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BIOFILM (BIO-CATALYST) FORMATION ON THE WORKING ELECTRODE IN A MICROBIAL FUEL CELL

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ABSTRACT

A microbial fuel cell (MFC) is a bioelectrochemical reactor in which microorganisms, feeding on organic matter, generate electricity. In such a reactor, microorganisms active on the anode form a biofilm, whose activity is a key factor determining the performance of the MFC. Biofilm also forms in water transfer installations, in substrate transfer installations in biogas plants, etc. However, in such cases such biofilm can be a source of microbiological infections or corrosion. In addition, such biofilm is composed of various microorganisms, not necessarily producing electrons. In the case of biofilm formed on the electrode in MFC, the most important thing is to build a thin layer of biofilm from electron-producing microorganisms.

This paper discusses the theoretical part of biofilm (bio-catalyst) formation and carries out the procedure of building a biofilm on a carbon electrode. It has been shown that to obtain a biofilm capable of generating electricity, at least three start-ups are necessary before the electrode reaches the appropriate operating parameters. After obtaining a ready-to-use electrode with an active biofilm, measurements of electricity generation in the MFC were carried out. The results demonstrated the effectiveness of performing multiple startups to achieve a suitable working electrode with an active biofilm.

Keywords*: biofilm, bio-catalyst, microbial fuel cell, electricity production, renewable energy sources, environmental engineering*

INTRODUCTION

Biofilms can form in various environments and may represent an undesirable phenomenon. One example of such a situation is the biofilm formed in water pipes, where microorganisms from water treatment facilities, as well as those introduced due to failures in the water supply network, play a significant role in the formation of biofilm (Mara and Horan, 2023). But such a situation (biofilm formation) also occurs on technological lines in food production and processing plants (Muhammad *et al.*, 2020). Initially, a chemofilm is created on the inner surfaces of the pipes, consisting of sand particles, glycoproteins, ions, polysaccharides, as well as humic and fulvic acids carried by the water. Subsequently, biofilm develops due to the adhesion of microorganisms. This adhesion to solid substrates results from Brownian

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motion, sedimentation, convective transport, and the motility of biofilm-forming organisms with cilia and fimbriae. The development of biofilm also depends on the type of substrate (Turhan *et al.*, 2020). As water flows over the biofilm, parts of the biofilm layer may be torn off due to cell death or shear forces. Biofilm cells are embedded in a dense matrix composed of extracellular polymeric substances (EPS), which are produced by the microorganisms themselves. This EPS matrix protects the microorganisms and, under certain conditions, makes them better adapted to the external environment than free-floating planktonic cells (Donlan, 2002; Muhammad *et al.*, 2020). Figure 1 illustrates the process of biofilm formation in water installations or on technological lines in food production and processing plants.

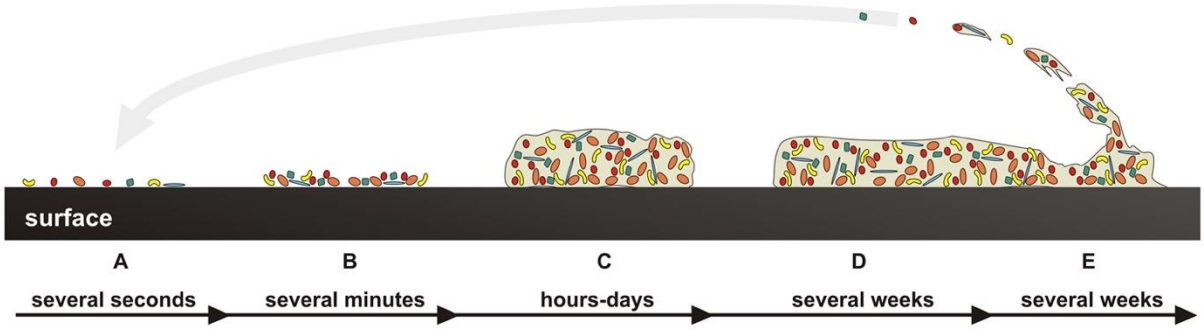

Figure 1. Stages of biofilm formation: A – reversible attachment to the surface, B – irreversible attachment to the surface, C – multiplication and production of EPS and formation of microcolonies, D – maturation, E – dispersion.

