Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.22880

128 (2018) 324–329
October

The application of *Saccharomyces cerevisiae* in the two-chamber microbial fuel cells

Anna Trusek*, Lukasz Janczewski, Michal Halon

Wroclaw University of Science and Technology, Division of Bioprocess and Biomedical Engineering, Norwida 4/6, 50-33 Wroclaw, Poland, Tel. +48 71 3202653; Fax: +48 71 3281318; email: anna.trusek@pwr.wroc.pl (A. Trusek)

Received 24 May 2018; Accepted 16 July 2018

ABSTRACT

An application of *Saccharomyces cerevisiae* in the two-chamber microbial fuel cells (MFCs) prototype was presented. Glucose was used as a carbon source. The influence of its concentration as well as the initial yeasts concentration on the obtained voltage was presented. Investigations were performed in the batch mode. Methylene blue at the concentration of 1 g/L was used as a mediator to enable electron transfer from the microbial cells. Easy accessible hydrogen peroxide was applied as an oxygen source. The voltage produced by the microorganisms in MFCs was monitored in real-time with Picolog 1012 from Pico Technology™. The voltage was obtained, when the substrate concentration was significantly higher than the yeasts concentration. The highest voltage of about 30–38 mV was achieved after 40 h of MFCs work for the initial glucose and yeasts concentrations 50 g/L and 30 g/L, respectively. The culture phase, in which energy was generated, was delayed in relation to the glucose uptake from the solution. As a summary of the research, the concept of an integrated process composed of membrane separation nodes and bioreactor processes was proposed.

Keywords: Two-chamber microbial fuel cells (MFCs); *Saccharomyces cerevisiae*; Exchange membrane; Integrated process; Voltage monitoring; Installation project

1. Introduction

Microbial fuel cells (MFCs) are an interesting alternative for obtaining energy from renewable energy sources. In many cases, wastewater from brewery, starch-processing, agricultural industry, petroleum as well as landfill leachates are used as the organic matter converted by microorganism. Basic compounds such as glucose or acetate are also applied [1–5].

Most of MFCs are built of following basic elements: cathode electrode, anode electrode, cathodic chamber, anodic chamber, chamber separator (usually an ion-exchange membrane), electrodes connection, and cathodic catalyst (optional). Inside the anodic chamber microorganisms decompose organic compounds. Electrons generated during this process are transported to the anode and further through an external electric circuit to the cathode (Fig. 1). Inside the cathodic chamber, the electrons are used in reduction processes. Both chambers of MFCs are separated by a cation exchange membrane that enables H^+ transport from the anodic to the cathodic chamber. In this type of MFCs, it is possible to use liquid oxidants, therefore other chemical compounds from oxidized ones can be obtained in the reduction process proceeded in the cathodic chamber.

Besides two-chamber MFCs, there are also one chamber MFCs (Fig. 1), which contain only the anodic chamber. In this type of MFCs, oxygen from air can play the role of the oxidant. At a full exposition of the cathode to atmospheric oxygen, a high active surface and a constant supply of oxygen can be obtained [6].

During MFCs assembling, it is important to protect the anodic chamber against oxygen. Otherwise oxygen catches protons and electrons that are created during substrates oxidization and in consequence, a redox reaction takes place in the anodic chamber. Hence, electrons are not transferred to the cathodic chamber via an electric wire.

Considering biocatalyst used in fuel cells, two basic concepts can be distinguished: enzymatic fuel cells (EFCs), which use redox enzymes in native or immobilized form for oxidation purposes and MFCs, in which electrochemically active

* Corresponding author.

Presented at the XII Scientific Conference "Membranes and Membrane Processes in Environmental Protection" – MEMPEP 2018 13–16 June 2018, Zakopane, Poland.

