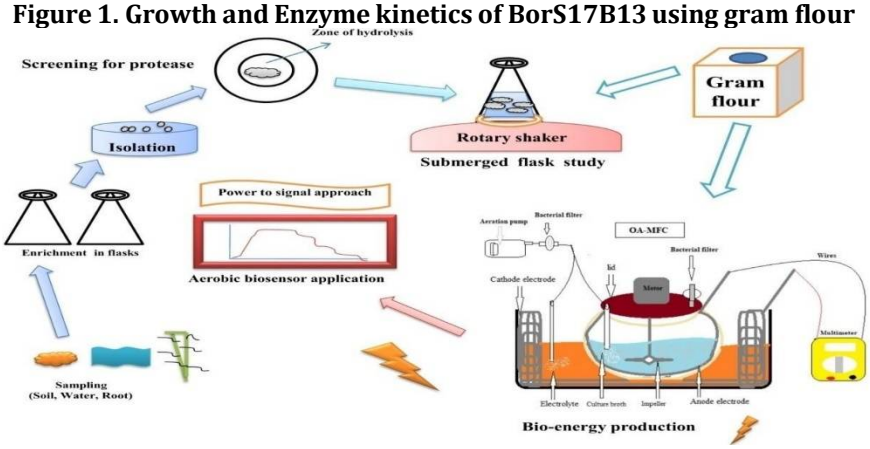

Figure 2. Oxy Anodic MFC (OA-MFC) Sketchmatic Flow

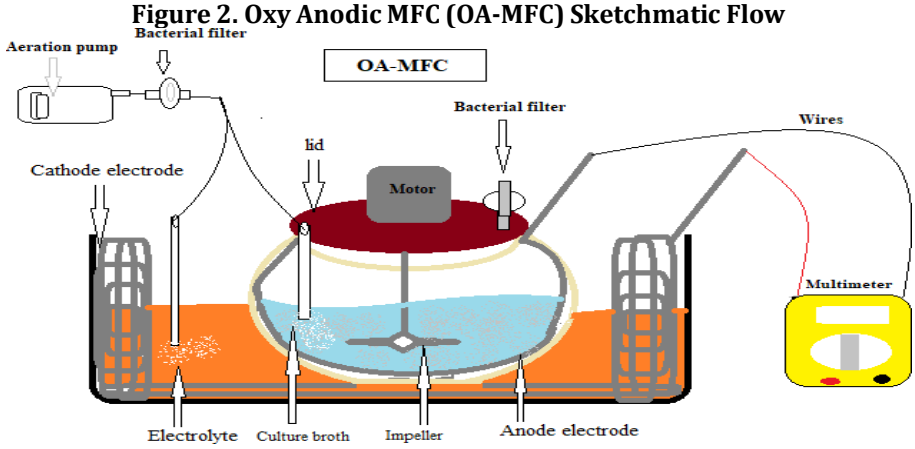

Figure 3. Oxy Anodic-MFC (OA-MFC)

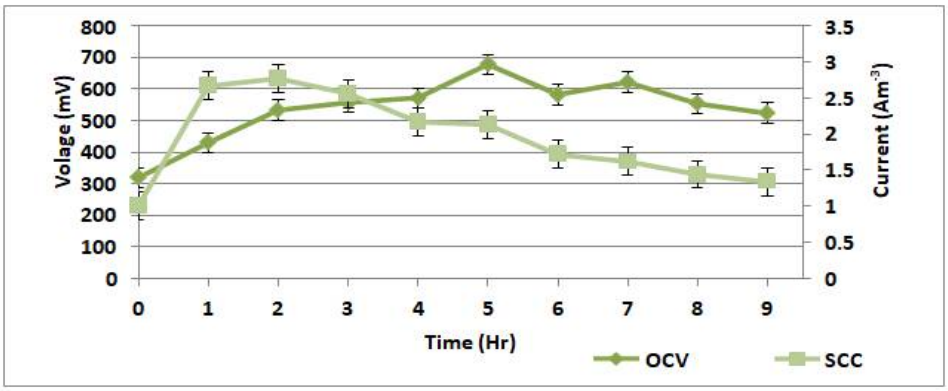

Figure 4. Open Circuit Voltage (OCV) Vs Short Circuit Current (SCC)

