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Conference paper

Characterization of Electrochemically Active Bacteria Utilizing Redox Response in Microbial Electrolysis Cell

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ABSTRACT

This study focuses on micro-electrochemical screening to select microbial strains capable of directly transfer electrons to the working electrode dependent on specific enzymatic machinery. The main objective of this work is to select and identify promising strains for allow the bioelectrolysis production of hydrogen. To achieve this goal, microbial composites (artificial biofilms), have been developed using *Escherichia coli CGE1 from LCPME. CNRS Fransh, Pseudomonas putrifisciens (CIP 69.13) (CIP, Collection Institut Pasteur, Fransh). Shewanella oneidensis MR-1 (ATCC 700550), and Thiobacillus denitrificans*, from (ATCC, American Type Culture Collection), each one enclosed in a matrix carbon nanotubes and protamine matrix, forming an artificial biofilm on buckypaper. Cyclic Voltammetry (CV) measurements were performed over a potential range of +0.4 to -0.7V at 5mV/s under 30°C, using a saturated KCl Ag/AgCl reference electrode and a stainless-steel grid counter electrode. For *E. coli and P. putrifisciens*, the measurement focused on the oxidation of 20mM glucose, while the former bacteria were growth with and without O_2. For S. *oneidensis* and *T. denitrificans* the focus was on the reduction of fumarate and 20 mM of $NaNOH_3^+$, respectively. As results, E. coli and P. putrifisciens species show no notable electrochemical activity, with no signal of glucose oxidation, due to the absence of type C cytochromes in the cytoplasmic membrane, unlike S. oneidensis and T. denitrificans, that demonstrate a direct electron transfer.

1. INTRODUCTION

Biofilms are complex structures consisting of microorganisms enveloped in a self-secreted matrix of extracellular polymeric substances (EPS) attached to a surface. Biofilms are considered as a

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predominant form of bacteria in the environment *(Potter, 1911)*. During the early stages of synthetic biofilm development technology, few bacteria could be used to attach the solid support. In the case of this research, this solid support is an electrode *(Bon et al. 2002)*. After an adhesion process that transitions from reversible to irreversible, the biofilm will evolve into a mature biofilm with a complex three-dimensional structure governed by the production of EPS by the bacteria *(Schroder, 2011; Gorby et al. 2006)*. The size and structure of the biofilm will then be regulated by erosion phases *(Schroder, 2011)*. Life in the form of a biofilm is interesting and essential for many bacterial species due to the numerous advantages it provides, such as resistance to biocides or the ability to store nutrients *(Gorby et al. 2006)*. An electroactive biofilm is composed of bacteria capable of performing extracellular electron transfer (EET) reactions. These bacteria can exchange electrons with each other, with electron shuttles present in the EPS matrix, and with solid electron acceptors or donors such as electrodes to which a biofilm may be attached. Today, the main applications of electroactive biofilms are microbial fuel cells, microbial electrocatalysis, and microbial electrosynthesis. For example, in a microbial fuel cell, energy is generated by the catabolism of nutrients, during which the generated electrons are transferred from the bacterium to the anode *(Reguera et al. 2005; Kizys et al. 2023)*. Conversely, energy is consumed when electrons are transferred from the cathode to the bacterium, which produces energy-rich compounds such as ethanol or dihydrogen; these are examples of microbial electrosynthesis systems *(Malvankar et al. 2014; Pirbadian et al. 2014)*. Additionally, these systems can also aid in the bioremediation of nitrogen species or sulfate through microbial electrocatalysis systems *(Steidl et al. 2016; Yates et al. 2016)*.

In this study, first two bacteria were chosen, *E. coli* and *P. putrifisciens*, known for their lack of electrochemical activity due to their membrane protein components. However, growth conditions such as anaerobiosis was chosen to try to study the electrochemical response. Then, the direct transfer of electrons in *S. oneidensis* and *T. denitrificans* during the reduction of fumarate and $NaNO_3$ was studied.

