Chapter 3

The Anode Biocatalyst with Simultaneous Transition Metals Pollution Control

Oleksandr Bilyy, Oresta Vasyliv and Svitlana Hnatush

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/58347

1. Introduction

The need for reducing dependence on fossil fuels and the promoting the use of renewable fuels requires the development of alternative sources such as waste biomass for environmental benefits and alternative global energy supplies [1]. Microbial fuel cells (MFCs) provide new opportunities for the sustainable production of energy from biodegradable and reduced compounds, and thus, have attracted substantial research efforts to develop various devices for generating electricity and removing wastes [1, 2]. The development of processes that can use bacteria to produce electricity represents a highly effective method for bioenergy production as the bacteria are self-replicating, and thus the catalysts of organic matter oxidation are self-sustaining [2]. The substrates used in MFCs range from carbohydrates (e.g. glucose, sucrose, cellulose, starch), volatile fatty acids (e.g. formate, acetate, butyrate), alcohols (e.g. ethanol, methanol), amino acids, proteins and even inorganic components such as sulfides or acid mine drainages [2, 3-9]. The type of substrate fed to a MFC potentially has an impact on the structure and composition of the microbial community. Untill now, no clear image of the effect of the type of substrate on electricity generation by the microbial fuel cells is available.

Analysis of external resistances, electron donor concentrations, cell densities, rates of electron transfer to electrodes at various voltages, and anode potentials can aid in understanding the power production capabilities using microorganisms [10]. A simplified model for the conversion of complex organic fuels to electricity is shown in figure 1 [10].

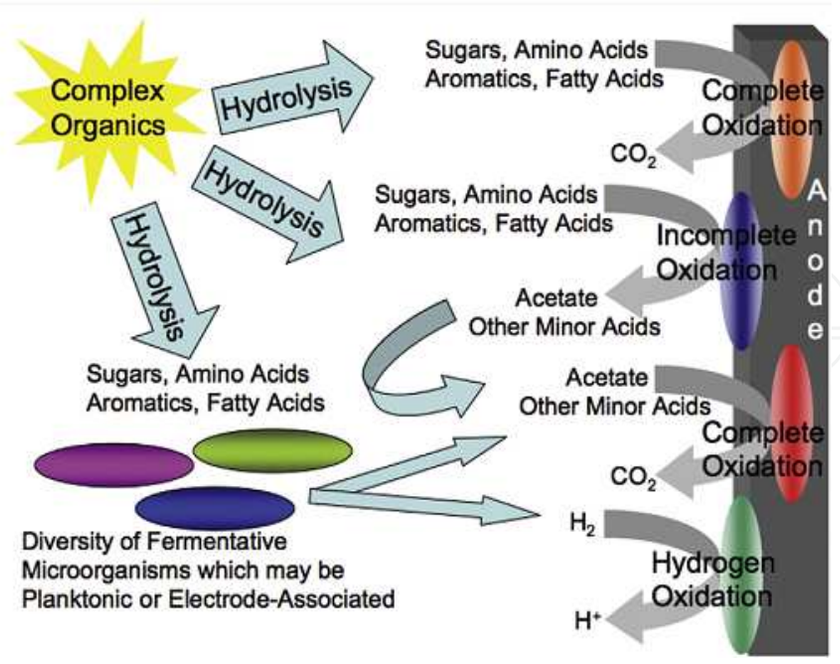

Figure 1. Usage of organic substrates as electron donors in microbial fuel cell [10]

Complex organic matter is hydrolyzed to constituents that in the most cases are primarily fermented, but there are microorganisms that can completely oxidize such compounds with an electrode serving as the sole electron acceptor or incompletely oxidize these substrates with electron transfer to an electrode [10]. Acetate and some other minor fermentation acids can be completely oxidized to carbon dioxide and it is typically the primary source of electrons for current production [10].

MFC is considered to be used also for hydrogen production from the generated potential of the organic matter electrolysis by bacteria [2].

Microbial fuel cell technologies also are a promising and yet completely distinct approach to wastewater treatment as the treatment process can become a method of capturing energy in the form of electricity or hydrogen gas, rather than a drain on electrical energy [2]. Wastewater treatment processes currently employ the biological activities of complex microbial biofilms to remove organic pollutants [11]. The most significant energy savings associated with the use of MFCs for wastewater treatment, besides electricity generation, result from savings in expenses for aeration and solids handling, because the major operating costs for wastewater treatment are wastewater aeration, sludge treatment, and wastewater pumping. The MFC process is inherently an anaerobic process, although, oxygen can diffuse into the system resulting in some aerobic organic matter removal [10].

