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Improvement of Yeast–Biofuel Cell Output by Electrode Modifications

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In this study, a methodology for electrodeposition of nickel nanostructures on carbon felt was developed on the base of pulse plating technique. Different in size, shape, and distribution, Ni-island nanostructures were deposited varying the potential, current, pulse duration, and cycle reiteration. The biocompatibility and nontoxicity of the newly created materials toward *Candida melibiosica* yeast cells was proven. The prepared Ni-nanomodified carbon felts were investigated as anodes in a two-chamber mediatorless yeast–biofuel cell. Maximum power density values of 720 and 390 mW/m² were achieved with the electrodes modified under galvanostatic and potentiostatic conditions, respectively, against 36 mW/m² for the nonmodified ones. The better biofuel cell performance obtained with the Ni-modified electrodes is assigned to an improved electron transfer.

1. Introduction

Biofuel cells, more popular as microbial fuel cells (MFCs), represent an innovative technology for simultaneous electricity generation and organic waste purification.^{1–4} The principle of the MFC is based on the direct conversion of the biochemical energy of living cells into electrical energy. The utilization of entire microorganisms as natural biocatalysts, the operation at ambient temperatures, and the use of neutral electrolytes and inexpensive carbon-type electrodes are the biggest advantages of the MFCs over chemical fuel cells. The low electrical output, however, is the major drawback for their wide application. The improvement of the electron transfer from the microorganisms, while oxidizing the biodegradable organic matter, to the anode is considered to be one of the most important factors for increasing the MFC efficiency. Physically, the extracellular transport of the electrons toward the anode can either occur through the use of soluble electron shuttles or through membrane-bound electron shuttling compounds.² An advance in the understanding of the electron transfer mechanisms has been recently achieved discovering that some metal reducing microorganisms like Fe(III) reducing bacteria can realize a direct electron transfer using the anode as a final electron acceptor.^{5,6} In the natural environment, these microorganisms produce energy for their growth and reproduction by coupling the oxidation of organic compounds to the reduction of insoluble metal and metalloid oxides.⁷ Unlike natural external electron acceptors such as Fe(III) or Mn(IV) oxides, the anodes in MFCs do not participate in mineral dissolution reactions, and the electron transfer rates can be estimated. The anodes also provide a stable source of electron acceptor and do not generate reduced products.⁸ The microorganisms, which are able to transfer electrons from reduced substrates to a solid electrode as part of their energy-generating respiration, are known as anode-respiring bacteria.⁹

The achieved up to now MFC power outputs are ranging within over 3 orders of magnitude—from milliwatts per square

meter to several watts per square meter.³ The obtained operational characteristics—cell voltage, current, and power density—depend on many variables, which may be divided into two groups: (i) MFC type (single or two-chamber), construction (batch or flow mode), and used components (electrodes, membranes) and (ii) used biocomponents—pure microbial culture or microbial society, cultivation conditions including medium and substrates, pH, temperature, use of artificial soluble electron mediator, etc.

As a novel technology developed to decrease the energy cost of the wastewater purification, the most of the reported results are obtained with prokaryotic strains associated to water reservoirs: from genera *Geobacter* (*Geobacter sulfurreducens*, *Geobacter metallireducens*), *Desulfuromonas* (*Desulfuromonas acetoxidans*), *Shewanella* (*Shewanella putrefaciens*), etc.^{2,3,6,10} Investigations with *Thermuncola* strain JR and *Geobacillus* strain S2E show that, members of *Firmicutes* produce current comparable or even higher than that of either *Geobacter* or *Shewanella* MFC communities.⁸ New strains like *Rhodoferax ferrireducens*,¹¹ capable of oxidizing various substrates while at the same time efficiently producing a continuous, usable electrical current, are on and on discovered.

The application of eukaryotic yeast cells in MFCs is still rare. Investigations with the yeast species *Saccharomyces cerevisiae*, *Hansenula anomala*, and *Arxula adeninivorans* have been reported.^{4,12–15} Recently, we have found that *Candida melibiosica* 2491, a special strain of Candida yeast, appropriate for purification of hard plant waste rich of phytate, possesses electrogenic properties and could be used as a biocatalyst in MFC.¹⁶ For improvement of the electron transfer in yeast MFCs, a supplement of exogenous mediator is usually needed. However, the addition of artificial mediators is not preferable as they are generally too expensive and they could be toxic at higher concentrations or can be degraded over longer time periods.^{12,16–18}

Of all components of MFC, the electrode materials play a crucial role in the electricity generation.¹⁹ Although much research has focused on cathode modification and optimization of bacteria inoculation,²⁰ according to Qiao et al. the anode modification results in considerable contribution to the overall MFC performance.²¹ The anode material and its structure can significantly affect bacteria attachment, electron transfer, and

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substrate oxidation.²² Up to now, various carbon materials such as cloth, felt, paper, and rods are most commonly applied as MFC anodes due to their biocompatibility, chemical stability in a microbial inoculum mixture, and good conductivity. However, these materials possess small electrocatalytic activity toward the anode microbial reactions. Thus, modification of the carbon electrodes is the main approach to improve their performance. It is worth noting that the electrocatalytic effects of different metal modified carbon materials have not been widely examined yet.