2. MATERIALS AND METHODS

2.1 Biologic materials

E. coli was cultured once on petri dish LBA medium for 24 hours at 37°C. In the second step, two perfect colonies were selected and each one was placed in LB under different conditions: one under anaerobic conditions and the other under aerobic conditions. E. coli was cultured once on LBA medium for 24 hours at 37°C. In the second step, two perfect colonies were selected and each one was placed in LB under different conditions: one under anaerobic conditions and the other under aerobic conditions, both at 37°C under orbital agitation at 160 rpm *(Lampa-Pastrirk et al. 2016)*. *P. putrifisciens* was cultivated in LB at 30°C in aerobic condition *(El-Naggar et al. 2010)*. *S. oneidensis t proliferated under the same operating conditions as E. coli and P. putrifisciens. T. denetrifican* were grouthe in mineral medium under conditions exposed by Abada et al. (2020). An assembly during the first 5 days of the bacterium's life was carried out on bacterial suspensions to ensure its viability and vitality. For this, a volume of 100 µl of a 5 mg/ml protamine solution, 100 µl of 5 mg/ml MWCNT, 100 µl of a bacterial suspension with a density adjusted to 5.10^9 CFU, and 200 µl of KCl (1 mM) are placed in a microtube (Eppendorf). The mixture is allowed to rest for fifteen minutes until a biphasic solution forms. Is using vacuum conditions to deposit their suspension on the buckypaper. Physical parameters were chosen after several trials and adjustments.

2.2 Electrolysis materials

Carbon-based materials are available in various forms such as plates, graphite rods *(Logan & Rabaey, 2012)*, granules, crosslinked vitreous carbon *(Lovley & Nevin, 2013)*, and fibrous materials like felt, fabric, paper, and carbon foam. These materials are commonly used as anodes *Abrevaya et al. 2015)*, due to their stability in microbial cultures, cost-effectiveness, ease of use, and large active surfaces, which lead to improved battery yields *(Pfeffer et al. 2012)*. As a result, current research is increasingly focusing on chemically modifying the surface of these electrodes to further enhance their specific surface area.in our study the electrode was made using a buckypaper connected by a carbon fiber, on which the bacterial composite was immobilized. The bacterial composite was made of Multi-Walled Carbon Nanotubes with carboxyl functional groups (MWCNT-COOH) and Protamine. The bacterial composite was made of Multi-Walled Carbon Nanotubes with carboxyl functional groups (MWCNT-COOH) and protamine. This mixture forms a mesh due to their opposite charges, in which bacteria are immobilized. In addition, an artificial biofilm is being created *Pfeffer et al. 2012)*. We are using stainless steel as the counter electrode and Ag/AgCl as the reference electrode+0.197 V vs. Standard hydrogen electrode).

2.3 Cell of electrolysis

The electrolysis cell is described in Figure 1. The electrolysis cell was fabricated in the laboratory workshop. The material from which it was made is resin.

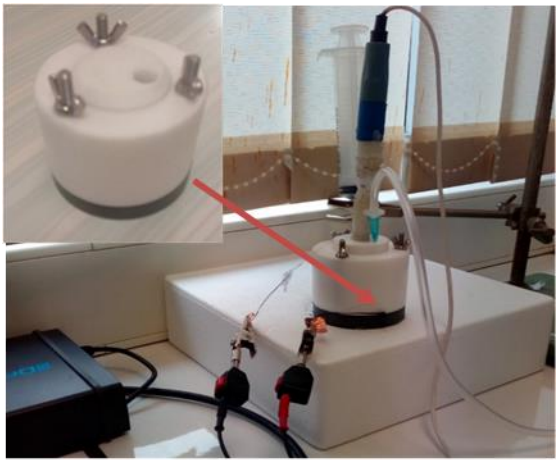

Fig. 1. The electrolysis cell under controlled atmosphere, N_2 gas.

