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Optimization of conditions for formation of electrochemically active biofilm on carbon felt anodes during operation of yeast-based biofuel cells

E.Y. Chorbadzhiyska[1*], M.Y. Mitov[1], Y.V. Hubenova[2]

[1]*Department of Chemistry, South-West University, Blagoevgrad, Bulgaria*

[2]*Department of Biochemistry and Microbiology, Plovdiv University, Plovdiv, Bulgaria*

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In this study, yeast-based biofuel cells using *Saccharomyces cerevisiae* as a biocatalyst were investigated under different operation conditions. The biofuel cells were operated under permanent load in a semi-batch regime. The increase of the anode mass as well as the improvement of the MFC outputs during operation indicates a formation of electrochemically active biofilm on the anode. The most active biofilm, resp. highest generated power, was obtained with the lowest load (100 Ω) applied. Besides the complexity of the system, a good reproducibility of the results was observed under controlled experimental conditions.

Key words: yeast-based biofuel cell, *Saccharomyces cerevisiae*, electrochemically active biofilm

INTRODUCTION

Microbial fuel cells (MFCs) are devices that convert the chemical energy of natural available organic substrates directly into electricity by using different microorganisms as bio-microreactors [1, 2]. In a typical MFC, electron donors, such as organic materials in wastewater, are oxidized by the electrochemically active bacteria mostly growing as a biofilm on the anode surface [3, 4]. The power generation is still insufficient for the practical applications. In order to improve the MFC performance, efforts have been made to enrich more electrochemically active bacteria [5, 6], to improve reactor configuration [7], to identify better electrode materials [7, 8, 9], as well as to optimize process parameters [7,10]. Many factors, such as nutrient supply, flow rate, pH, temperature [4, 7, 11, 12, 13], have been found to strongly affect the MFC performance and start-up time. Optimizing the growth conditions for the electrochemically active bacteria on the anode is also an important consideration for improving the performance of MFCs.

One of the most important and most investigated factors is the anode potential at which the MFC is operated, as it controls the theoretical energy gain for microorganisms [14]. Finkelstein et al. [15] reported that a larger and earlier maximum current was obtained at a more positive applied potential due to the increased energy yield for microbial colonization. Besides the anode potential, the effect of external resistance applied to the electrical circuit also received wild attention since controlling the growth condition for the electrochemically active bacteria by changing the external resistance is more feasible than poising the anode potential in MFC applications. In general, MFC performance improves with decreasing the applied external resistance. Liu et al. [16] demonstrated that the lower external resistance was applied, the higher maximum power output was obtained.

Bacteria such as *Escherichia coli* [17, 18] *Geobacter sulfurreducens, Pseudomonas aeruginosa* [17, 18, 19], *Rhodoferax ferrireducens, Shewanella oneidenis, Shewanella putrefaciens* [17, 18], *Enterobacter cloacae* [2, 18], etc., have been most frequently studied for application in MFCs. Eukaryotes, e.g. yeasts, are still rarely investigated for this purpose.

In this study, the influence of the experimental conditions (load resistance value, temperature, purging with nitrogen) on the formation of yeast anodic biofilm on carbon felt electrodes and its impact on the performance of yeast-based biofuel cell were investigated.

MATERIALS AND METHODS

Baker's yeast *Saccharomyces cerevisiae* was applied as a biocatalyst in double-chamber MFC. 1g dry yeast biomass was suspended in 80 ml of a modiñed minimal M9 salts nutrient medium [20], prepared as follows:

* To whom all correspondence should be sent:
E-mail: elli_e1@abv.bg

M9 salts solution consisted of 64 g/l $Na_2HPO_4.H_2O$, 15 g/l KH_2PO_4, 2.5 g/l NaCl and 5.0 g/l NH_4Cl was prepared and sterilized by autoclaving. 0.489 g/l $MgSO_4$, 0.011 g/l $CaCl_2$ and 4 g/l glucose as a carbohydrate source were add to 200 ml of M9 solution, and the volume was adjust to 1 l with distilled water. The final nutrient solution was sterilized again by autoclaving.