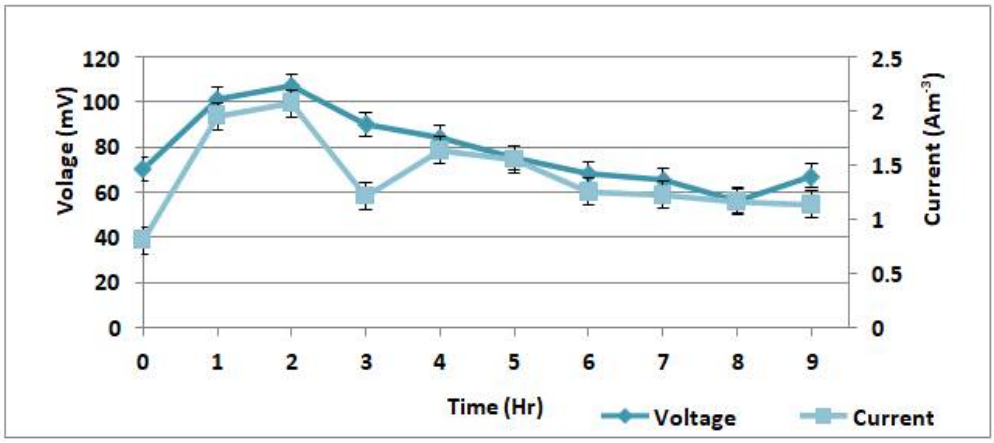

Figure 5. Voltage (mV) Vs Current (Am^{-3}) with Rex 47 Ohm

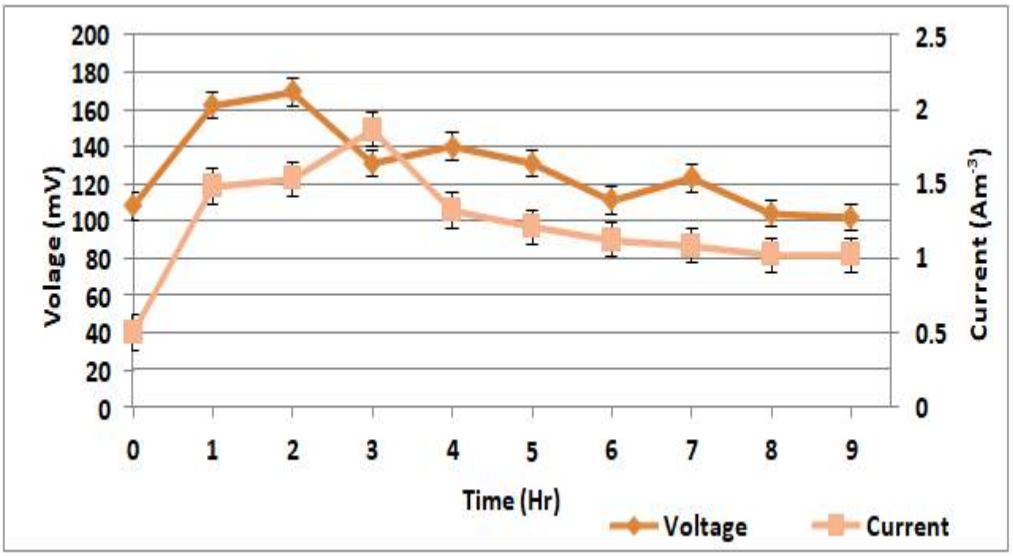

Figure 6. Voltage (mV) Vs Current (Am^{-3}) with Rex 100 Ohm

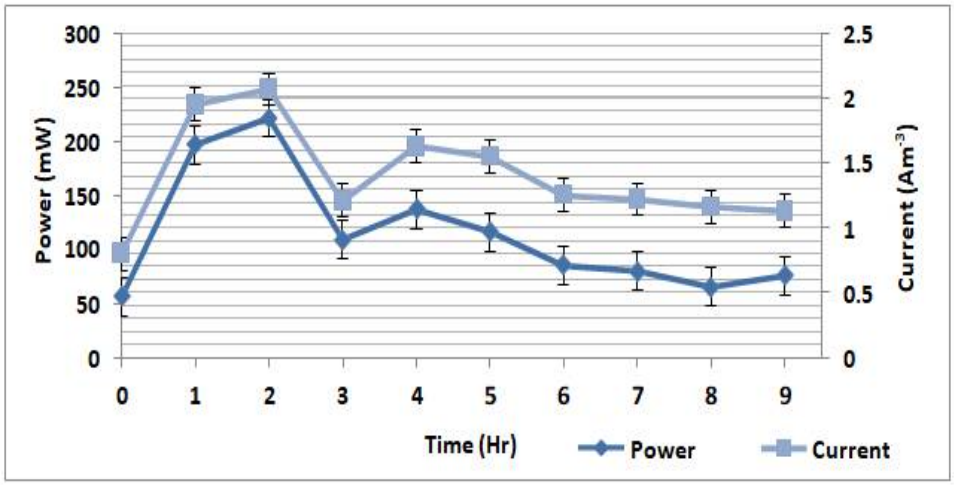

Figure 7. Power (mW) Vs Current (Am^{-3}) for Rex 47 Ohm

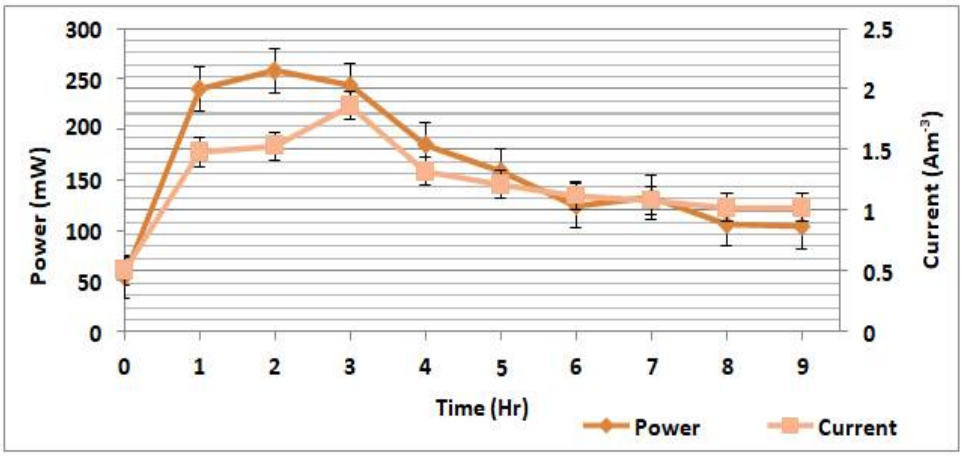

Figure 8. Power (mW) Vs Current (Am^{-3}) for Rex 100 Ohm

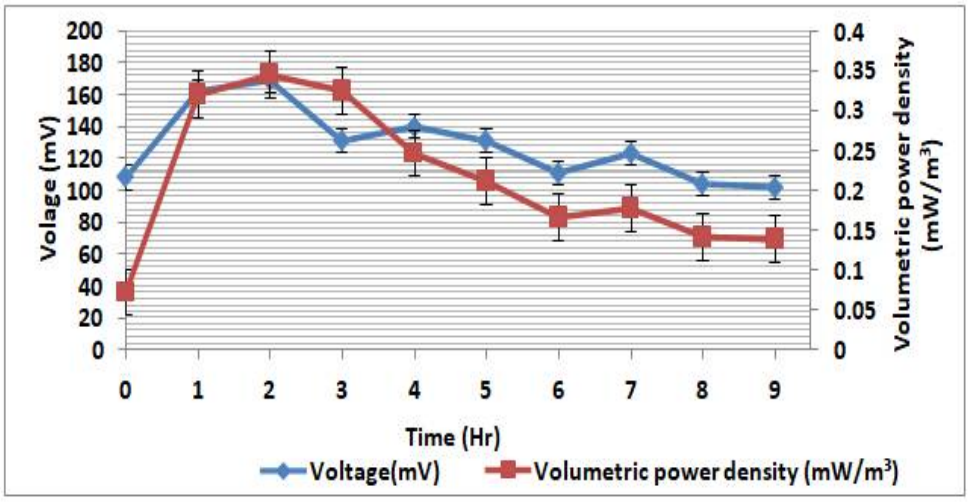

Figure 9. Voltage (mV) Vs Volumetric power density (mW/m^3) with Rex 100 Ohm

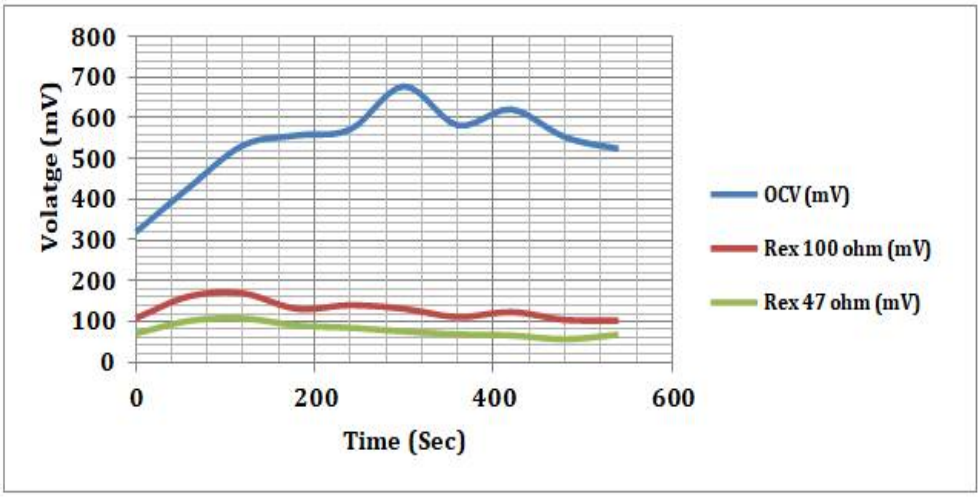

Figure 10. Voltage (mV) Vs Time (s)

CONCLUSION

The biota abundant in mangroves, was used for the purpose of isolating bacteria that were halotolerant or halophilic. These bacteria were extensively examined for