Nickel is one of the essential microelements participating in several metabolic pathways as a cofactor for the enzymes involved. Ni-containing enzymes catalyze five distinct biological activities including reversible hydrogen oxidation, interconversion of carbon monoxide and carbon dioxide, methane generation, urea hydrolysis and superoxide dismutation.²³ Like other transition metals, however, excess Ni is toxic to cells; thus, synthesis of these Ni-enzymes requires the presence of carefully controlled Ni-processing mechanisms that range from selective transport of Ni into the cells to productive insertion of Ni into the apoproteins.²⁴ From another point of view, nickel-based materials have found important industrial applications as electrocatalysts in batteries, fuel cells, electrolyzers, and electrosynthesis devices due to the well-established surface oxidation properties of nickel.^{25–27} Up to now, however, nickel-based or modified electrodes have been rarely tested in MFCs.²⁸

The aim of this study was to develop methods for electrodeposition of nickel-island structures on carbon felt and to examine the performance of the prepared nanomodified materials as anodes in mediatorless yeast–biofuel cell. In order to evaluate the electrocatalytic properties of modified materials, the recorded MFC operational characteristics—open cell voltage (OCV), maximum power, and current density—were compared with those obtained with nonmodified carbon felt anodes.

2. Materials and Methods

2.1. Electrodeposition of Nickel Nanostructures on Carbon Felt.

Nickel was electrodeposited on carbon felt (SPC-7011, 30 g/m², Weiβgerber GmbH & Co. KG) by a pulse plating technique, varying the voltage, current, and pulse duration. A nickel sulfamate electrolyte containing 80 g/L Ni²⁺, 35 g/L H₃BO₃, and 10 g/L NiCl₂ (pH 4) was used. The electrolysis was carried out in a conventional three-electrode cell with platinum–titanium mesh counter electrode and saturated calomel electrode (SCE) as a reference using a Gamry G750 potentiostat–galvanostat (Gamry Instruments, US). The electroplating bath temperature was kept constant at 50 °C by thermostat. The following three regimes were determined as appropriate for deposition of nickel island structures:

- (i) double potentiostatic pulse ($E_1 = -1.8$ V vs SCE for 0.5 s and $E_2 = -0.8$ V vs SCE for 10 s);
- (ii) multiple potentiostatic pulse ($E_1 = -1.8$ V vs SCE for 0.25 s and $E_2 = -0.2$ V vs SCE for 0.25 s, 10 cycles);
- (iii) multiple galvanostatic pulse ($i_1 = -40$ mA/cm² for 1 s followed by 1 s pause, 10 cycles).

Scanning electron microscopy (SEM) using Leo 1455VP and Leo Supra 55VP microscopes with energy dispersion X-ray (EDX, Oxford Inca 200 instrument, Software INCA-Vers.4) was applied for characterization of the surface morphology of modified carbon felt materials. The samples were examined before and after being used as electrodes in MFC. In the latter case, 1 nm-thick Pt/Pd layer was sputter-coated onto the specimens using a Cressington Ressington Sputter Coater 208 HR system.

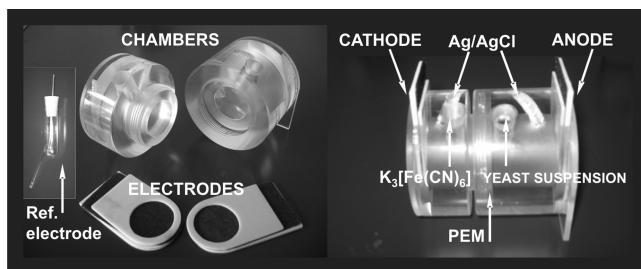


Figure 1. Two-chamber MFC used in the bioelectrochemical experiments.

2.2. Cell Cultivation and Cytotoxicity Test.

Candida melibiosica 2491 was cultivated in YP_{fru} medium in an incubator at 28 °C using an orbital shaker, 120 rpm, for receiving of cell biomass quantity. The YP_{fru} medium consisted of 0.5% yeast extract, 0.8% peptone, 167 mM fructose, pH 7. The cells were collected by centrifugation at 5000g for 10 min and washed twice, and the concentrate was kept under distilled water at low temperature (4 °C) for culture synchronization under starving conditions. The cell vitality was examined microscopically using 0.4% trypan blue dye solution and a Trinocular Microscope Magnum-T equipped with an Si-3000 camera. The yeast biomass quantity was determined spectrophotometrically by measurement of optical density at $\lambda = 600$ nm. A unified quantity of yeast concentrate, corresponding to 1 g/L yeast cells, was used as inoculum for biofuel cells experiments.