At the same time, wastewater contains high concentrations of xenobiotics, such as heavy metal ions that have an overwhelming harmful effect towards all living organisms. These substances even in small concentration in the environment cause the increasing inhibition of physiological and biochemical properties of the most bacteria. Despite that, some genera of bacteria possess high resistance according to toxic heavy metals influence because of functioning of highly-

http://dx.doi.org/10.5772/58347

efficient mechanisms of antioxidant defense system, ion efflux transport enzyme complexes etc. Examples of these genuses are *Feroplasma*, *Streptomyces*, *Thiobacillus*, *Desulfuromonas* etc [12]. The specific metal-transport systems that were found in gram-negative bacteria which support transport of iron, zinc and manganese, copper, nickel and cobalt are schematically presented in figure 2 [13].

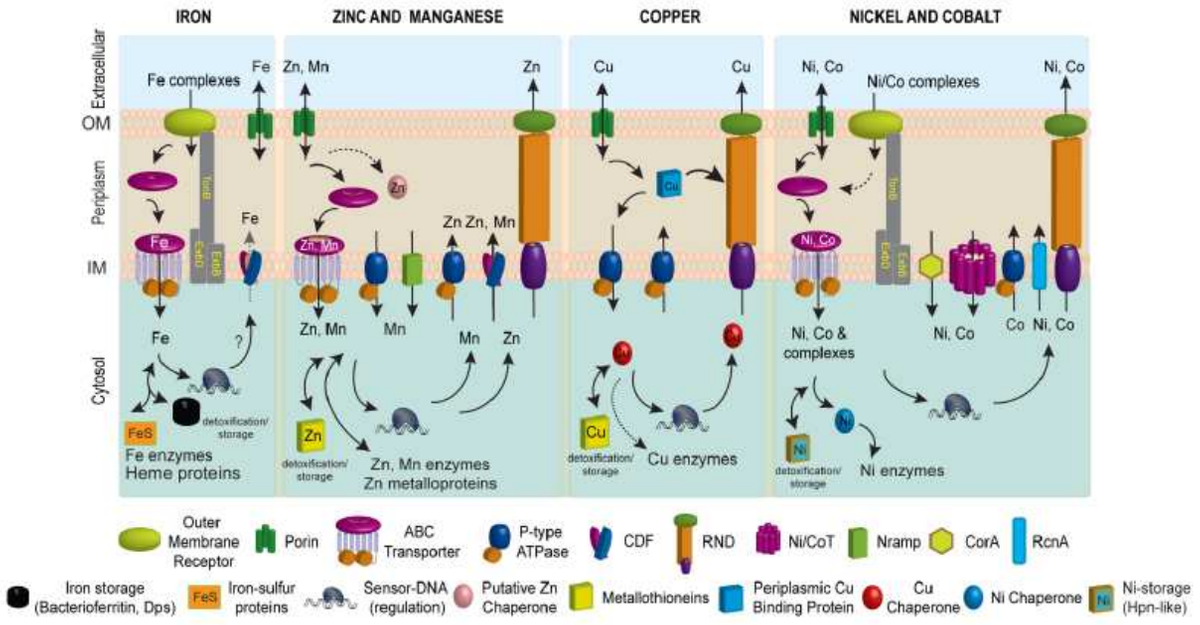

Figure 2. Schematic metal homeostasis models for iron, zinc and manganese, copper, nickel and cobalt in gram-negative bacteria [13]

The variety of ion transport systems in gram-negative bacteria represents sophisticated mechanisms of bacterial cell metal homeostasis regulation. This is possible because of the formation of specific protein-metal coordination complexes used to effect uptake, efflux, intracellular trafficking within compartments, and storage [12].

Previous research has shown that gram-negative bacterium *Desulfuromonas acetoxidans* possesses resistance to copper, cadmium, lead, zinc, manganese, iron, etc. [14, 15]. Bacteria of *Desulfuromonas* genus are also shown to be highly effective in term of electricity generation [2, 16, 17]. However the research on its application as the anode biocatalyst in MFC is inadequate. *Desulfuromonas* sp. can be very promising for MFC development because of inexpensive cultivation medium, high survival rate and resistance to toxic xenobiotics such as the various metal ions [2].

