BIOELECTROCHEMICAL BEHAVIOR OF WILD TYPE BACILLUS CEREUS IN DUAL CHAMBER MICROBIAL FUEL CELL

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ABSTRACT: A microbial fuel cell (MFC) is a bioelectrochemical system that uses living microbes as biocatalyst to oxidize organic substrates as well as release electrons that can be harvested in an external circuit to produce electrical energy. In this study, a proteolytic biocatalyst, *Bacillus cereus,* has been employed for the first time in a microbial fuel cell (MFC). The wild type pure culture was isolated from municipal wastewater and identified using Biolog Gen III analysis. The MFCs were fueled with palm oil mill effluent (POME) and attained a maximum power density of about 3.88 W/m^3. The electrochemical behavior of the MFC was evaluated using a polarization curve, electrochemical impedance spectroscopy (EIS), and cyclic voltammetery (CV) analysis. The CV and EIS results suggest that the predominant electron transfer occurred through the electron shuttle mechanism. The electron shuttle mediators excreted by *B. cereus* significantly reduced the anode charge transfer resistance (52.95%). The FESEM result shows that *B. cereus* has the capability to form an effective biofilm on the anode electrode surface. These results revealed the electrocatalytic potentiality of *B. cereus,* making it a promising candidate to be used in MFCs. Therefore, this biocatalyst can be used to generate electricity through wastewater valorization.

ABSTRAK: Sel bahan api mikrob (MFC) adalah sistem bioelektrokimia yang menggunakan mikrob hidup sebagai bio pemangkin untuk mengoksida substrat organik bagi menghasilkan tenaga elektrik dengan membebaskan elektron mengunakan litar tertentu. Kajian ini telah menggunakan bio pemangkin proteolitik *Bacillus cereus* buat pertama kali dalam sel bahan api mikrob (MFC). Kultur asli jenis liar telah diasingkan daripada sisa air kumbahan dan dikenal pasti dengan menggunakan analisis Biolog Gen III. MFC ini telah diberi bahan bakar bersama efluen kilang minyak sawit (POME) dan didapati ketumpatan kuasa maksimum adalah sebanyak 3.88 W/m^3. Tindak balas elektrokimia MFC ini dinilai menggunakan analisis lengkung polarisasi, Elektrokimia Impedan Spektroskopi (EIS) dan kitaran voltammetri (CV). Keputusan CV dan EIS menunjukkan dominasi pemindahan elektron telah berlaku menerusi mekanisme pemindahan elektron. Pengantara pemindahan elektron ini yang terhasil daripada *B. cereus* telah menurunkan rintangan pemindahan cas anod dengan ketara (52.95%). Hasil FESEM menunjukkan bahawa *B. cereus* mempunyai keupayaan membentuk biofilem yang berkesan pada permukaan elektrod anod. Keputusan ini menunjukkan potensi

elektrokatalitik *B. cereus* sebagai calon terbaik dalam MFC. Ini kerana bio pemangkin ini boleh digunakan untuk menjana tenaga elektrik melalui volarisasi air kumbahan.

KEYWORDS: *microbial fuel cell; Bacillus cereus; biofilm; palm oil mill effluent; cyclic voltammetry; electron transfer mechanism*

1. INTRODUCTION

The microbial fuel cell (MFC) is an important type of bioenergy source for the future because it can use a wide variety of substrates, including soluble or dissolved complex organic wastes and various types of artificial or real wastewater [1]. In MFCs, the use of pure cultures may not be suitable due to high costs and its inefficiency to utilize the complex substrates; therefore, it is not efficient for practical operation such as treatment of industrial effluents. On the other hand, mixed cultures from natural sources such as soil, wastewater, and anaerobic sludge (AS) have been widely used as inoculum in MFCs since they are more readily obtainable in large quantities, more tolerant to environmental fluctuations, and more accommodating to a variety of substrates [2, 3]. However, AS could not achieve a significant performance because of the presence of non-electrogenic bacteria (i.e., methanogenic bacteria and denitrifying bacteria) in mixed cultures that consume organic substrates without generating electricity [4]. Therefore, this problem can be solved using wild type pure culture bacteria that can utilize a wide variety of substrates as inoculum in MFCs.