The newly synthesized nickel–carbon felt materials were analyzed for cytotoxicity toward yeast cells by applying the agar diffusion assay,^{29,30} but with the following modification: instead paper discs, specimens electrodeposited under both galvanostatic and potentiostatic conditions of modified Ni–carbon felt as well as Ni-wire and nonmodified carbon felt were used. The samples were put in a contact with the yeast monolayer on agar medium and then incubated at 28 °C for at least 48 h. The cells’ growth was comparatively characterized by morphology, viability, and proliferation. At the same time, the potential cytotoxicity of the Ni²⁺ ions to the yeast cells was also tested. The yeast cultivation was carried out as upper described with addition of Ni²⁺ (NiSO₄) in concentrations up to 100 nmol/L, corresponding to the quantity of the electrodeposited nickel on carbon felt. The cell development in the presence and absence of Ni²⁺ ions was followed up and compared by measuring the yeast culture optical density OD₆₀₀ with time.

2.3. Yeast–Biofuel Cell Studies.

The electrocatalytic effects of the island type nickel modifications were studied by using the nanomodified carbon felt materials as anodes in a recently designed two-chamber MFC (Figure 1).

The tested carbon felt sample was fixed in a plastic holder and assembled in the anodic compartment of the fuel cell. The one-side round-shaped hole of the holder assured a projected surface area of 4.5 cm², exposed to the anolyte. A sheet of nonmodified carbon felt with the same dimensions as the anode was assembled in the cathodic compartment. For simplification of the next expose, the electrodes modified by galvanostatic pulse deposition, potentiostatic pulse technique, and nonmodified ones will be denoted as GME, PME, and NME, respectively. A buffered suspension of *Candida melibiosica* 2491 yeast cells in YP_{fru} medium (pH 7) was used as an anolyte in a batch operation mode and 0.1 M potassium ferricyanide served as a terminal electron acceptor in the cathode compartment. The anodic and cathodic chambers, each with a volume 13 cm³, were separated by a proton-exchange membrane (Nafion 117, DuPont).

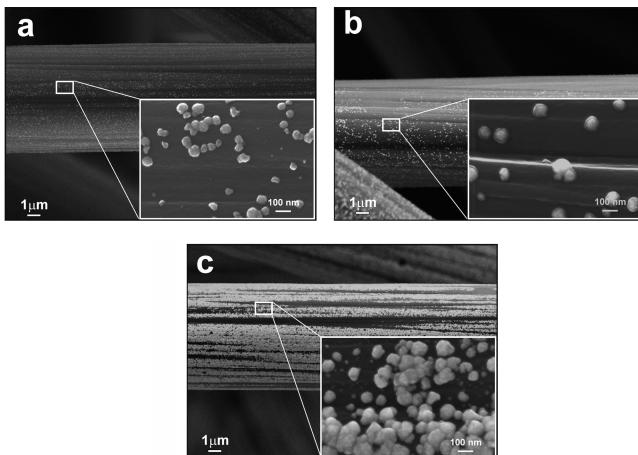


Figure 2. SEM images of nickel-modified carbon felt obtained under: (a) double potentiostatic pulse; (b) multiple potentiostatic pulse; (c) multiple galvanostatic pulse conditions.

In the progress of the yeast culture development, polarization measurements under different loads ranging from $100\text{ k}\Omega$ to $10\text{ }\Omega$ were carried out at each 6 h by using a decade resistor box. The external resistances were changed through 2 units at each decade. The cell voltage was recorded 5 min after switching a given resistance by using digital multimeter DMM2700 (Keithley Instruments Inc., US). Current density, i (A/m^2), was calculated as $i = V/RA$, where V (V) is the cell voltage recorded, R (Ω) is the external resistance applied, and A (m^2) is the projected electrode surface area. For each pair $V-i$, the power density, P (W/m^2), was estimated according to $P = iV$. The obtained experimental data were plotted as polarization curves V vs i and power curves P vs i .

Fed-batch operation MFC experiments with continuously switched constant load resistance ($1\text{ k}\Omega$) were also carried out. Every 24 h, half of the anolyte suspension was replaced with a fresh yeast-free YP_{fru} medium. During these experiments, the anode potential was monitored with time.

Cyclic voltammetry (CV) experiments were performed by switching the MFC anode as a working and the cathode as a counter electrode. The working electrode potentials were measured against Ag/AgCl reference. CV measurements of Ni-modified carbon felt as well as massive nickel in a phosphate buffer (Phi, pH 7) and YP_{fru} medium without yeast cells were also carried out. At the end of the MFC experiments, the yeast suspension (anolyte) was centrifuged at 5000 g for 10 min, and the obtained fractions—the supernatant, containing exhausted medium and excreted metabolites, and the cell pellet, washed twice and resuspended in the same volume fresh YP_{fru} medium, were analyzed by means of CV. Usually, a scan rate of 2 mV/s was applied. CV experiments were carried out using a PJT 35-2 potentiostat-galvanostat (Radiometer-Tacussel) with an IMT 101 electrochemical interface and VoltaMaster 2 data acquisition system.