their ability to produce protease enzyme from an economical source such as gram flour. Further, a cutting-edge idea regarding the application of an oxygen-rich anodic chamber within a microbial fuel cell to produce electricity through aerobic bacteria

has the potential to revolutionise the field of biosensors, leading to the development of an innovative concept known as the "Aerobic-Biosensor" in the upcoming years. The empirical evidence presented in our study supports the feasibility of utilising varied communities of microbes in biotechnological applications. Furthermore, our findings highlight the potential benefits of combining multiple technologies to achieve beneficial synergistic effects. The application of Oxi-Anodic Microbial Fuel Cells (OA-MFCs) poised to revolutionize the field of microbial fuel cell (MFC) technology by enabling the expansion of MFC applications to include aerobic bacteria, in addition to anaerobic bacteria. A plethora of aerobic bacteria are employed for diverseapplications in the realm of human well-being. The utilisation of aerobic bacteria, which were obtained from geographically uncommon mangrove swamps, as biosensors through the implementation of MFC techniques served as the fundamental concept behind OA-MFCs, which seeks to draw inspiration from future advancements.

CONFLICT OF INTEREST

Author (Sawant and Thumar) declares no conflict of interest.

COMPETING INTERESTS

There are no financial or non-financial conflicts related to the submitted work.

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