More than 95% of microorganisms in water installations are found within biofilms. Such biofilms can often be the cause of drinking water contamination supplied to consumers, as well as a source of microbiological corrosion. Therefore, biofilms in water systems represent a highly undesirable phenomenon, leading to secondary contamination of drinking water and gradual degradation of water installations (Batte *et al.*, 2003; Chowdhury, 2012).

The same situation occurs on technological lines in food production and processing plants. Microbial contamination in these environments can lead to the growth of undesirable microorganisms that spoil food, posing a risk to life and public health (Donlan, 2002). In technological lines, high humidity and the accumulation of organic material on working surfaces, especially in hard-to-reach areas such as tanks, pipelines, centrifuges, and meat processing equipment, create an ideal environment for biofilm formation. The multiplying biofilm cells secrete various enzymes, such as lipases and proteases, which can alter the organoleptic properties of products and cause equipment failures due to corrosion, increased friction between machine components, restricted pipe flow, reduced heat exchange, and other issues. Biofilms produced by bacteria such as *Campylobacter jejuni*, *Escherichia coli*, Listeria monocytogenes, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, and yeasts of the *Candida genus* pose a significant threat to public health. These pathogens can form mature biofilm structures within just a few hours of colonizing surfaces such as synthetic materials,

glass, or stainless steel (Donlan, 2002; Obe *et al.*, 2021). Many biofilm-forming microorganisms carry antibiotic resistance genes, which makes these pathogens even more hazardous to human health.

Although biofilm is most often associated with an undesirable phenomenon, in some cases it is the basis for the functioning of the system. An example of such use of biofilm are microbial fuel cells (MFCs). In the case of MFCs, it is crucial to develop a thin biofilm layer (acting as a bio-catalyst) composed of electron-producing microorganisms on the electrode. The anode chamber of MFCs contains a diverse inoculum, representing multiple microbial species. Alongside numerous unidentified microorganisms, the identified bacteria in the anode compartment belong to various classes, such as *Clostridia, Nitrospirales, Deferribacteres, Alphaproteobacteria, Deltaproteobacteria, Bacteroidetes, Spirochaetes, Betaproteobacteria, Sphingobacteria, Flavobacteria, Planctomycetes,* or *Gammaproteobacteria*. Additionally, fungal cells, including those from the genera *Saccharomyces* or *Pichia*, are also present (Chiao *et al.*, 2006; Lee *et al.*, 2003; Mitra and Hill, 2012; Prasad *et al.*, 2007). But bacteria are the primary microorganisms utilized in the MFCs currently under study (Schaetzle *et al.*, 2008). The diversity of the microbial community in an MFC is influenced not only by the source of the inoculum but also by factors such as the type of fuel powering the MFC, the presence of redox mediators, and the oxygen conditions within the bioreactor (Logan, 2008; Schaetzle *et al.*, 2008). Fuels for MFCs can include pure substances like acetate, glucose, cysteine, and ethanol, as well as mixtures of organic compounds such as wastewater, liquid animal waste, landfill leachate, and liquid agricultural or industrial waste (Logan and Regan, 2006; Wang *et al.*, 2008; Włodarczyk and Włodarczyk, 2018).

Electrons generated by microorganisms are conveyed to the anode (within the anode chamber) either through direct or indirect electron transfer mechanisms (Lonag, 2008; Prasad *et al.*, 2007; Das, 2017). Only certain bacterial genera and species, such as *Shewanella* or *Geobacter*, exhibit electrochemical activity through direct electron conduction mechanisms (Kim *et al.*, 1999; Gorby *et al.*, 2006; Lovley, 2008). These bacteria are capable of transferring electrons even through multiple layers of cells via a dense network of appendages, known as bacterial nanowires, which exhibit metal-like conductivity (Malvankar *et al.*, 2011). Electron transfer can occur from cell to cell over distances exceeding 10 mm, ultimately delivering electrons to the electrodes (Yang *et al.*, 2017). On the other hand, bacteria such as *Pseudomonas* or also *Shewanella* can transport electrons using soluble mediators, both natural (e.g., sulfate-reducing species) and synthetic (e.g., methylene blue or neutral red) (Malvankar *et al.*, 2011; Yang *et al.*, 2017; Wrighton *et al.*, 2011).