3. Results and Discussion

Nickel island nanostructures different in size and distribution were produced by using the above described three regimes of electrodeposition. According to the double pulse method,³¹ the first pulse $E_1 = -1.8\text{ V}$ vs SCE was sufficient to create nuclei and the subsequent second one was essential for the growth of the already existing nuclei. Under such conditions, the size of deposited nickel crystallites varied between 20 and 70 nm (Figure 2a). Decreasing the amplitude and the duration of the

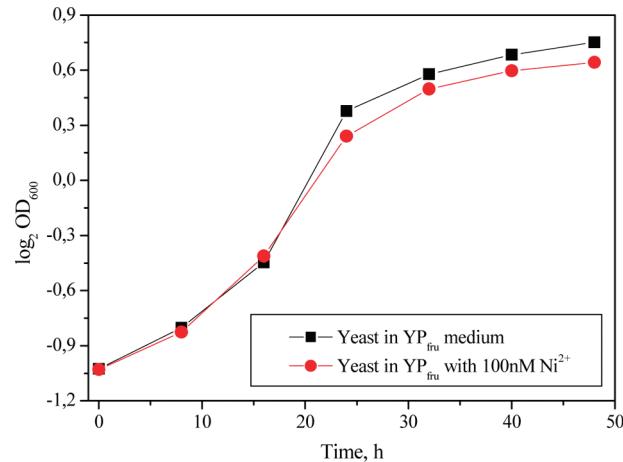


Figure 3. Development of *Candida melibiosica* in YP_{fru} medium in the presence and absence of Ni^{2+} ions.

second pulse and increasing the number of reiterations, bigger size crystallites (about 100 nm) disposed on rare occasions have been obtained (Figure 2b). A formation of dense deposit from separate nanocrystallites was achieved under galvanostatic conditions by alternation of cathodic current pulses ($-40\text{ mA}/\text{cm}^2$) and power breakdowns (Figure 2c).

The specific application of new materials in MFCs requires an implementation of preliminary cytotoxicity analysis. The applied agar diffusion assay showed that the yeast cells, which were in a contact with the Ni-modified carbon felt materials on agar medium, grew unchanged. No zones of inhibition of cells' proliferation were observed, which proved the yeast viability in the presence of the examined materials. In addition, the development of *Candida melibiosica* 2491 yeast strain in YP_{fru} medium without and with the addition of Ni^{2+} ions was followed up by means of spectrophotometric analyses. The presence of Ni^{2+} in the yeast suspension does not disturb significantly the cell development, and it is commensurable to the growth of the yeast/ YP_{fru} control (Figure 3). The observed subtle distinction is in the limits of the normal diversion of culture's development. The resistance of *Candida* yeast to nickel ions could be explained with the known ability of fungi and microorganisms to prevent heavy metals' toxicity by production of metal-binding proteins, organic and inorganic precipitation, active transport, and intracellular reorganization.³² At high nickel concentrations, *Candida* species are able to switch on mechanisms for restriction of metal entry into the cells by (i) reducing metal uptake or increasing metal efflux; (ii) metal immobilization, e.g., cell wall adsorption, extracellular precipitation of secondary minerals; (iii) extracellular metal sequestration by exopolysaccharides and other extracellular metabolites.³³ On the basis of the obtained results from the conducted cytotoxicity tests, it has been concluded that the prepared Ni-modified carbon felt materials are nontoxic and biocompatible toward *Candida melibiosica* 2491 yeast cells and may be applied as anodes in a yeast–biofuel cell.

The examination of the prepared nanomodified Ni-carbon felt materials as anodes in a mediatorless yeast–biofuel cell has shown that in the progress of MFC tests the operational characteristics (OCV, maximum power, and current density) increase, reaching maxima at the late log-phase and the beginning of stationary phase (24th to 30th hour) of the yeast culture development. Despite the observed variations in nickel islands' size and distribution, the results obtained with the anode materials modified under the both potentiostatic regimes show unsubstantial differences (less than 10%), which determines the later on presentation and discussion of only one type of them

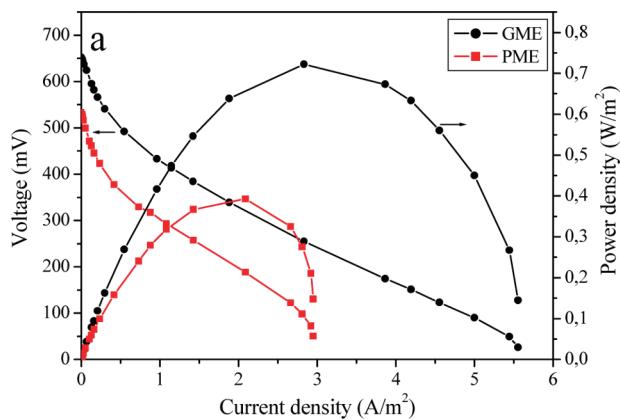


Figure 4. Polarization and power curves of a *Candida melibiosica* yeast–biofuel cell by using (a) GME, PME and (b) NME, NME + 0.9 mM Methylene Blue, as anodes.