In the past decade, a number of new strains of bacteria have been reported to be capable of generating electrical current through MFCs. Although many new strains have been identified, only a few strains are capable of producing significant power using complex wastewater as a substrate [5, 6]. One of them is *Bacillus cereus.* It is a Gram-positive, soil-native bacteria, widespread in different habitats around the world. Some studies showed that Gram-positive bacteria especially *Bacillus spp.* increased current generation through the redox shuttles in MFC [7, 8]. Besides that, it has the capability to degrade toxic waste compounds and efficiently utilize them as a carbon sources [9, 10]. These degrading skills shown by *B. cereus* could be improved if it grows in adverse environmental conditions [11, 12]. Therefore, *Bacillus cereus* can be considered as a potential biocatalysts to be used in MFC for simultaneous power generation and wastewater treatment.

Bacteria are, so far, known to transfer electrons to anode surfaces via two mechanisms. The first is a biofilm mechanism, where exoelectrogens form a biofilm on the anode surface. Some microorganisms can even produce nanowires carrying electrons from the cell to anode surface. The second is the electron-shuttle mechanism, where the microorganism itself can produce and use low molecular weight compounds, soluble mediators (e.g. phenazine, quinones) that eliminate the need for direct contact between the cell, and electron acceptors [13]. The understanding of electron shuttles in these electron-generating processes is emergent to effectively harvest the electricity from MFCs. A pure exoelectrogen strain is required for studying the mechanism of electricity production in MFC. Currently, quite a number of pure exoelectrogens are demonstrated to have electrochemical activity, such as *Geobacter* and *Shewane1la* [14]. However, only a few strains were capable of producing electron shuttles, such as pyocyanin by *Pseudomonas aeruginosa* and riboflavin by *S. oneidensis* MR-1 [15].

The purpose of this study is to investigate the electrochemical activity as well as electron transfer mechanism of wild type *B. cereus* in microbial fuel cells. The

electrochemical activity was evaluated using the polarization measurement, biofilm formation capability, electrochemical impedance spectroscopy and cyclic voltammetry analysis.

2. MATERIALS AND METHODS

2.1 Source of Microorganism and Sample Collection

Palm oil mill effluent (POME) samples were obtained from a local palm oil mill industry located in Pahang, Malaysia. Municipal wastewater was collected in a sterilized bottle from a drainage discharge point in Kuantan, Pahang, Malaysia. All samples were stored at 4 ^{o}C to minimize the degradation of samples by indigenous microbial activities, thus preserving the quality of the samples.

2.2 Isolation of Microorganism

The source of microorganisms was municipal wastewater. Enrichment of the cultures was carried out by preparing an overnight culture in LB broth (10% v/v) incubated at 35^{o} C with shaking at 150 rpm. The overnight culture (10% v/v) was used as primary inoculum in the anode compartment. After 13 days of MFC batch operation, the anode was taken out and placed in 0.1 M phosphate buffer and shaken vigorously to detach bacteria that grew as biofilm on the anode. The side of the anode was also carefully scraped and resuspended in phosphate buffer. The bacterial suspension was serially diluted and the pure culture bacteria were obtained using the spread plate technique. The predominant colony was identified using Biolog GEN III, specifically *B. cereus*.

2.3 MFC Configuration

The dual chamber MFC was fabricated with a Plexiglas cube with a dimension of 5 cm x 5 cm x 5 cm (Shanghai, Sunny Scientific, China) and a total working volume of 25 mL. A carbon brush was used as an electrode in all experiments. Nafion 117 membrane was used to separate the anode and cathode compartments of MFC (Dupont Co., USA). Prior to use, the Nafion membrane was pretreated using diluted H_2SO_4 for 1 hour followed by washing with DI (de-ionized) water several times. After the whole MFC set up was tightened up with screws, the anode compartment was filled with sterilized 50% POME (20mL) and subsequently the pure culture bacteria (1mL) were inoculated into it while the cathode chamber was filled with $KMnO_4$ solution. The anode chamber was purged with N_2 for 30 min and then tightly closed to maintain the anaerobic condition. The whole experiment was conducted in batch mode. The anode and cathode electrodes were connected using titanium wires with a rheostat (Crotech DRB-9, UK) to form a circuit.