The electrons subsequently flow through the external load to the cathode (located in the cathode chamber), where they undergo reduction by an oxidizing agent, primarily oxygen (Ibrahim *et al.*, 2022; Sun *et al.*, 2012). In MFCs microorganisms serve as catalysts for this process (Schaetzle *et al.*, 2008). Consequently, the rate at which electrons are produced is determined by the metabolic activity of the microorganisms (Sarma *et al.*, 2022). The effectiveness of the microorganisms relies on the consortium they form, particularly the biofilm that develops on the anode (Logan and Regan, 2006; Yang *et al.*, 2012; Patil *et al.*, 2009; Saratale *et al.*, 2017a; Saratale *et al.*, 2017b; Markowska *et al.*, 2013).

During biofilm formation, various stages can be observed: the development of non-electroactive bacteria (non-EAB), nonspecifically electroactive bacteria (nonspecific EAB), and specifically electroactive bacteria (specific EAB). Both types of electroactive bacteria (nonspecific and specific EAB) are capable of extracellular electron transfer (EET). In microbial fuel cells (MFCs), the focus is on the development of EAB, as conditions are provided to support electricity generation (Pinck *et al.*, 2020; Greenman *et al.*, 2021). When biofilms form on MFC electrodes, two main phases of bacterial community development on the anode biofilm can be distinguished. The first phase lasts approximately four days, during which all types of bacteria initially colonize the electrode. Over time, EAB begin to dominate over non-EAB, leading to a stage where almost exclusively EAB are present (both specific and nonspecific). At this point, the second stage begins, characterized by a steady increase in the proportion of specific EAB (adapted to EET) within the biofilm. Biofilm formation in MFCs concludes when the number of specific EAB significantly surpasses that of nonspecific EAB, marking the readiness of the electrode (with biofilm) for use in MFCs (Godain *et al.*, 2022). Figure 2 illustrates the stages of biofilm formation on the electrode.

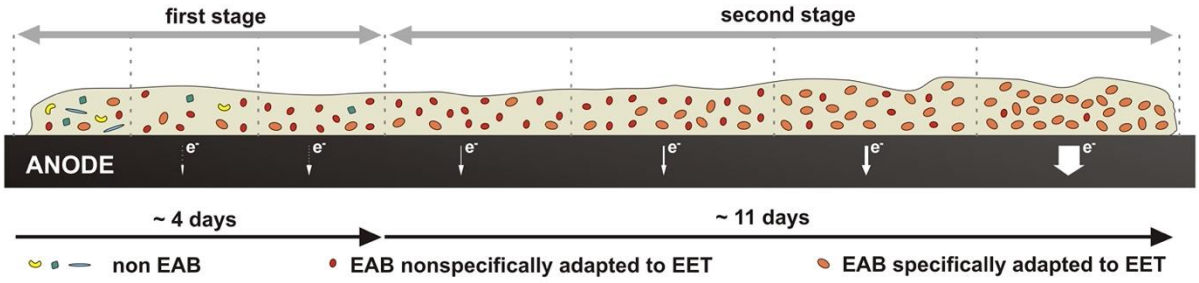

Figure 2. Stages of biofilm formation on anode in MFC.

In bioelectrochemical systems (BES) such as microbial fuel cells (MFCs), creating optimal conditions for microorganism development is critical (Saratale *et al.*, 2017a). It is essential to maintain proper temperature, pH, and other environmental factors, as well as to provide a suitable surface for biofilm growth (Saratale *et al.*, 2017b), which requires the use of an appropriate anode (Markowska *et al.*, 2013). The anode plays a key role in the performance of MFCs, as its materials and structure can significantly influence microbial biofilm formation and, as a result, the efficiency of electron transfer. The anode must meet several primary requirements: it should be biocompatible, have good electrical conductivity, and offer a large surface area (Logan and Regan, 2006; Markowska *et al.*, 2013). Additionally, it is important that the anode be cost-effective (Das, 2017). Typically, metals and carbon-based materials are used for electrodes in MFCs (Logan, 2008; Das, 2017). Tested metals include stainless steel, copper, nickel and titanium (Baudler *et al.*, 2015; Zhou *et al.*, 2016), as well as metal alloys like Ni-Co, Cu-B, and Cu-Ag (Włodarczyk and Włodarczyk, 2023). Although metals and their alloys provide high electrical conductivity and electrocatalytic activity, they are prone to corrosion, especially in environments containing chloride ions. Furthermore, metal ions released from metal anodes can decrease the efficiency of microorganisms or