D. acetoxidans, which belongs to the class ∂-*Proteobacteria,* are uncolored obligate anaerobes that inhabit sulfur containing aquatic environments [18]. *D. acetoxidans* supports reductive stage of sulfur cycle in the nature, but it can not possess an ability to reduce sulfate or other oxoanions of this element. Sulfur is a crucial component of various biological active substances, such as

vitamins, coenzymes, several amino acids etc. This is an element with variable valence, which participates in the different chemical and biochemical redox-reactions [19]. Sulfur deposits formation is tightly bound with the process of sulfate-reduction, which is carried out exclusively by microorganisms. $SO_4^{2-}$is the prevalent hydrogen acceptor in the processes of organic matter destructions under anaerobic conditions. Majority of strains can use unspecific electron acceptors, such as L-malate or fumarate, instead of sulfur [20]. S^0-reduction by *D. acetoxidans* causes hydrogen sulfide production. Using metal-resistant strains of these bacteria also helps overcome H_2S toxicity since divalent cations will interact with sulfide ions, forming insoluble precipitates in form of metal sulfides. *D. acetoxidans* contains NiFe-hydrogenase [20], which catalyses hydrogen uptake and production; polysulfide reductase [19], which supports sulfur reduction with hydrogen sulphide formation, and specific metaloreductase that reduce $Fe^{3+}$and Mn^{4+} with formation of magnetite (Fe_3O_4), siderite ($FeCO_3$) and rhodochrosite ($MnCO_3$) as final products of Fe (III)-and Mn (IV)-dissimilative reductions [21].

Several redox-proteins have been elucidated of the cells of *D. acetoxidans* [22]. This bacterium, similarly to *Desulfovibrio* sp., contains huge amount of cytochromes *c*-type, which possibly are involved in the electron transport to the elemental sulfur. Multiheme cytochromes *c*-type are shown to possess metaloreductase activity, which possibly could have practical application in metal ions bioremediation from the environment. It was found that electric current production in MFCs operated with different organisms such as *Shewanella oneidensis*, *Pelobacter carbinolicum* or *Geobacter sulfurreducens* requires the presence of multiheme cytochromes *c* [23, 24]. Given the clear interest in *D. acetoxidans* for alternative processes of energy generation, the identification and understanding of the role of the macromolecular components responsible for these metabolic capabilities becomes a priority. Recently the final draft genome of *D. acetoxidans* was made available by the Joint Genome Institute [25] coding for "cytochromome" of 47 putative multiheme cytochromes *c*-type. Of those, up to now only the triheme cytochrome c_7 was characterized in detail. Its structure in the fully oxidized and fully reduced states, its thermodynamic and kinetic redox properties and its thermodynamic stability has been reported in the literature [22]. It shows high similarity to tetraheme cytochrome c_3, extracted from sulfate-reducing bacteria [26]. Cytochrome synthesis by bacteria is observed under usage of insoluble extracellular electron acceptors, such as sulfur.

D. acetoxidans can obtain energy as a result of sulfur respiration and complete acetate oxidation via the citric acid cycle [27]:

$$Acetate^- + 4S^0 + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2S;\ \Delta G = -39kJ \times mol^{-1}$$

This is the first investigated microorganism, which obtains energy by the complete acetate oxidation under the anaerobic conditions. It was shown that only 4% of consumed acetate by bacteria was assimilated into the cell material [28]. *D. acetoxidans* contains a succinyl-CoA: acetate CoA transferase instead of an acetyl-CoA synthetase and a succinyl-CoA synthetase. The succinyl-CoA: acetate-CoA transferase couples the formation of succinate from succinyl-CoA with the activation of acetate. The enzymes required for the assimilation of acetate and CO_2 into pyruvate are acetyl-CoA synthetase and pyruvate synthase [27]. C^{14}-labeling experiments shown that *D. acetoxidans* metabolism includes synthesis of oxaloacetate from acetate

http://dx.doi.org/10.5772/58347

and $2CO_2$ as anaplerotic reaction while most organisms oxidizing acetate to CO_2 by using the glyoxylate bypass for this function. Besides acetate, *D. acetoxidans* can completely oxidize L-malate, fumarate, propionate, ethanol etc as electron donors. *D. acetoxidans* was one of the first electrogenic bacterium described, a microorganism performing complete oxidation of an organic substrate with electron transfer directly to the electrode. It was calculated that in the fuel cell that contains acetate as the sole electron donor up to 82% of acetate is oxidized by *D. acetoxidans* with an electrode as the terminal electron acceptor [29]. Therefore, *D. acetoxidans* accumulates energy for growth by electron transfer to the electrodes. Similar results were obtained with *Geobacter metallireducens*, oxidizing aromatic compounds, and the predominantly freshwater *G. sulfurreducens* [17, 30].