3. RESULTS AND DISCUSSION

3.1 Cyclic Voltammetry

The technique involves recording the current density generated by an electrochemical system while scanning the potential over a given range. The CV measurements were recorded over a range from -0.8V to +0.4 V, with a scan rate of 5mV/s and a step of 0.001V. These parameters were used for measurements of nitrate and nitrite reduction. The temperature was maintained at 30°C under a continuous flow of nitrogen. After stabilizing the current at the beginning of the CV cycle, a concentration of 20mM nitrate or nitrite was injected for the first measurement. It should be noted that all potentials in this communication are provided vs Ag/AgCl (sat. KCl; +0.197 V vs. Standard hydrogen electrode).

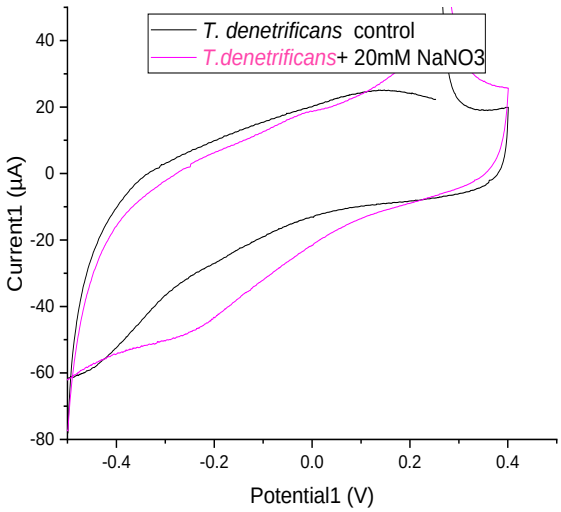

Fig.2. Control experiment with the blank and composite with *T. denetrificans* at 20 mM $NaNO_3$.

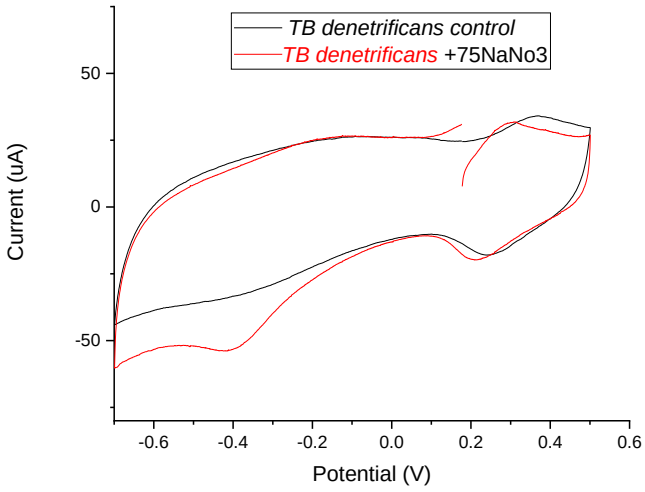

Fig 3 Control experiment with the blank and composite with *T. denetrificans* at 75mM $NaNO_3$

It is demonstrated from the results of cyclic voltammetry that the potential at which nitrate is reduced lies between -0.29V and -0.35V, and that of nitrite is in the range of -0.65V and -0.7V.

Fig 4. Variation of the current at 0mM, 20mM, and 75mM $NaNO_3$ during a CV between +0.4V and -0.8V

The work of Narcis Pous et al., published in Electrochemistry Communications in 2014, revealed the potentials at which direct electron transfer sites occur in Thiobacillus sp.; -0.34V for nitrate reduction and -0.7V for nitrite reduction. They also noted that the high concentration of nitrite inhibits nitrite reductase, so even if nitrate is added, catalysis does not occur, which is the phenomenon of negative regulation. These results are consistent with our findings.