The prepared yeast suspension was used as an anolyte in the MFCs. 1 ml (0.1%) methylene blue was added to the anolyte suspension as an exogenic mediator. 100 mM $K_3[Fe(CN)_6]$ dissolved in 67 mM phosphate buffer (pH 7.0) was applied as a catholyte and terminal electron acceptor. Rectangular carbon felt samples (5 cm height, 3 cm width; SPC-7011, 30 g/m^2, Weibgerber GmbH & Co. KG) were used for both anodes and cathodes. Prior to use the electrodes were sonicated in ethanol-acetone mixture (1:1) for 15 min. Samples with equal specific resistance were applied as electrodes. The anode and cathode chambers of the MFCs were connected with a salt bridge – Fig.1.

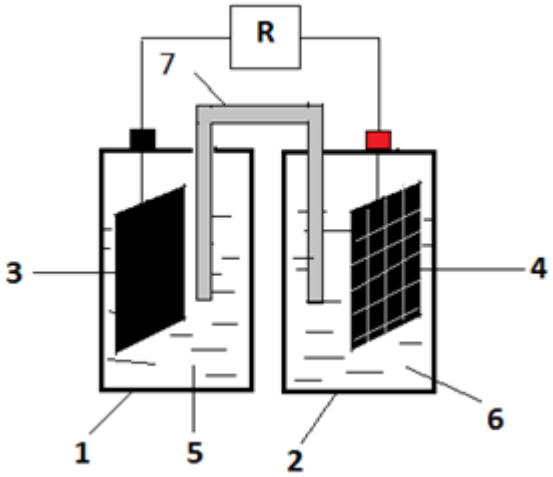

Fig. 1. Scheme of double-chamber MFC: 1-anode chamber (volume 100 cm3); 2-cathode chamber volume 100 cm3); 3-anode; 4-cathode; 5-anolyte; 6-catholyte; 7-salt bridge.

The fuel cells were operated at a load of 100, 500, 1000 or 5000 Ω for at least one week. During these experiments, the terminal voltage as well as the anode and cathode potential, measured against Ag/AgCl reference electrode, were monitored with time. After the 3rd day from the beginning of experiments every day the anolyte was replaced with a fresh cultivation medium. Before each medium replacement, polarization measurements under a variable resistor load were carried out using resistor box. The voltage was measured by digital mutimeter and the current was calculated by using the Ohm's law. At each load the voltage was allowed to stabilize for at least 2 minutes before a reading was taken. The results were plotted as polarization curves *U=f(I)*. The generated power at each load was estimated by equation *P = U.I* and plotted as power curves *P = f(I)*.

In a series of experiments the yeast biofuel cells were operated at the same conditions, but part of them were incubated in a thermostat at a constant temperature (22±1 °C) and the rest were cultivated at a temperature varying between 15 and 25 °C. In another series of experiments part of the MFCs were operated with purging of nitrogen in the anode chamber to create strict anaerobic conditions, and the rest - under normal conditions (without purging with nitrogen).

During the MFC-experiments the optical density of the anolyte suspension was measured at the wavelength 600 nm (OD600). The spectrophotometric studies were performed by using Agilent Hewlett-Packard 8453A UV-VIS-NIR Spectrophotometer.

After the end of MFC operation, the anodes were dried overnight and weighted by using Mettler AE 100 analitycal balance. The mass of the anodic biofilm was calculated as a difference of the masses of used anodes before and after the polarization tests in yeast-biofuel cell.

Each MFC-experiment at identical conditions was carried out in triplicate.

RESULTS AND DISCUSSION

Relatively high values of the open circuit voltage (above 500 mV) were recorded few hours after the start-up of the investigated MFCs. After 3 days operation the OCV grew up to values exceeding 800 mV, but after the first replacement of the anolyte with a fresh medium a drop of about 100 mV was observed (Fig. 2).

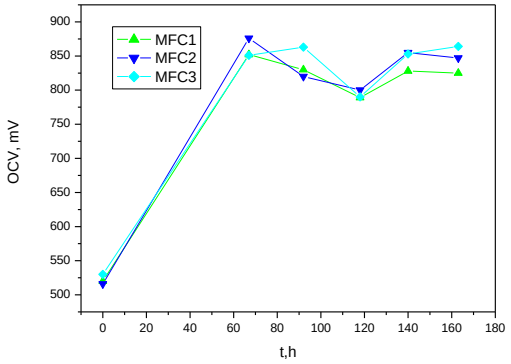

Fig. 2. Variation of open circuit voltage (OCV) of the studied yeast biofuel cells with time

Possible explanation of this decrease is that electrochemicaly active compounds, produced under polarization by yeast, are removed from the

system with the exhausted medium. Stabilization of the OCV at higher values was achieved after six days operation of the MFCs at constant load.