Thus, *D. acetoxidans* has a crucial role in the biosphere and shows prospect of prosperous development of microbial fuel cell with simultaneous control of toxic metals environmental pollution because of formation of insoluble precipitates of metal sulfides. Although profound analyses of *D. acetoxidans* physiology and its linkage with power generation in MFC and organic matter consumption need to be established for its efficient application as the anode biocatalyst in microbial fuel cell.

2. Methodology

2.1. Microbial strains, medium and cultivation

Microbial strain *D. acetoxidans* IMV B-7384, which was applied in these investigations, belongs to the Ukrainian Collection of Microorganisms of D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine. Bacteria have been cultivated under the anaerobic conditions in the modified Postgaite C medium [20] in which sterile sulfur (10 g/l) and biotin (20 μg) were added before cultivation. Biotin served as a growth factor. Optimal pH for growth was 6.8-7.5 and optimal temperature was 30 ^{0}C.

2.2. Cell size distribution and relative content measurement

Bacterial growth commonly can be investigated by the registration of bacterial suspension turbidity or by the methods of dynamic or static light dispersion. The new method of rapid measurement of cell size distribution and their relative content, which is based on cell light scattering changes [31, 32] is proposed in this study. It includes the sounding of flow suspended bacterial cells by monochromatic coherent light, the registration of cooperative signals of sounding radiation and the explored microbiological objects by detecting of amplitudes and duration of scattered light impulses. The distribution of particles in size is determined on the basis of the measured functional dependence of the number of registered particles upon the amplitude and duration of corresponding electric impulses on the photoreceiver output by solving integral equation of Fredholm of the first kind (1):

$$F(U,t) = \int_{r_{min}}^{r_{max}} K(U,t,r) n(r) d(r), \quad (1)$$

where r_{min} and r_{max} – upper and lower limits of particle size distribution, which is registered;

n(r) – the function of particle size distribution;

K(U,t,r) – the function of distribution of normalized values of amplitude and duration of registered impulses of scattered light by the calibrating particles, which is a result of the previous probing of liquid flow by the monochromatic coherent light of the polymeric latex with set sizes and known refractive index.

However, presence of bacterial metabolism products in the growth medium could lead to errors in cell size distribution measurement because of additional light scattering. These errors were eliminated in the next way. Bacteria were cultivated in the liquid growth medium. Dependence between quantities of microbial cells and background particles in the growth medium had been determined during the time of bacterial cultivation. Liquid growth medium with and without bacterial cells was diluted in the same proportions by using highly purified liquid (deionized water). Then were registered separately the total quantity of cells and background particles in the highly purified liquid, which contains cells, and the total size distribution of background particles in the growth medium without cells. Then, relative content of bacterial cells was determined in the chosen interval of sizes, which equaled 0.3-1.9 μm. The cells relative content was measured by the calculation of quantity ratio of the set size cells to their total quantity. Specimens for determination of cell size distribution were prepared by dilution of 1 ml of bacterial suspension in 100 ml of deionized water [31, 32]. Measurements have been carried out by using the equipment PRM-6M, which was constructed at the Laboratory of Optical-Electronic Device of Faculty of Electronics of Ivan Franko National University of Lviv. The errors of cell size distribution measurement of constructed equipment are 5%.

2.3. Electron microscopy

After the third day of *D. acetoxidans* IMV B-7384 growth, the cells were harvested by centrifugation (2500 g, 30 min, 4°C) and washed three times in a buffer (50 mM potassium-phosphate buffer, pH 7.5). Intact cells were fixed at 1.5% $KMnO_4$ solution during 20 min under the room temperature (20 ^{0}C). Post-fixation was carried out with 1% OsO_4 in cacodylic buffer during 90 min under 0 ^{0}C. Fixed cells were washed and dehydrated in solutions with gradient concentrations of ethanol and propylene oxide. Specimens were fixed in epoxy Epon 812. Ultrathin cross-sections were obtained with the ultramicrotome UMTP-6 and contrasted by lead citrate [33]. Electron photographs were obtained by transmission electron microscopes UEMB-100B and PEM-100 at acceleration voltage 75 kV. Final photographs magnification – 10000 times.