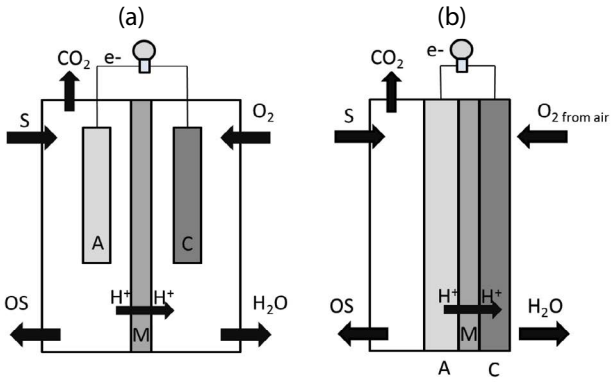


Fig. 1. Scheme of MFCs: (a) two-chamber, (b) one-chamber; S – substrate, OS – oxidized substrate, M – ion exchange membrane, A – anode, and C – cathode.

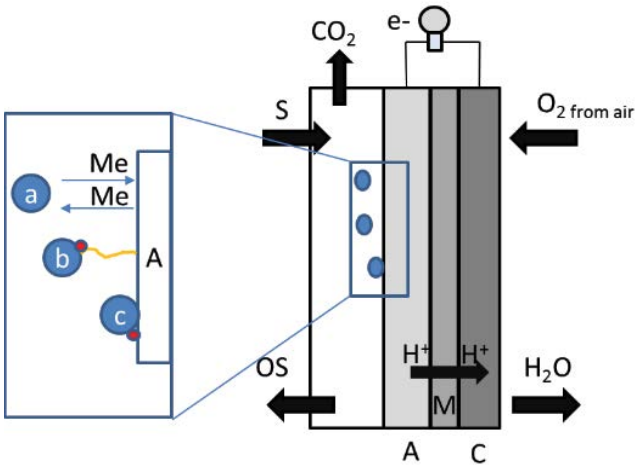


Fig. 2. Simplified scheme of two-chamber MFC with possible mechanisms of electron transfer: (a) electron transfer with the help of a mediator; (b) electron transfer with the help of nanowires, and (c) direct electron transfer with the help of external cell cytochromes.

bacteria play a role of a biocatalyst. Only some enzymes used in EFCs, for example, cytochrome c, laccase, oxidase bilirubin, hydrogenase, and selected peroxiydase can directly transfer electrons from the reaction place to the electrode. In another case, the electron mediators have to be used or an enzyme has to be immobilized exactly on the electrode surface. EFCs work usually more predictably than MFCs, however, with the process duration their efficiency decreases as the effect of an enzyme inactivation. Additionally, many enzymes' inhibitors can be found in a wastewater used as a substrate medium.

A broad range of electrochemically active microorganisms, both anaerobic ones, that oxidize organic matter in the anodic chamber, and aerobic ones, that reduce an oxidant in the cathodic chamber, can be used in the process. At appropriate process conditions microorganisms can simultaneously produce electricity and grow, what relevantly increases efficiency of such fuel cells.

1.1. Mechanism of electron transfer in MFCs

There are two basic mechanisms of electron transfer from microbial cells to the electrode (Fig. 2):

- A direct transport: electrons are transported from cells to the electrode (in situation when substrates are oxidized) or from the electrode to microbial cells (in situation when oxidant is reduced) without indirect steps of reduction and oxidation;
- An indirect transport, that is, a transport with mediator's help. The mediator can be an additional substance added to the chamber or it can be produced by the microorganisms. Additionally, microorganisms that can produce mediators by themselves are called electrochemically active microorganisms [7].

A special case is the electron transport by bacterial nanowires. Bacterial nanowires are electrically conductive appendages produced by a number of bacteria most notably (but not exclusively) from the Geobacter and Shewanella genera [8,9]. Nanowire networks have been shown to enhance the electricity output of MFCs with efficient and long-range conductivity.

An externally introduced mediator has to be able to perform reduction and oxidation to the initial form, however, mediators produced by the microorganisms do not have to transform back to the initial form after reduction and oxidation, because microbial cells will produce new mediator molecules [10].

Mediators of electrons, both the exogenous and the endogenous should have following properties [11]: low molecular mass, easy conversion from oxidized to reduced form and vice versa, easy permeation through the membrane, disability to adsorption on microbial cells, and the membrane and chambers material.