In parallel, a shift of the anode potentials from 160±30 mV to -530±20 mV was observed in a contrary to the cathode potentials, which values remain relatively constant (Fig. 3).

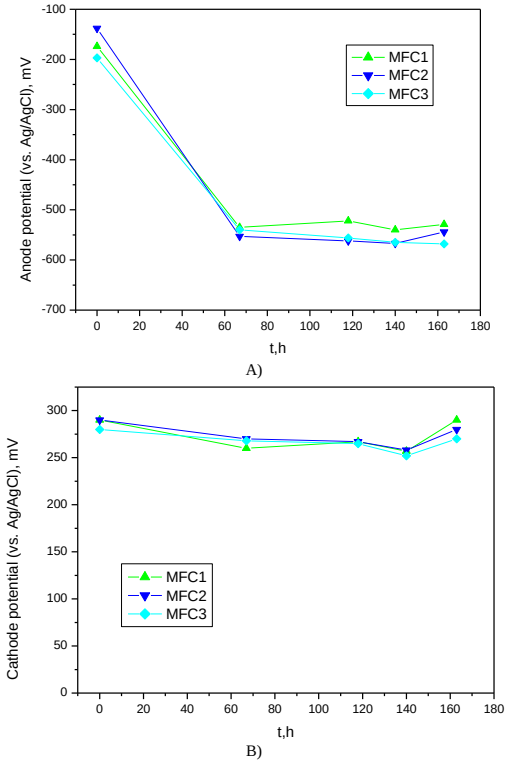

Fig. 3. Variation of anodic (A) and cathodic (B) potential of the yeast - biofuel cells with time

These results show that the processes taking place on the bioanode have predominant role for the performance of the examined yeast biofuel cells. Such abrupt changes of the anode potential in a negative direction by more than 300 mV is often associated with the formation of an active anodic biofilm [21]. The change in the optical density OD600 of the anolyte suspension, presented in Fig. 4, is in accordance with such suggestion. Three days after the start-up of the MFCs the measured optical density of the cell suspension drastically decreased in comparison to the initial one and the subsequent refreshments of the nutrient medium practically did not change its values. The observed decrease of the optical density of the anolyte can be connected with a lack of yeast cells in suspension due to formation of anodic biofilm.

After stabilization of the anode potential referred to a biofilm formation the achieved terminal voltage values under a load also showed relatively constant and high values - Fig. 5.

Very close electrical outputs (open circuit voltage, short circuit current, maximum power) can be derived from the polarization (Fig. 6A) and power curves (Fig. 6B) of MFCs operated under the same load and other conditions.

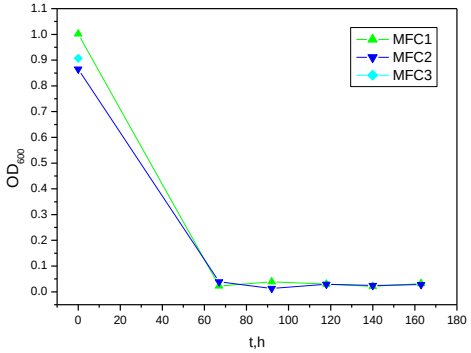

Fig. 4. Variation of the optical density OD600 of the anolyte cell suspension with time