2.4 FESEM Analysis

Field emission scanning electron microscopy (FESEM) was used to observe the microorganisms grown on the electrode material. A small portion of the electrodes was cut off for FESEM observation. The electrode sample was immediately immersed in 3% glutaraldehyde and 0.1 M phosphate buffer solution and then dehydrated with increasing ethanol concentration (30–100%) [16]. Thereafter, samples were freeze dried and sputter coated with a thin layer of platinum under vacuum to neutralize the charging effects prior to scanning in the FESEM. The morphology was observed under a scanning electron microscope (Hitachi, Japan, Model: 3400N) with an acceleration voltage of 5 kV during the scanning.

2.5 Measurement and Analyses

The voltage (V) and current (I) across an external resistor (1 kX) in the MFC circuit was monitored using a digital multimeter with data logger (Fluke 289 True RMS Multimeter, USA) connected to a computer through a USB cable adapter. To obtain polarization data, the external resistance was varied from 50 to 20,000 X. Power density normalized by surface area (P_A, W/m^3) was measured and calculated using the following equations.

$$P_A = UI \tag{1}$$

$$P_v = R_v I^2/V \tag{2}$$

where V is the volume of the MFC (m^3), U is the voltage of the cell (V) and R_v is the external resistance (Ω), and I is the current (A).

2.6 EIS and CV Analysis

Electrochemical impedance spectroscopy (EIS) was carried out using an electrochemical workstation (AUTOLAB 2273, PAR, USA). A conventional three-electrode system was employed with the anode as the working electrode, the cathode as the counter electrode, and an Ag/AgCl reference electrode that was placed as close as possible to anode electrode [17]. The impedance data were fitted to the equivalent electrical circuit to obtain key parameters such as the electrochemical charge transfer resistance for the anode. The Nyquist plots of the impedance spectra were analyzed using NOVA 1.9 (NOVA Software). The CV was run at a scan rate of 30 mV/s with a scan range of -1.0 to +1.0 V versus Ag/Agl and the voltammogram was compared for different days in identical configuration and operational conditions.

3. RESULTS AND DISCUSSIONS

3.1 Performance of MFC

The performance of MFC fed with 50% of POME (COD=18,260 mg/l) and inoculated with 1 ml of liquid culture was investigated. The MFC performance was recorded for 13 days, as shown in Fig 1.

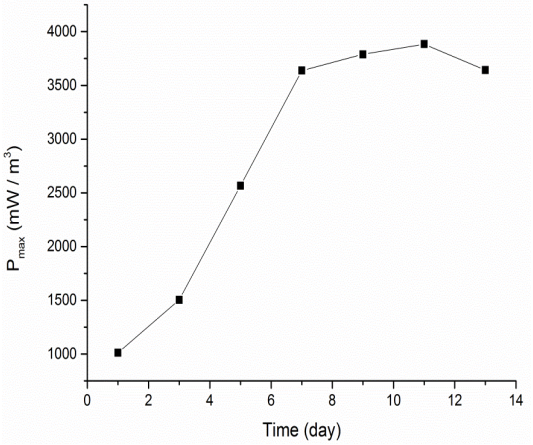

Fig. 1: Maximum power densities of the MFC versus time.

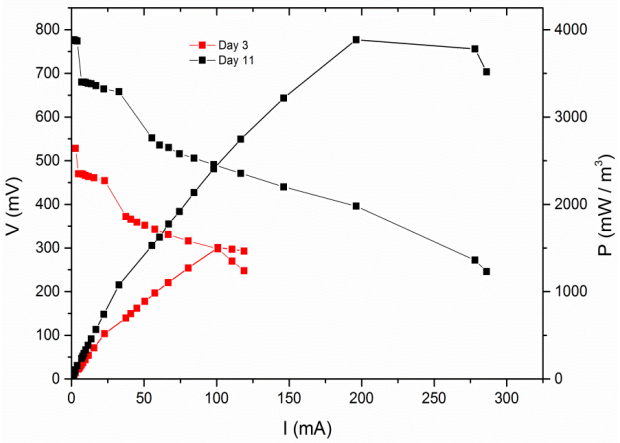

Fig. 2: Polarization curves of the MFC on the 3rd and 11th day using *B. cereus*.