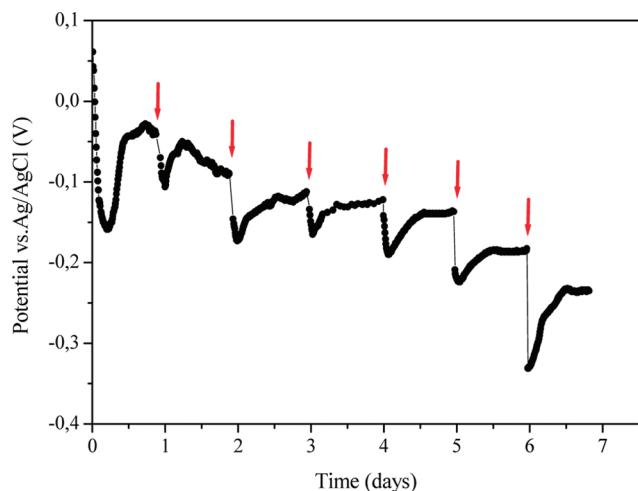
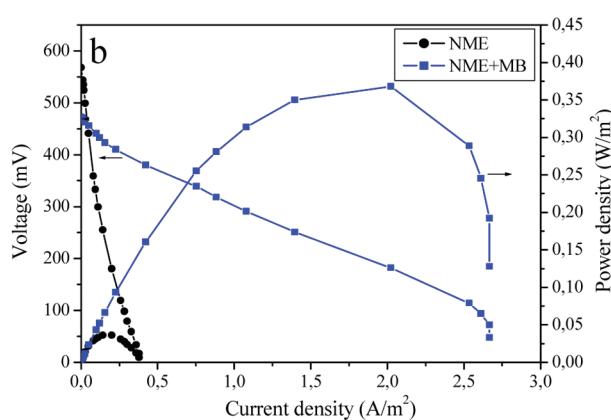


Figure 5. Variation of potential of modified MFC anode (GME) with time in a fed-batch operation mode. The arrows indicate the refreshment of the anolyte.

(related to Figure 2a). Comparing the MFC performance using GME and PME, higher operational characteristics were achieved with the anodes modified under galvanostatic conditions. Typically, after inoculation of the MFC the OCV values stabilized at about 400 mV for the both types of modified anodes. In the progress of the experiment, the OCV grew up to 660 and 540 mV when using GME and PME, respectively. The recorded polarization curves, presented in Figure 4a, reveal that the use of former electrodes results in lower internal cell resistance, which may be assigned to the higher nickel loading and better conductivity of the modified carbon felt in this case. The better performance of GME also finds expression in almost twice higher short circuit current density of 5.5 A/m^2 in comparison with 3 A/m^2 for PME and fifteen times higher than 0.38 A/m^2 for NME (Figure 4b). The highest maximum power density values of 720 and 390 mW/m^2 were achieved with GME and PME, respectively, against 36 mW/m^2 for the NME. These values exceed even those obtained when the artificial mediator MB was added to the anolyte suspension and nonmodified carbon felt anode was used (Figure 5b).

The MFC outputs grew up to the 30th hour of inoculation, then the power and current densities began to diminish. Because the experiments were carried out in a batch mode, the possible explanation of the performance deterioration may be assigned to an exhaustion of nutritious ingredients in the medium as well as to a cell density inhibition. In order to eliminate the effect of these factors, we performed experiments in a fed-batch

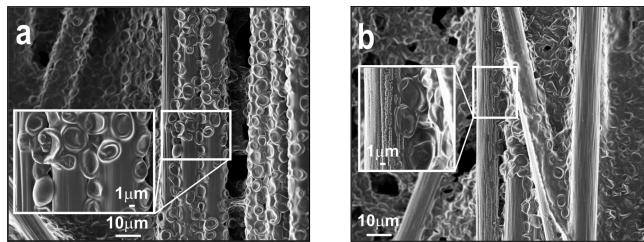


Figure 6. SEM images of *Candida melibiosica* biofilm on the surface of (a) NME and (b) GME.

operation mode, in which the MFC was continuously loaded with $1 \text{ k}\Omega$ external resistance and half of the yeast suspension was replaced with a fresh medium every 24 h. When working with a fixed external resistance, the evolution of the electrode's potential as a function of microorganisms' kinetics can be monitored.³⁴ Usually, at the beginning of such an experiment, a steep drop of the anode potential in the negative direction was observed (Figure 5). Similar decreases of up to 500 mV of the anode potential in an MFC have been observed within just a few hours,^{35,36} which indicates that communities adapt quickly to large fluctuations in the potential of the electron acceptor. The subsequent opposite shift and stabilization of the potential may be connected with the ability of the microorganisms to drive the system toward an attainable activity that allows energy capture for growth and maintenance.³⁷ The repetitive half-replacement of the anolyte with fresh yeast-free medium results in a similar variation of the anode potential with a tendency toward decrease of the steady-state values, which is a precondition for long-term MFC operation.