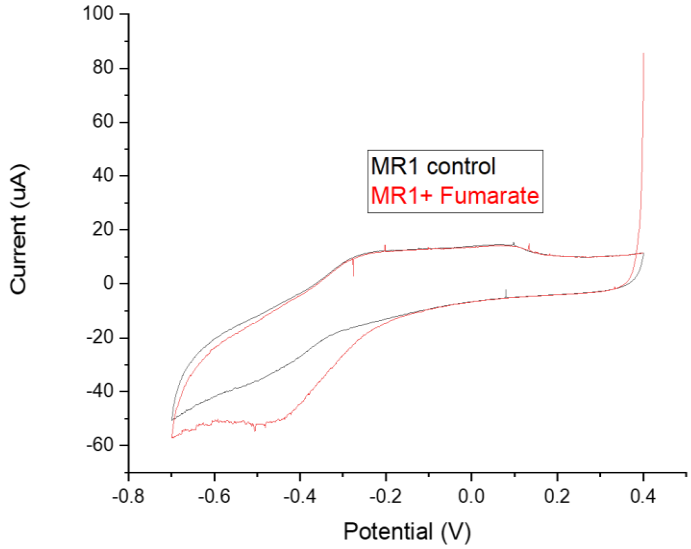

Fig.5. Voltammetry cyclic control experiment with the blank and composite with MR1 at 20 mM Fumarate.

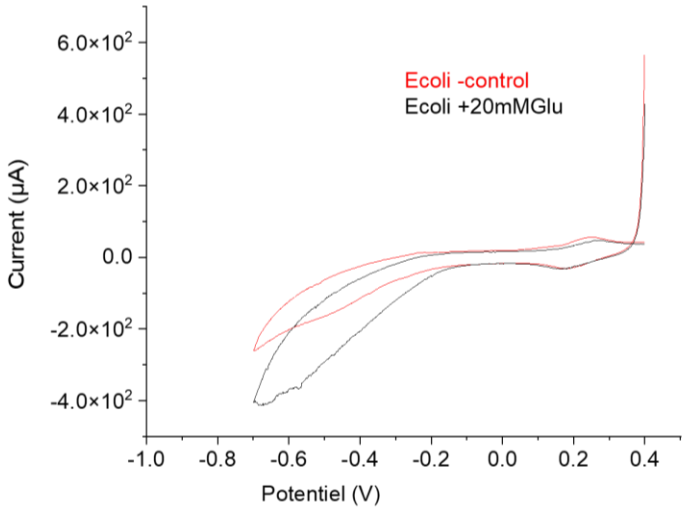

Fig 6. Voltammetry cyclic of control experiment with the blank and composite with *E .coli* at 20 mM glucose from aerobic culture

3.2 Assembly Mechanisms of Biocomposite Constituents

The importance of electrostatic forces in the formation of the artificial biofilm has been mentioned several times. This observation comes from various experiments conducted with proteins that have different charges at neutral pH, such as negatively charged Multi-Wall Carbon Nanotubes (MWCNT) and positively charged protamine (Figure 9).

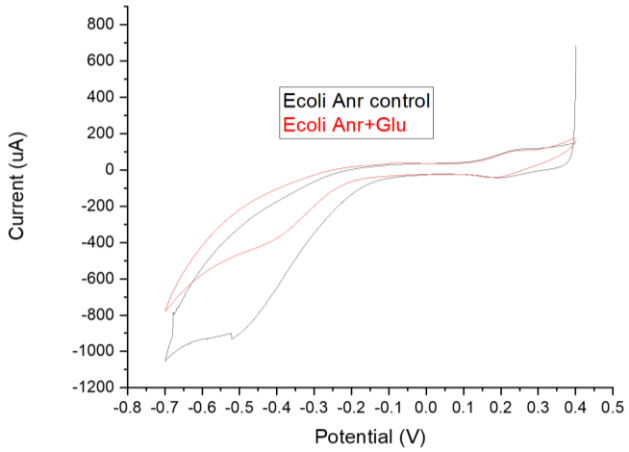

Fig 7. Cyclic voltammetry of control experiment with the blank and composite with *E. coli* at 20 mM glucose from anaerobic culture.