1.2. Application of Saccharomyces cerevisiae in MFCs

An influence of a mediator type on an electricity production efficiency was investigated in work of Permana et al. [12]. Within the research, methylene blue was applied as the mediator in the anodic chamber of MFCs to transfer electrons from *S. cerevisiae* cells to the anode. Glucose was used as a substrate. It produced current of 5.5×10^{-5} A, voltage of 0.886 V, power density of 4.48×10^{-3} W/m^2, and energy 4.14×10^{-3} J. Glucose was utilized in 95.0% and, additionally, 0.74% (v/v) bioethanol was produced in the same time. In comparison in MFCs, in which no mediator was used, current was 4.5×10^{-5} A, voltage 0.689 V, power density 2.12×10^{-3} W/m^2, and maximal energy of 1.96×10^{-3} J. Glucose was utilized in 96.3% and 0.74% (v/v) of bioethanol was produced. Power density yields in both compared cases were very small and did not differ relevantly.

Results of whey degradation with *S. cerevisiae* (PTCC 5269) in two-chamber MFCs and resulted current values are presented in paper [13]. Experiments were performed with and without mediator. Two types of mediators were used: methylene blue and natural red. Voltage of 500 mV was achieved in the open circuit without mediators at temperature of 25°C ± 2°C. In about 2 d, stable voltage values were

achieved. Natural red was the better mediator in comparison with methylene blue. It was possible to achieve power of 50 μW and current of about 470 μA.

In work [14], a laboratory scale MFC, in which the yeast *S. cerevisiae* was used to utilize different substrates and produce electrical energy, was presented. Nafion membrane 117 (Lyntech, USA) and graphite cathode of 1.46×10^{-3} m^2 were used. The cathodic chamber was filled with a mixture of electrolyte compounds $K_3Fe(CN)_6$ and buffer solution. During experiments, different parameters (pH, O_2) were controlled, when microbes effectively produced electricity. Application of riboflavin as an electron mediator increased the MFC efficiency by 53.9%.

Detailed studies of the effect of a substrate and microorganisms concentrations on the amount of energy produced and determination of the production phase presented in this paper are necessary for continuous MFCs design. Widely available and resistant to infection *S. cerevisiae* was applied. Glucose was used as the organic matter source due to the possibility of easy measurement of its concentration in time. However, the dependencies obtained in studies can also be transferred to other strains and waste carbon sources.

2. Materials and methods

A freeze-dried *S. cerevisiae* cells (as lyophilisate) from ALCOTEC™ (Poland) were used to produce electrical energy from glucose in the self-made two-chamber MFC (Fig. 3). Investigations were performed in a batch mode. To enable electron transfer from the microbial cells, methylene blue (POCh, Poland) at the concentration of 1 g/L was used as the mediator. Methylene blue was used in previous researches with different efficiency [12,13,15,16].

Atmospheric oxygen is the most available and, simultaneously, the cheapest electron acceptor, however, its concentration in air (21%) is relatively small. Therefore, in performed experiments hydrogen peroxide (POCh, Poland) at concentration of 0.75 % v/v was applied.

The electrodes (anode and cathode) were made of carbon. Their area was approximately 74 cm^2 for each. To enable the regulation of the distance between the electrodes and the diaphragm, stainless steel elements with a composite electrode were used. The electrodes were anchored in easily removable side walls of half-cells, and they were externally connected to the ports enabling the connection of electric wires.

Working volume of anodic and cathodic chambers was 150 mL for each chamber. The anodic and cathodic chambers were built of PCV. It was important to keep hermetic conditions in the anodic chamber. Therefore, chambers were protected with rubber sealing. Additionally, the anodic chamber was equipped with a venting port and a sampling port. The venting port was connected with test tube filled with water by a hose to enable degassing of a liquid in the anodic chamber (bubbling). The port in the cathodic chamber served to replace an oxidizing agent solution. A proton exchange membrane (Nafion 117, Sigma-Aldrich) was placed between two chambers. Fig. 3 presents these connections.