The performance of the MFC gradually increased until 7 days of operation and reached maximum power density of 3.52 mW/m^3. After 7 days of operation, it showed that stable power generation was achieved for a few days. Maximum performance of 3,88 mW/m^3 achieved on the 11^{th} day and started decreasing after 11 days of operation. Our previous study [12] showed that *B. cereus* possesses electrogenic properties and could form an effective biofilm. Moreover, usually Gram-positive bacteria develop viable biofilm compared to gram negative bacteria and that could also be a reason for achieving a higher performance in MFC_3 [7]. However, the drastic decrease in power generation could be due to the excessive bacterial colonization on the anode surface, which might have limited the substrate access to the inner layer of the biofilm [18]. Hence, it reduced the number of viable cells in the inner layer which in turn declined the power generation. The polarization curve for the 3^{rd} day and 11^{th} day has been shown in Fig. 2. As can be seen from the Fig. 2, the power generation on 11^{th} day was around 3 times higher than the 3^{rd} day. The higher performance on 11^{th} day was attributed to the formation of an effective biofilm on the anode surface that resulted in higher kinetics of the bio-electrochemical reaction on the surface of the MFC anode [19].

3.2 Biofilm Analysis

The formation of a biofilm is vital for the MFC because it significantly influences the electron transfer through the extracellular polymeric substances [20]. FESEM analysis was done to visualize the biofilm on the anode electrode surface before and after operation (on the 3^{rd} and 11^{th} days) of the MFC, and the results have been presented in Fig. 3a and 3b. On day 3, as shown in Fig 3a, less bacterial colonization was scattered around the electrode and loosely associated microbial clumps were observed in some locations on the electrode surface. However, on day 11, it can be seen that the significant bacterial cells, that colonized the electrode surface and formed the mature biofilm, are embedded in a self-produced matrix of extracellular polymeric substance (EPS) (Fig. 3b). Hence maximum performance was achieved by the MFC. Read et al. [7] reported that a biofilm is a crucial component of the MFC that allows considerable conversion capacity and opportunities for extracellular electron transfer. Moreover, the cells attached on the electrode surface need less of a diffusion path for the transport of electrons from the microbes to the electrode [21]. The results suggest that an effective biofilm, comprising of higher active microorganisms might have produced more redox compounds that enhanced the electron transfer efficiency as well as the performance of the MFC.

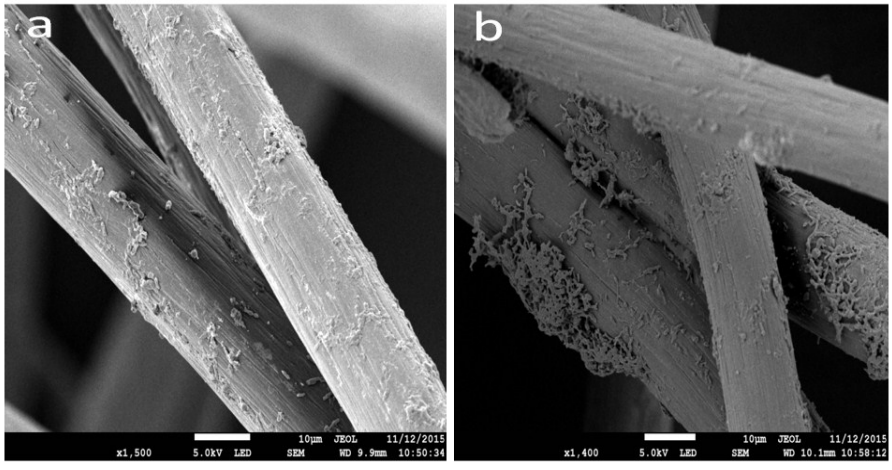

Fig. 3: FESEM images on anode carbon brush in the MFC fed with POME as substrate, (a) day 3 and (b) day 11.