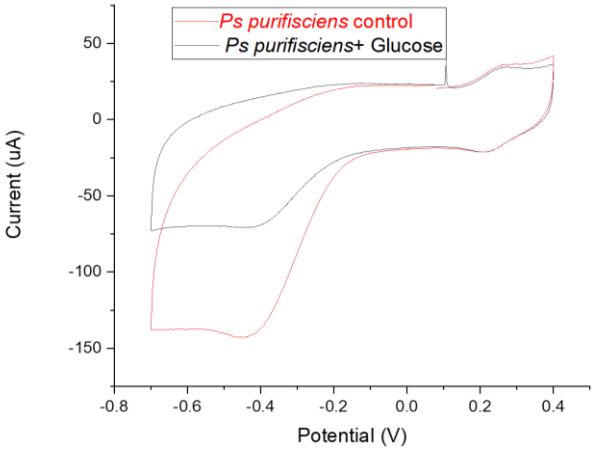

Fig 8. Cyclic voltammetry of control experiment with the blank and composite with *P. putrifisciens* at 20 mM glucose.

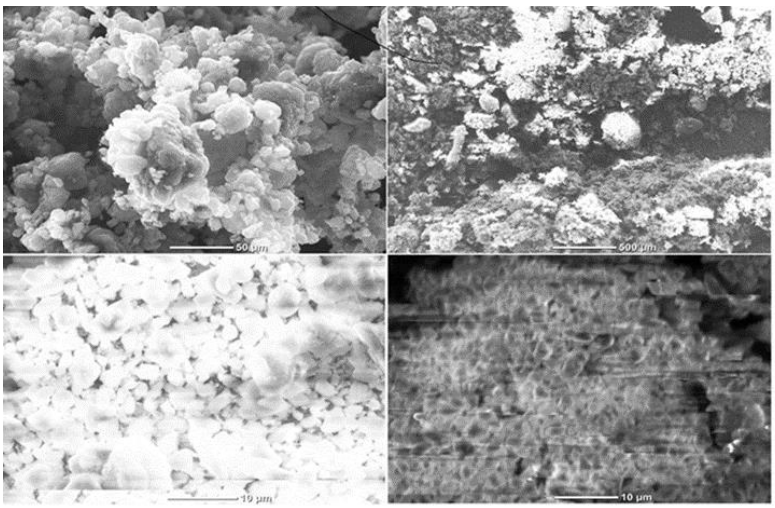

Fig 9. The importance of electrostatic forces for the formation of artificial biofilm at neutral pH with different charges.

Scanning electron microscopy was then used to describe the aggregates formed, exhibiting a structure resembling natural biofilms where MWCNTs replaced the matrix of extracellular polymeric substances. The aggregates were deposited on carbon felt and Buckypaper discs, forming a composite material based on MWCNTs and protamine in which the bacteria were embedded. The cell density of the formed films was 1 cell 2µm-. The presence of protamine improved viability, likely due to the organization of MWCNTs aggregating with each other and with protamine, limiting the mechanical cytotoxicity of MWCNTs on the bacteria. All experiments conducted with these proteins confirmed that the biocomposite aggregates were formed only in the presence of positively charged proteins and rarely with negatively charged ones.

4. CONCLUSION

The Cyclic Voltammetry (CV) results conclusively demonstrate the total absence of direct electron transfer in *E. coli* and *P. putrifisciens*. Results in the presence of 20mM glucose show no variation in oxidative current density, despite Api20 gallery results confirming glucose oxidation. Therefore, these two bacterial species show no notable electrochemical activity, with no signal of glucose oxidation, due to the absence of type C cytochromes in the cytoplasmic membrane, unlike *S. oneidensis* and *T. denitrificans*.

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