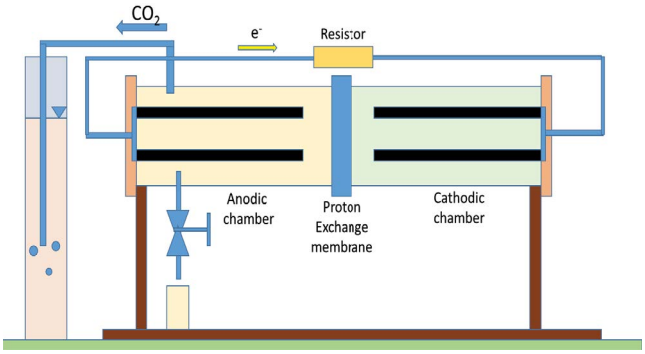
To maintain the temperature (37°C), MFC was placed in a thermo-isolated chamber equipped with a water jacket joined to thermostat (Bionovo) – Fig. 4.

The voltage produced by the microorganisms was monitored in real-time with Picolog 1012 from Pico Technology™. In the presented MFC, 100 Ω resistor (Elektronikom) was used. Glucose concentration was measured with application of DNS test [17]. Yeasts concentration was measured spectrophotometrically at 600 nm. 1 g/L of dry weight of yeasts was an equivalent to an optical density of 0.708 and a total cell count of $1{\cdot}34 \times 10^{10}$ cells/L [18]. The plan of experiments is presented in Table 1.

3. Results and discussion

3.1. Substrate concentration effect

In Fig. 5, voltage and substrate concentration changes in time for the initial glucose concentration in range 10–50 g/L at concentration of yeasts 30 g/L, dye 1 g/L and 0.75% H_2O_2



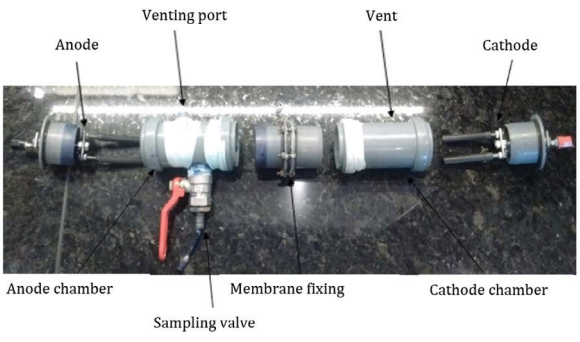


Fig. 3. Elements used in tested MFC.

Fig. 4. Two-chamber MFC used in experiments.

Table 1
Plan of performed experiments

Experiment number	Glucose concentration (g/L)	Yeasts lyophilisate concentration (g/L)	Methylene blue concentration (g/L)	H_2O_2 concentration (%) v/v
1	50	30	1	0.75
2	30	30	1	0.75
3	10	30	1	0.75
4	30	10	1	0.75
5	30	50	1	0.75

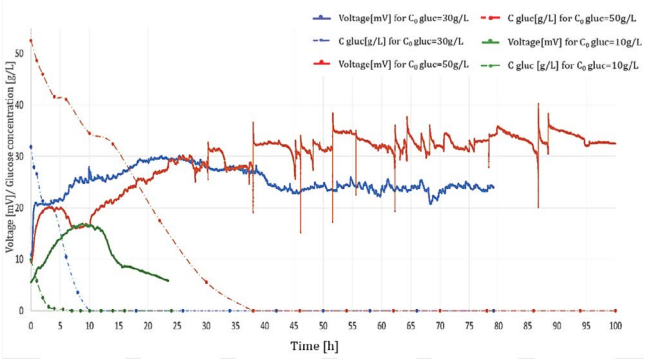


Fig. 5. Voltage and glucose concentration changes in time for the initial glucose concentrations in range 10–50 g/L (at the yeasts concentration 30 g/L).