The anode biofilm formation is considered to contribute to more effective electron transfer mechanism.^{1–3,6,10} The SEM observations of modified and nonmodified carbon felt specimens that have been applied as anodes in MFC confirmed the formation of yeast biofilm on the electrode surface. The SEM images, shown in Figure 6, illustrate the aptitude of *Candida melibiosica* rapidly to create biofilm on a carbon surface. At the 30th hour of cultivation, the biofilm is not in a matured phase yet and the yeast cells are keeping on germinating and spreading on the electrode surface. An oval shape and a size of *Candida melibiosica* cells up to $7 \mu\text{m}$ have been determined. It should be pointed out that the yeast cells grow well and gemmate both on nonmodified and Ni-modified carbon felt anode. The observed budding cells on the GME surface (Figure 6b) demonstrate once again the biocompatibility of this strain to the electrodeposited nickel. Harrison et al. supposed that the biofilm formation may be one of the strategies for metal

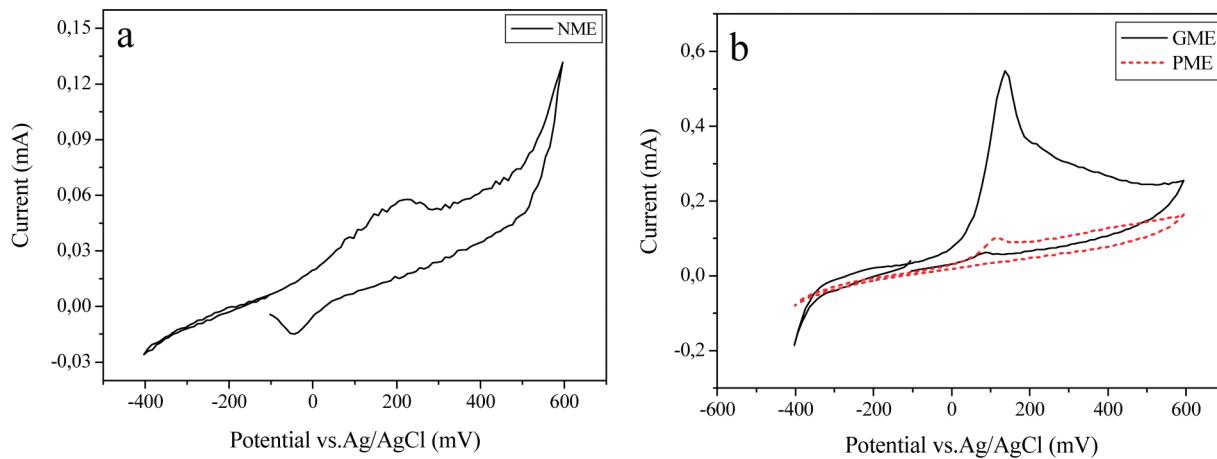


Figure 7. Cyclic voltammograms of *Candida melibiosica* yeast/YP_{fru} suspension after 24 h yeast-MFC operation obtained with (a) NME; (b) GME and PME. The scan rate was 2 mV/s.

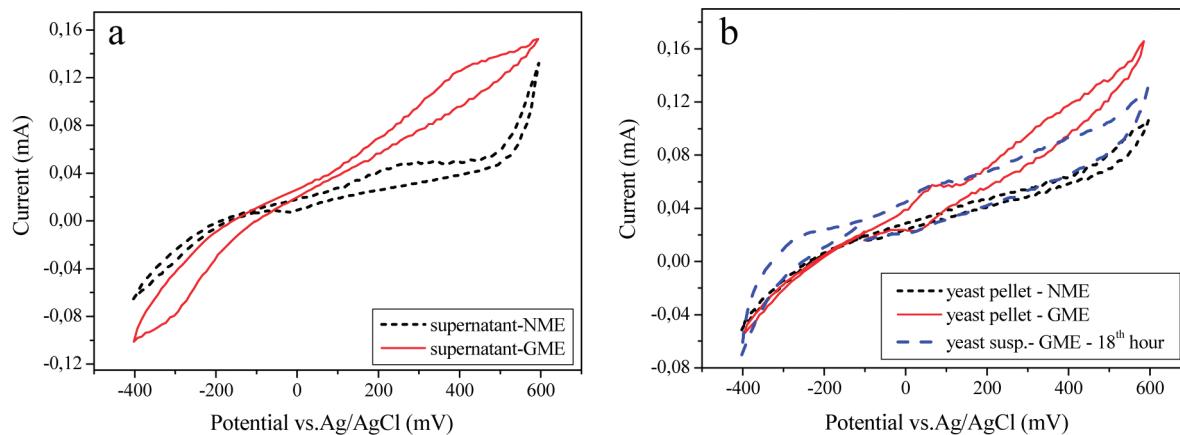


Figure 8. Cyclic voltammograms of (a) the supernatant obtained by centrifugation of the anolyte (*Candida melibiosica* yeast/YP_{fru} suspension); (b) resuspended yeast cells after 30 h MFC operation with NME and GME anodes. The CV of yeast suspension with GME recorded at the 18th hour of MFC operation is shown for comparison. A scan rate of 2 mV/s.

resistance and/or tolerance of yeasts.³⁸ Metal–chelator precipitates could be formed in biofilms following exposure to the heavy metals like Ni²⁺ and Cu²⁺.³⁸ This suggests that *Candida* cells may adsorb metal cations from their surroundings and that sequestration in the extracellular matrix may contribute to resistance. Moreover, this natural ability of *Candida melibiosica* probably assists the achieved improvement of MFC outputs.