even be harmful to them (Logan, 2008). These ions may also pose environmental risks (Saratale *et al.,* 2017b). As a result, other types of electrodes, such as composites (Zheng *et al.,* 2015) or carbon-based electrodes, are also employed in MFCs, with carbon-based materials being the most widely used. This preference stems from the high biocompatibility of carbon electrodes, a crucial factor when choosing electrodes for MFCs. Materials such as carbon felt, carbon paper, and carbon fiber are commonly used as carbon-based electrodes (Hutchinson *et al.,* 2011; Włodarczyk and Włodarczyk, 2024; Hidalgo *et al.,* 2016; Deng *et al.,* 2010). The carbon-based electrodes are characterized by low catalytic activity, which can limit the overall performance of MFCs, but has good electrical conductivity and resistance to corrosion.

The aim of this study was to analyze biofilm formation on a carbon electrode using the start-up method. The start-up process was carried out in a two-chamber MFC (with a carbon anode and a stainless-steel cathode) to obtain a biofilm that ensures a stable voltage value. The number of required start-up cycles was analyzed to achieve satisfactory cell voltage values and determine the success of the start-up process.

MATERIALS AND METHODS

For analyzing of biofilm (bio-catalyst) formation time on carbon electrode the two chamber MFC was used. A carbon-based gas diffusion electrode was used as the anode (working electrode). In the electrode used, the carbon layer was pressed into a steel mesh (matrix). As material for the cathode, steel springs (SSC) was applied. The springs used in the study were made of AISI 304 alloy. Before filling the cathode chamber, the springs were washed in sodium hydroxide solution and then rinsed thoroughly with water several times. To obtain high conductivity of the cathode, the steel springs were tightly wound around a steel wire made of the same material. The sintered glass was used as chambers separator. To ensure faster startup of the MFC, microorganisms from a previously operating MFC were used. During the operation of the MFC, the cathode chamber (with SSC) was constantly aerated (5 $L \cdot h^{-1}$) by stone air bubbler. The electrical circuit of the MFC was constantly connected with a 100 Ω resistor. For feeding the MFC, the molasses decoction from yeast production (MDYP) was used. MDYP is a thick liquid, so before using it in the MFC, it was mixed with water in a ratio of 1:5. The acidity of the feeding solution was 6.8 pH, and its conductivity was 2.13 $mS \cdot cm^{-1}$. All measurements were performed at temperature of 25 °C. Electrical measurements of the ML-MFC were conducted using a Fluke 8840A multimeter (Fluke Corporation, Everett, WA, USA) and a PGSTAT302N potentiostat (Metrohm-Autolab BV, Utrecht, The Netherlands). For temperature measurements, a UNI-T UT33C multimeter (UNI-Technology, Hongkong, China) was used.

RESULTS AND DISSCUSSION

A sequence of start-up tests was conducted to determine the time required for biofilm formation. Throughout these start-ups, cell voltage was monitored. The cell voltage measurements were taken in comparison to the voltage of a cell operating without microorganisms (i.e., an MFC with electrodes

containing a diluted MDYP solution). Figure 3 illustrates the cell voltage recorded over the course of three successive MFC start-up phases.

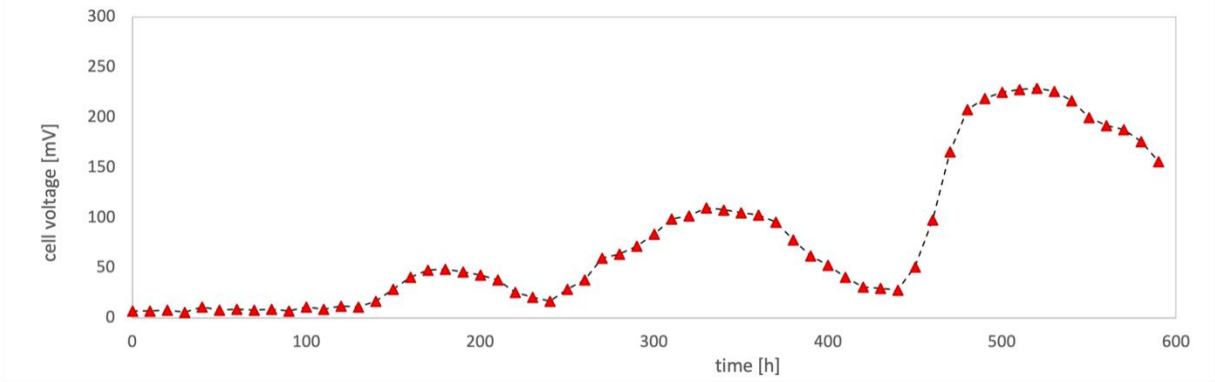

Figure 3. Cell voltage during MFC start-ups.