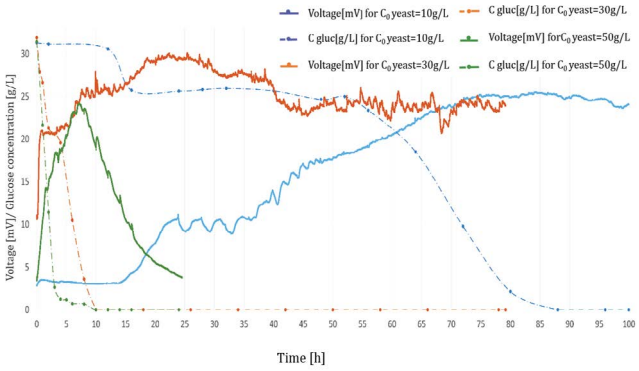


Fig. 6. Voltage and glucose concentration changes in time for the initial yeasts concentrations in range 10–50 g/L (for initial glucose concentration 30 g/L).

are presented. In the case, when the substrate concentration was higher than the yeast concentration, significant voltage was only obtained.

The maximum voltage of about 30–38 mV was achieved after 40 h of the MFC work for the initial glucose concentration 50 g/L. The moment of the maximum voltage occurred after the complete glucose consumption. It was possible that in the initial stage of the process yeasts transformed glucose with a high efficiency to the by-product/products, which were further used in a respiratory chain. This high value of the voltage holds for the next 50 h.

At lower (10 g/L) glucose concentration, an efficient electricity production phase was not observed. Glucose was utilized after about 5 h of the process. After this time the voltage shortly increased to 16 mV and after few hours it started to decrease. Probably, the concentration of glucose 10 g/L was too low for 30 g/L of dry yeast to enable the long-term, stable operation. Microorganisms could not store enough of carbon and therefore, they could not generate electrical energy effectively.

Yeasts concentration did not increase significantly. Probably, it was caused by the deficiency of micro – and microelements, which were supplied only with lyophilisate. Remarks from this step of research are summarized in Table 2.

Based on the results, the initial glucose concentration of 30 g/L was selected as the most efficient for further experiments. However, the process at the initial glucose concentration of 50 g/L was not stable; voltage values fluctuated relevantly in comparison with the process with initial glucose concentration of 30 g/L. After reaching the maximum value 30 mV, the voltage values decreased to 23–24 mV and this level hold during the next 50 h at least.

3.2. Yeasts concentration effect

In Fig. 6, voltage and substrate concentration changes in time for the yeasts concentration in range 10–50 g/L at glucose 30 g/L, dye 1 g/L and 0.75% H_2O_2 are presented.

For initial yeast concentration of 10 g/L, three phases could have been identified. The growth was the longest and the highest yeast concentration increase was observed (Table 2). In the first phase, which took about 14 h, the voltage was about 4 mV and the glucose concentration slowly decreased. In this phase, yeasts had to adapt to the process conditions (at a relatively high substrate concentration in refer to the yeasts concentration). In the second phase, the logarithmic increase in the produced potential with simultaneous linear decrease of the glucose concentration in time was observed. In this phase, which took about 60 h, cells grew up to the moment until glucose concentration in the solution fell below 5 g/L. After this moment, an effective energy production started.

As a comparison, for the initial yeasts concentration of 50 g/L, only two rapidly occurring phases were observed. In the first phase, the voltage increased from 4 to 24 mV in about 8 h. In this time, glucose concentration fell to about 1 g/L. In second phase, which lasted about 16 h (from 8 to 24 h of the process), the generated potential decreased below 4 mV.

Table 2
Results summarizing the effect of the glucose initial concentration (initial yeasts concentration 30 g/L)

Initial glucose concentration (g/L)	Maximal activity phase	Range of generated voltage during the maximal activity phase (mV)	Time of complete substrate utilization (h)	Final yeasts concentration (g/L)
50.0	Exists	30–38	38	42.6
30.0	Exists	23–30	10	33.1
10.0	Exists for a very short time	15–16	5	30.9

Table 3
Results summarizing the effect of the yeasts initial concentration *i* (initial glucose concentration 30 g/L)

Initial yeast concentration (g/L)	Maximal activity phase	Range of generated voltage during maximal activity phase (mV)	Time of complete substrate utilization (h)	Final yeasts concentration (g/L)
10	Exists	24–26	88	20.2
30	Exists	23–30	10	33.1
50	Does not exist	20–25	8	43.2

The initial glucose concentration was too low in refer to the yeasts concentration to sufficient collect energetic reserves inside the yeast cells. Thus, the stable phase of electrochemical activity did not occur in this case.