Recently, cyclic voltammetry is more frequently used to study the complex electron transfer phenomena between living microorganisms and electrodes in MFCs.^{39,40} In this study, CV measurements were performed to elucidate the differences in the performance of Ni-modified and nonmodified carbon felt materials as MFC anodes. Cyclic voltammograms recorded at times corresponding to the optimum MFC characteristics achieved are presented in Figure 7. The CV patterns obtained with *Candida melibiosica* yeast suspension/nonmodified carbon felt electrode are characterized by appearance of anodic and cathodic peak at +225 and -45 mV vs Ag/AgCl, respectively (Figure 7a). Similar quasi-reversible behavior has been also observed on graphite electrodes.¹⁶ In a contrast, when Ni-nanomodified carbon felt electrodes were applied, only an anodic peak with a long shoulder after the maximum appeared in the voltammograms (Figure 7b). The position of this anodic peak is at more negative potentials than that obtained with NME, which indicates a participation of different electroactive species in the electrochemical reaction in the case of modified and nonmodified electrodes. At the same time, the anodic peak

heights achieved with the different electrodes decrease in the order GME–PME–NME corresponding to their performance as MFC anodes.

To get more information concerning the observed electrochemical behavior, CV measurements of the fractions, obtained by centrifugation of the anolyte suspension after the end of MFC experiments, were also carried out. CV plots recorded with the collected supernatants and resuspended cell pellets are presented in Figure 8. An anodic and a cathodic peak at the same potentials as those associated with the quasi-reversible behavior of the yeast suspension/NME system (Figure 7a) appeared only in the voltammograms obtained with the correspondent supernatant (Figure 8a), but not with the resuspended yeast pellets (Figure 8b). This suggests that the electrochemical activity of the NME bioanode is due to oxidation/reduction of excreted metabolite (endogenously generated mediator) in the medium. The cyclic voltammograms obtained with the fractions from anolytes contacted with Ni-modified electrodes, however, have not given clear evidence for the electron transfer mechanism. A not well-shaped anodic peak at more positive potentials than that of yeast suspension/Ni-modified carbon felt appeared in the voltammograms recorded with supernatant (Figure 8a). Surprisingly, a reversible electrochemical behavior, characterized by the appearance of oxidation and reduction peaks at +85 and +25 mV (vs Ag/AgCl), respectively, was observed for the yeast pellets (Figure 8b). A similar CV performance of the yeast suspension/Ni-modified anode has been noticed at earlier periods (between