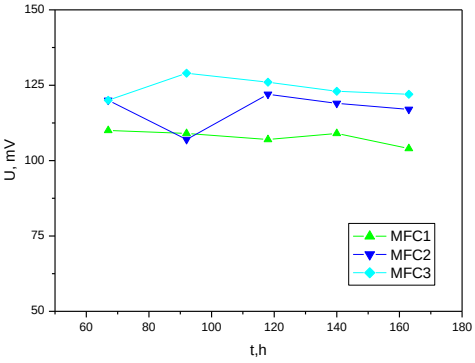

Fig. 5. Variation of the terminal voltage of studied MFCs under a load of 1000 Ω

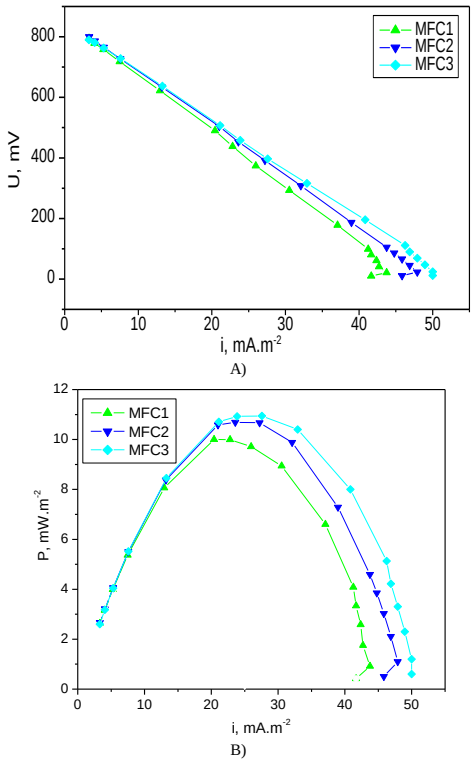

Fig. 6. (A) Polarization curves and (B) power curves obtained at the 3rd day after the start-up of the yeast biofuel cell

This indicates that reproducible characteristics could be obtained at controlled conditions besides the complexity of the system.

The maximum power, obtained from different MFC operated under the same conditions, also shows same tendencies of variation with time and close values (Fig. 7). Connecting these results with those received for open circuit voltage (Fig. 2), anode potential (Fig. 3) and optical density (Fig. 4), it can be concluded that six days are optimal for the formation of an active anode biofilm, and the periodic replacement of the medium contributes to its stability and activity for a longer period of time.

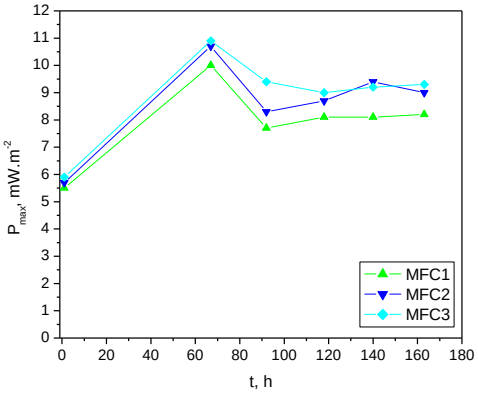

Fig. 7. Variation of generated maximum power from yeast biofuel cells with time

Table 1 presents the results from the weight analysis of the formed biofilm. It is obvious that the mass of the formed biofilm on the anodes of three MFCs, operated under the same conditions, is quite close and the observed deviations of the values are insignificant. These results explain the similar electrochemical behaviour of the studied yeast biofuel cells and confirmed the suggestion that the behaviour of the MFC strongly depends on the formed anodic biofilm.

Table 1. The mass of biofilm, formed on the anode of three yeast biofuel cells, operated for one week (load resistance 1000 Ω)

No MFC	Mass of the biofilm, g
1	0.0325
2	0.0346
3	0.0362

Set of experiments aiming to clarify the factors that contribute for the optimum of the operating characteristics were performed. The results presented on Fig. 8 show that maintening a strict constant temperature is not essential for the electric outputs of the studied yeast biofuel cells.

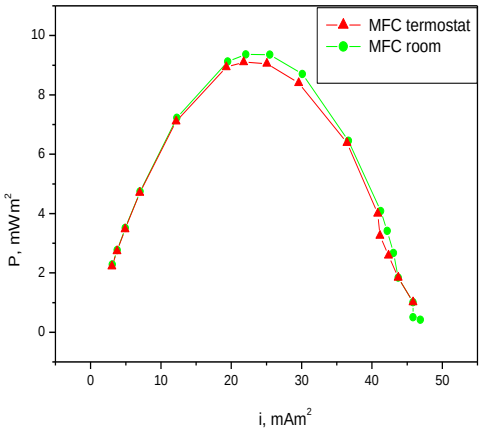

Fig. 8. Power curves obtained with the yeast biofuel cells operating in a thermostat and at ambient temperature

In contrary, the maintenance of strict anaerobic conditions results in significantly lower operating characteristics than those obtained under normal conditions (without purging with nitrogen) - Fig. 9. This is associated with the fact that in aerobic conditions yeast catabolizes the substrate (glucose) through the processes of cellular respiration, in which the total number of generated electrons is much bigger than those of anaerobic fermentation. Such results have been also reported for other types of yeasts [22].