3.3 Electrochemical Impedance Analysis

Nyquist plots for the anode configuration on different days of operation are shown in Fig. 4. The measurement of EIS spectra for the anode demonstrates the key information of resistance created by electrode and bacterial metabolism. The impedance at the high frequency region is attributed to the ohmic resistance and the diameter of the semicircle represents the polarization resistance (or charge transfer resistance), which is affected by the kinetics of electrode reactions [22]. The charge transfer resistance (R_{ct}) values using *B. cereus* on the 3rd and 11th day were found to be 89.7 Ω, and 42.37 Ω, respectively. The higher R_{ct} using *B. cereus* (89.7Ω) on the 3rd day might be due to the absence of mature biofilm on electrode surface (Fig. 3a). However, on the 11th day, the R_{ct} was reduced by 52.95% (42.37 Ω) indicating that the *B. cereus* formed an effective biofilm on the anode. Thus, it decreased the anode activation losses by enhancing the kinetics of the bio-electrochemical reactions [19]. These results revealed that the effective biofilm is necessary to facilitate the anodic electron transfer as well as power generation in the MFCs.

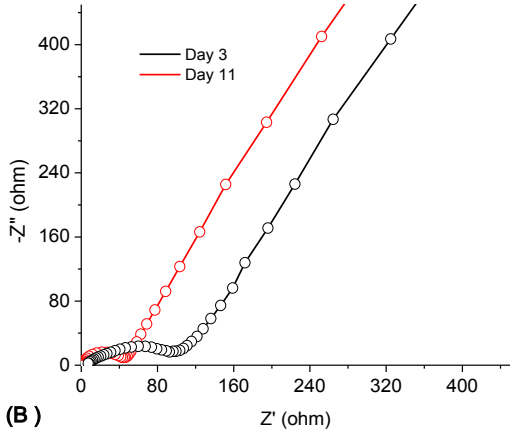

Fig. 4: Nyquist plots of *B. cereus* on the 3rd and 11th day of operations.

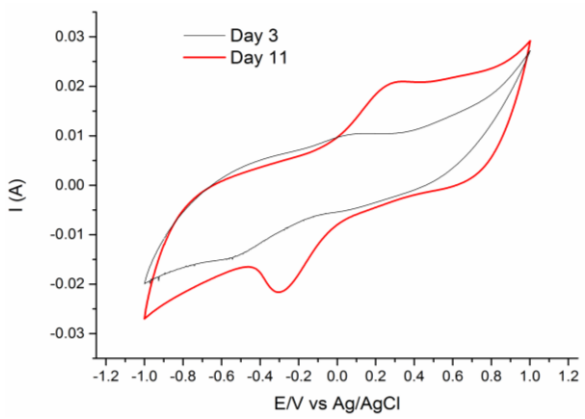

Fig. 5: Cyclic voltammetry curves of *B. cereus* on the 3rd day and 11th day of operations.

3.4 Cyclic Voltammetry Analysis

CV analysis has been extensively used to evaluate the bioelectrocatalytic activity of anodic biofilms and to determine the electron transfer mechanisms [23]. The CV was conducted before inoculation and after inoculation on day 3 and 11, as shown in Fig 5. Marked variations of catalytic behavior between the 3rd and 11th day are clearly observed by CV analysis, as shown in Fig. 5. The observation that the maximum current reached up to 0.23 A on day 11, which is about 2 times higher than that of 3rd day (0.01 A), might be due to the excretion of large amounts of electron shuttle compounds in the anode [24]. Moreover, a reversible CV peak apparently demonstrates the redox transformation of electro chemically active species [21]. The abundance of electron shuttle compounds decreased the charge transfer resistance in the MFC by around 52%. The catalytic measurements demonstrated that the presence of a recyclable electrochemical active compounds produced by *B. cereus* would have mainly contributed to the electricity generation in the MFC [12]. Moreover, the electrochemical activity of *B. cereus* behaved distinctly different during different stages, suggesting that suspension cells were growing or adhering onto the anode to form a biofilm in the MFC [25]. However, further study is

needed in order to identify the redox compound produced by *B. cereus*.

4. CONCLUSION

The outcome of this study suggests that *B. cereus* is an efficient microorganism to be used as inoculum in an MFC. *B. cereus* showed significant power production of about 3.9 W/m^3 using POME as substrate. The reversible CV peak confirmed that *B. cereus* produced electron shuttle compounds that enhanced the electron transfer efficiency. The anode charge transfer resistance decreased by 52%, indicating that the presence of an efficient biofilm formation. The visualization of the biofilm by FESEM further confirms the formation of effective biofilm after 10 days of operation. Further developments can be made by mixing different kinds of microorganisms with *B. cereus* in order to possibly improve the power generation of the MFC.

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