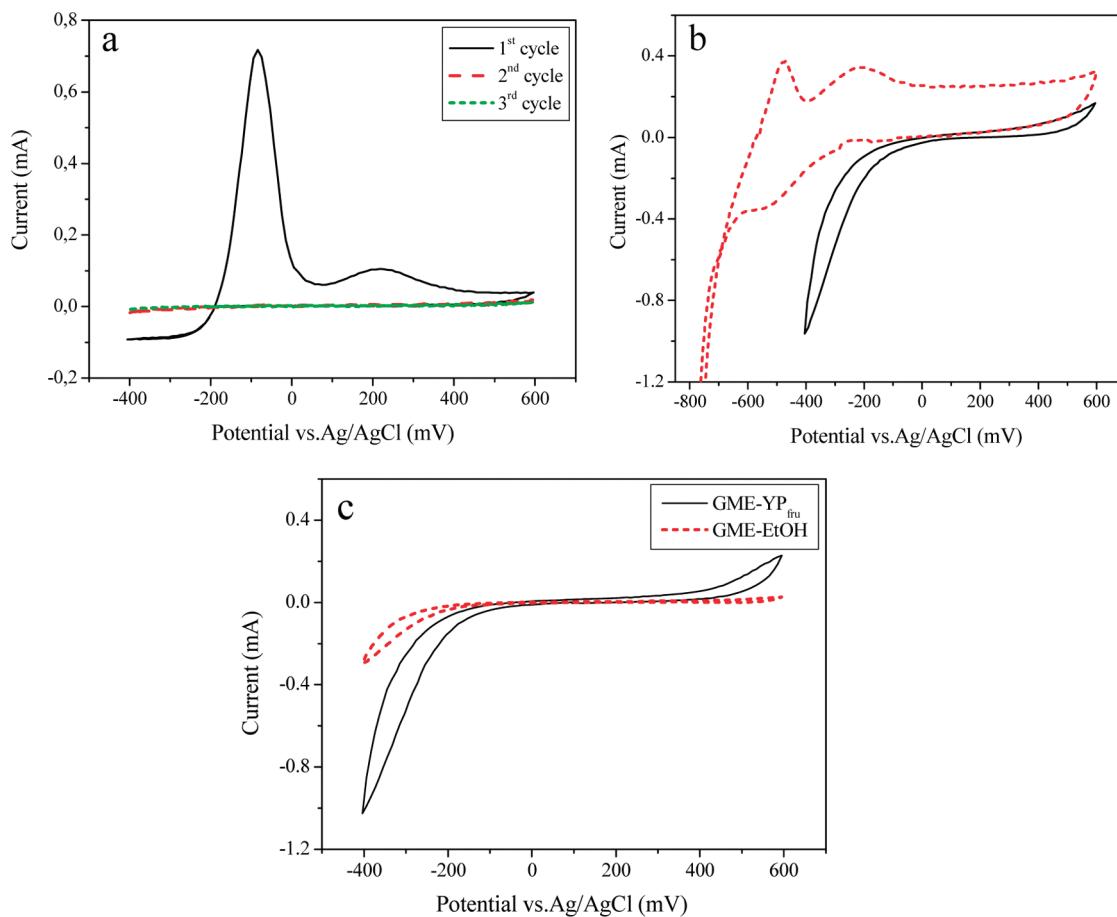


Figure 9. Cyclic voltammograms of (a) massive nickel in phosphate buffer (pH 7); (b) GME in phosphate buffer (pH 7) with different cathodic switch potentials; (c) GME in YP_{fru} and 5%EtOH/Phi, pH 7. The scan rate is 2 mV/s.

the 12th and 18th hour) of yeast-MFC operation, but at the 24th hour and later, the system is characterized by the irreversible behavior, shown in Figure 7b.

To examine the hypothesis for participation of the electrode-deposited nickel in the anodic reaction, the CV behavior of Ni-modified carbon felt as well as massive nickel electrodes in neutral phosphate buffer solution and YP_{fru} medium without yeast cells was studied. The obtained voltammograms are shown in Figure 9.

Two anodic peaks, corresponding to electrooxidation of metallic nickel most probably to Ni(II) and Ni(III), appear in voltammograms obtained with massive nickel during the first scan in the potential range between -400 and +600 mV vs Ag/AgCl (Figure 9a). In the subsequent cycles, these peaks disappear, which indicates a formation of an insoluble protective film on the nickel surface. At the same potential range, no peaks are present in the voltammograms obtained with the electrode-deposited Ni-carbon felt electrodes (Figure 9b, solid line). The possible explanation of this behavior is that the dispersed nickel has already been oxidized, while exposed to the air. When the potential sweep starts from more negative values, however, two anodic and one cathodic peak appear in the voltammograms (Figure 7b, dashed line). We consider that while passing through the potential region where hydrogen evolution reaction takes place, the evolved hydrogen reduces the oxidized nickel on the surface, and then, when the potential is swept in the anodic direction the reduced nickel undergoes subsequent electrooxidation to different oxidation states. The cathodic peak in the next reverse scan corresponds to reduction of oxidized Ni from higher to lower oxidation state. Similar differences in the CV

behavior of massive and deposited nickel (peak potentials, peak separations, and peak current ratios) have been often observed and contributed to the thickness and dispersion of the deposition, depending on the method of preparation as well as the nature and surface area of the support.⁴¹ It is worthwhile to note, however, that none of the observed peaks in the voltammograms recorded with Ni-modified electrodes in the presence of yeast cells (Figures 7b and 9b) coincide with those obtained in buffer solution (Figure 9b). Therefore, the electrochemical behavior of the yeast suspension/Ni-modified electrode system could not be assigned to a direct electrooxidation of the electrodeposited nickel. Furthermore, the absence of peaks in the CVs obtained with modified carbon felt electrodes in the YP_{fru} medium or buffered solution of alcohol without yeast cells (Figure 9c) indicates that neither the medium ingredients nor the product of fructose fermentation undergo electrochemical oxidation on this type of anode.

The observed differences in the electrochemical activity of modified and nonmodified electrodes more probably suggest a change of the yeast metabolism in the presence of nickel resulting in the expression of specific substance(s) and formation of outer-membrane associated metalloprotein(s).⁴² We suppose that nickel in different oxidation states improves the electron transportation via two possible mechanisms: acting as an electron acceptor, similar to the interaction between metal reducing bacteria and insoluble metal oxides,⁷ and/or due to adaptive mechanisms as a response to Ni²⁺, resulting in facilitated electron transfer across the cell membrane. Other studies also indicate the capability of anode-respiring cultures to adapt their metabolism and the mechanisms of electron

transfer to changes in the anode potential in order to maximize the biological energy gain.⁴³

4. Conclusion

On the basis of the obtained results in the present study, it may be concluded that the application of a pulse plating technique provides the opportunity for preparation of new biocompatible nickel-nanomodified carbon felt materials, possessing high electrocatalytic activity as anodes in a yeast–biofuel cell. The generated power densities using Ni-modified carbon felt anodes exceed over an order of magnitude those obtained with nonmodified ones and are comparable or even higher than values achieved by other yeast–biofuel cells reported until now. The improved performance of the modified electrodes is associated with the presence of nickel on the anode surface, switching over specific cell metabolic processes, facilitating the electron transfer mechanism.

Further investigations, aiming at optimization of electrode-deposits' composition and structure, electrode conductivity and stability, better understanding of the possible mechanisms of interactions between the microorganisms and the modified materials, and identification of secreted metabolites as well as long-term MFC-experiments, are in progress.

Acknowledgment

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