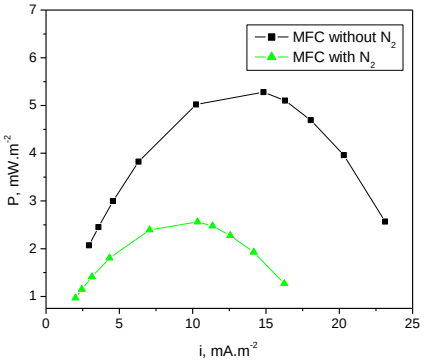

Fig. 9. Power curves obtained with the yeast biofuel cells operating with and without purging of nitrogen

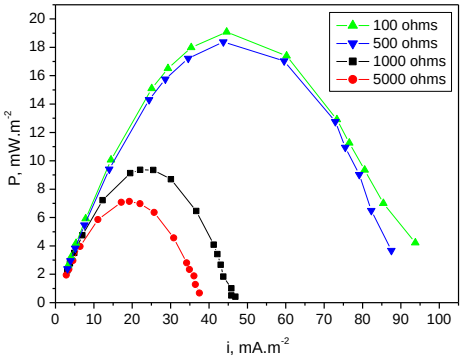

Fig. 10. Power curves obtained with the yeast biofuel cells polarized with different load resistances one day after the first change of the medium

The role of the load resistance on the activity of the formed anode biofilm, resp. MFC outputs, was also examined. The highest maximum power of 19±3 mW/m2 was achieved with MFCs operated under the lowest load of 100 Ω and the generated power decreased with an increase of applied external resistance – Fig.10. The same tendency for formation of more active biofilms at lower loads was also reported by other researchers [23, 24].

CONCLUSION

A long-term operation of yeast-based biofuel cells using *Saccharomyces cerevisiae* as a biocatalyst can be accomplished by periodical replacement of the anolyte with a fresh nutrient medium. The formation of electrochemically active biofilm on the anode has a predominant role for the MFC-performance. From all studied factors, the major impact on the activity of the formed anode biofilm has the load resistance, by which the MFC is polarized. The lower resistance is applied, the more active biofilm is formed. The maintenance of strictly anaerobic conditions diminishes the MFC-outputs due to the fact that at such conditions the facultative yeasts catabolize the substrate through fermentation, which generates quite less electrons in comparison with the processes of respiration.

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ОПТИМИЗИРАНЕ НА УСЛОВИЯТА ЗА ПОЛУЧАВАНЕ НА ЕЛЕКТРОХИМИЧНО-АКТИВЕН БИОФИЛМ ВЪРХУ ВЪГЛЕРОДНИ АНОДИ В ДРОЖДЕН БИОГОРИВЕН ЕЛЕМЕНТ

Е. Й. Чорбаджийска[1*], М. Й. Митов [1], Й. В. Хубенова[2]

[1]*Катедра „Химия“, Югозападен университет „Неофит Рилски”, Благоевград, България*

[2]*Катедра „Биохимия и Микробиология“, Пловдивски Университет „Паисий Хилендарски“, България*

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(Резюме)

В настоящата разработка са изследвани дрождени биогоривни елементи, използващи дрожди *Saccharomyces cerevisiae* като биокатализатор, при различни условия. Биогоривните елементи бяха тествани в полунепрекъснат режим при постоянно приложено товарно съпротивление. Увеличаването на масата на анода, както и подобряването стойностите на операционните характеристики на микробиологичните горивни елементи свидетелства за образуването на електрохимично-активен аноден биофилм. Най-активен биофилм, съответно най-голяма електрическа мощност, бяха получени с най-малкото приложено товарно съпротивление (100 Ω). Въпреки сложността на системата, поддържането на постоянни експериментални условия води до получаването на добре възпроизводими резултати.