In the first start-up phase, it is notable that the MFC did not generate a substantial voltage for a prolonged period, producing only a minimal voltage range of 3–12 mV for approximately 120 hours. After this period, a gradual increase in cell voltage was observed, though it remained modest, reaching only about 25–50 mV. This stage aligns with the initial phase of biofilm formation on the anode (refer to Figure 2). During the second start-up, the cell voltage increased further to 51–110 mV; however, this level was still considered insufficient. Consequently, a third start-up was initiated, after which the cell voltage rose significantly to 230 mV. Subsequent start-ups showed no further increase, indicating voltage stabilization. Thus, the third start-up was considered successful, signaling the complete formation of the biofilm on the anode, corresponding to the final biofilm development phase depicted in Figure 2 (second stage).

Next, the cell voltage of the MFCs (after third start-up) was measured both in a single cycle and over six cycles (continuous/cycling feeding of the MFC). Figure 4 shows the cell voltage of the MFCs fed by the MDYP in (A) one cycle and (B) six cycles.

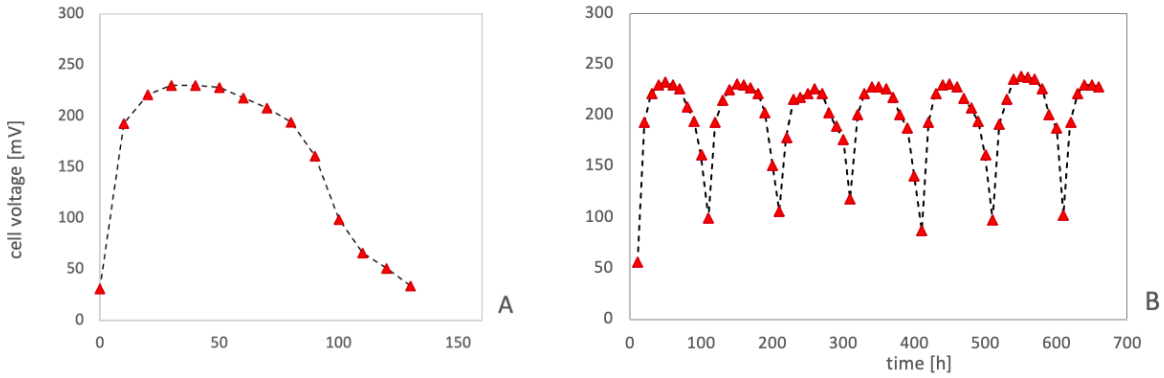

Figure 4. Output cell voltage of the MFC in one cycle (A) of feeding the MDYP to MFC and in six cycles (B) of feeding the MDYP to MFC.

The analysis of cell voltage during one cycle after biofilm stabilization (Figure 4A) showed an average voltage of 245 mV. It is important to note that the substrates for microorganisms (MDYP) are consumed rapidly, causing a sharp decline in cell voltage after about 100 hours of operation. Therefore, when conducting measurements across multiple MFC feeding cycles (Figure 4B), it was decided to feed the biofilm every 100 hours to maintain consistent performance.

CONCLUSIONS

The study investigated the biofilm formation time in a laboratory-scale MFC fed with diluted MDYP. Due to the use of microorganisms from a previously operating cell, the MFC was successfully initiated during the first start-up. However, this initial start-up did not result in achieving the appropriate cell voltage. Only after the third start-up were satisfactory values achieved, and the start-up was considered successful. For this reason, it can be concluded that the initiation of the MFC requires at least three start-ups to obtain the appropriate operating parameters. This also suggests that the majority of EAB cells specifically adapted to EET in the biofilm were obtained.

ADDITIONAL INFORMATION

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