At 30 g/L of yeasts, the process was the most stable. The energy production started fast and a relatively high value of voltage (25 mV) hold for long time. Remarks from this step of the research are summarized in Table 3.

The substrate supplied for cultivation is always firstly used for the basic metabolism of cells, then for biomass growth and then for the production of secondary metabolism products if any. In the case of high biomass concentrations in the bioreactor, further biomass growth is limited. In the case of energy production, this is a desirable phenomenon.

3.3. The concept of the continuous process

Fig. 7 presents the concept of an integrated process composed of membrane separation nodes and bioreactor processes, considering the obtained data.

In the case, when the organic matter transformed in MFC origins from a wastewater of a low content of organic compounds, the UF/NF membrane separation node can be used. The concentrated stream (retentate) is fed to the MFC, and the permeate with organic material residues is directed to a stirred tank microbial bioreactor (MBR). The number of MFC tanks and their volume depends on the required residence time. This time will be significantly longer than the residence time in other units of the installation.

The purpose of using MBR is to renew the biomass used in MFCs. As shown in the studies, the increase in biomass in MFCs is negligible. It is then very easy to degenerate the strain. Depending on the permeate composition, it can be necessary to add external organic matter as well as macro- and micronutrients to the MBR.

The biomass from MBR is concentrated on a membrane unit (MF). The permeate obtained in this stage should be a clean water without organic matter and cells. The retentate

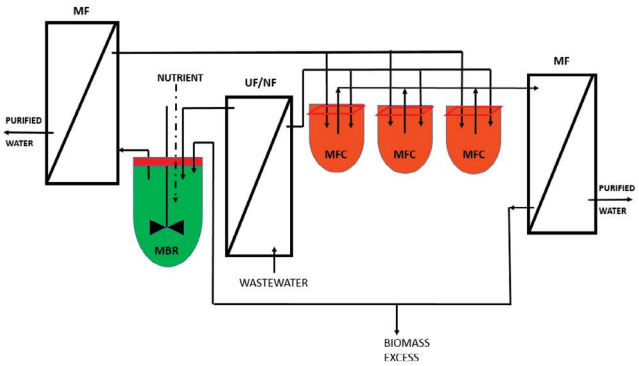


Fig. 7. The concept of the continuous process of energy production.

with cells is directed to MFCs. The effluent from MFCs contains mainly biomass. It can be separated on a membrane unit (MF). A small part of the retentate stream from this stage is only directed to the MBR; the rest is removed as a so-called excess biomass.

As a result of the installation operation, the energy and the purified water are obtained from wastewater. The waste stream is the stream of the excess biomass, but it can also be converted into energy in the combustion process. The presented technology, thus, fully complies with the principles of clean technology. The values of particular streams and apparatus volumes should be calculated for a given technology after microbial kinetics checking and membrane units testing.

4. Conclusion

As shown in the research, voltage obtained in MFCs is strongly dependent on substrate concentrations in relation to the microbial cells concentration. MFCs should work at a high biomass concentration (20–30 g/L) and substrate concentration even higher than the biomass concentration (30–50 g/L).

In the conducted studies, a high (≥ 10 g/L) concentration of yeast was used. However, lyophilized yeasts were not effective energy producers. This stage could be improved probably by using liquid inoculum and supplying, except for organic source, required minerals to bioreactor.

References

[1] D. Gong, G. Qin, Treatment of oilfield wastewater using a microbial fuel cell integrated with an up-flow anaerobic sludge blanket reactor, Desal. Wat. Treat., 49 (2012) 272–280.

[2] A. Fogg, V. Gadhamshetty, D. Franco, J. Wilder, S. Agapi, S. Komisar, Can a microbial fuel cell resist the oxidation of tomato pomace?, J. Power Sour., 279 (2015) 781–790.

[3] D. Pant, G. Van Bogaert, L. Diels, K. Vanbroekhoven, A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production, Bioresour. Technol., 101 (2010) 1533–1543.

[4] P. Pushkar, A.K. Mungray, Real textile and domestic wastewater treatment by novel cross-linked microbial fuel cell (CMFC) reactor, Desal. Wat. Treat., 57 (2016) 6747–6760.

[5] Z. Ye, B. Zhang, Y. Liu, Z. Wang, C. Tian, Continuous electricity generation with piggery wastewater treatment using an anaerobic baffled stacking microbial fuel cell, Desal. Wat. Treat., 55 (2015) 2079–2087.

[6] J. Philips, K. Verbeeck, K. Rabaey, J. Arends, Electron tranasfer mechanisms in biofilms. Microbial Electrochemical and Fuel Cells: Fundamentals and Applications, Woodhead Publishing Series in Energy, 2015, pp. 67–113.

[7] C. Rojas, I.T. Vargas, M.A. Bruns, J.M. Regan, Electrochemically active microorganisms from an acid mine drainage-affected site promote cathode oxidation in microbial fuel cells, Bioelectrochemistry, 118 (2017) 139–146.

[8] G. Reguera, K.D. McCarthy, T. Mehta, J.S. Nicoll, M.T. Tuominen, D.R. Lovley, Extracellular electron transfer via microbial nanowires, Nature, 435 (2005) 1098–1101.

[9] N.S. Malvankar, M. Vargas, K.P. Nevin, A.E. Franks, C. Leang, B.C. Kim, K. Inoue, Tunable metallic-like conductivity in microbial nanowire networks, Nat. Nanotechnol., 6 (2011) 573–579.

[10] S.A. Patil, C. Hägerhäll, L. Gorton, Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems, Bioanal. Rev., 4 (2012) 159–192.

[11] A. Sikora, R. Sikora, Mikrobiologiczne ogniwa paliwowe, Biotechnologia, 2 (2005) 68–77 (in Polish).

[12] D. Permana, D. Rosdianti, S. Ishmayana, S.D. Rachman, H.E. Putra, D. Rahayuningwulan, H. Hariyadi, Preliminary investigation of electricity production using dual chamber microbial fuel cell (DCMFC) with *Saccharomyces cerevisiae* as biocatalyst and methylene blue as an electron mediator, Procedia Chem., 17 (2015) 36–43.

[13] G. Najafpour, M. Rahimnejad, N. Mokhtarian, A.A. Ghoreyshi, Bioconversion of whey to electrical energy in a biofuel cell using *Saccharomyces cerevisiae*, World Appl. Sci. J., 8 (2010) 1–5.

[14] R. Arbianti, H. Hermansyah, T.T. Utami, N.C. Zahara, I. Trisnawati, E. Kristin, The usage of *Saccharomyces cerevisiae* in microbial fuel cell system for electricity energy production, Chem. Chem. Eng., 6 (2012) 814–819.

[15] M. Rahimnejad, G. Najafpour, A.A. Ghoreyshi, H. Zare, Methylene blue as electron promoters in microbial fuel cell, Inter. J. Hydrogen Energy, 36 (2011) 13335–13341.

[16] A.L. Popov, J.R. Kim, R.M. Dinsdale, S.R. Esteves, A.J. Guwy, G.C. Premier, The effect of physico-chemically immobilized methylene blue and neutral red on the anode of microbial fuel cell, Biotechnol. Bioprocess Eng., 17 (2012) 361–370.

[17] C.N. Miller, Use of dinitrosalicyle acid reagent for determination of reducing sugar, Anal. Chem., 81 (1959) 426–428.

[18] E. Li, R.M. de Orduna, A rapid method for the determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance, Lett. App. Microbiol., 50 (2010) 283–288.