2.4. Measurement of catalase, superoxide dismutase activity and intracellular reduced glutathione content

Antioxidant defense system activity has been measured in the cell-free extract after the second, third and fourth day of bacterial growth. Cells were washed by 0.9% NaCl solution and disintegrated on the ultrasonic homogenizer at 22 kHz at 0ºC during five minutes. Cell debris were sedimented by centrifugation at 5640-8800g at 4 ºC during 30 minutes. Catalase activity was measured spectrophotometrically at λ=410 nm by the degree of breakdown of hydrogen peroxide in the cell-free extract [34]. Superoxide dismutase activity was measured by the level of inhibition of 2,3,5-triphenyltetrazolium chloride reduction that follows formazan formation (absorbance maximum λ=405 nm) [35, 36]. Reduced glutathione content has been measured by the degree of dithionitrobenzoic acid reduction in cell-free extract (absorbance maximum λ=412 nm) [37].

2.5. Microbial fuel cell construction and maintenance

In this study two chamber microbial fuel cell has been constructed, in which *D. acetoxidans* IMV B-7384 was applied as the anode biocatalyst. Bacteria were cultivated in the modified Postgaite C medium [20] without sulfates under the anaerobic conditions and temperature 25-28 ^{0}C during twenty days in MFC. 0.1% potassium permanganate solution served as catholyte and bacterial suspension with 0.30±0.05 g/l dry cell weight/liter initial biomass served as anolyte respectively. 0.1% KMnO4 was replaced after 14 days of bacterial growth in MFC. Anode and cathode chambers with 0.3 l volume were separated by proton-exchange membrane (Millipore) with surface area of 2.5 cm^2. Graphite rods with the surface area of 130 cm^2 were applied as electrodes. Graphite is electrically conductive and conforms to the requirements of electrodes in MFC: non-corrosive, highly conductive, large surface area, high porosity etc [2]. Bacteria were cultivated periodically in the anode chamber of constructed MFC under separate addition of such electron donors as acetic, lactic and fumaric acids in form of sodium salts in concentrations 6 and 9 g/l. Electrode material served as the sole electron acceptor. All experiments were conducted under strictly sterile conditions.

2.6. Power output measurements of the microbial fuel cell

Electric current and voltage generation in constructed MFC were determined from the measured voltage drop across the resistor by multimeter DT-830C. The external load resistor with value 2.2 kΩ, which was shown to be the most optimal in constructed microbial fuel cell, was applied. The power output of an MFC was calculated from the measured voltage, E_{MFC}, across the load and the current as (2):

$$P = I \times E_{MFC} \quad (2)$$

Power generation by *D. acetoxidans* IMV B-7384 was investigated during twenty days of bacterial growth under the application of lactic, acetic and fumaric acids in form of their sodium salts.

3. Results and discussion

3.1. Particularities of *D. acetoxidans* IMV B-7384 growth physiology

Biomass accumulation of *D. acetoxidans* IMV B-7384, its cell size distribution and relative content changes have been carried out [14, 15]. Investigated bacterium has been shown to accumulate biomass the most intensively during third-fourth days of its growth. (fig. 3). Since bacterial growth processes were shown to be maximal during third-fourth days of cultivation, possibly, the highest power output in microbial fuel cell should be observed during this period.

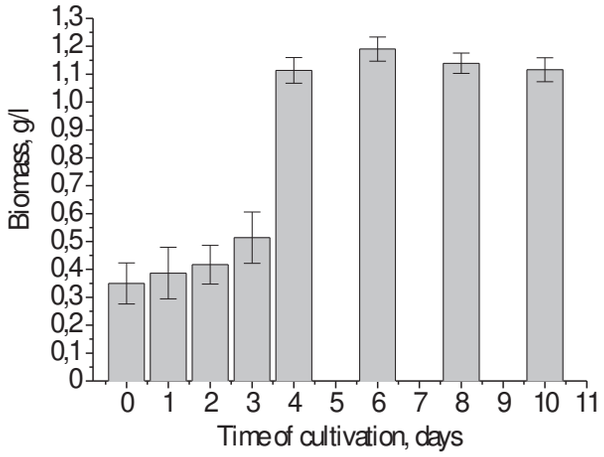

Figure 3. Changes of *D. acetoxidans* IMV B-7384 biomass during ten days under normal cultivation conditions (addition of lactic acid (6 g/l) and elemental sulfur (10 g/l) respectively as electron donor and acceptor)

Electron micrographs of *D. acetoxidans* IMV B-7384 cross-sections are presented on the fig. 4. Cells were obtained on the third day of their growth when maximal bacterial biomass accumulation was observed. These are rod shaped or slightly curved cells.

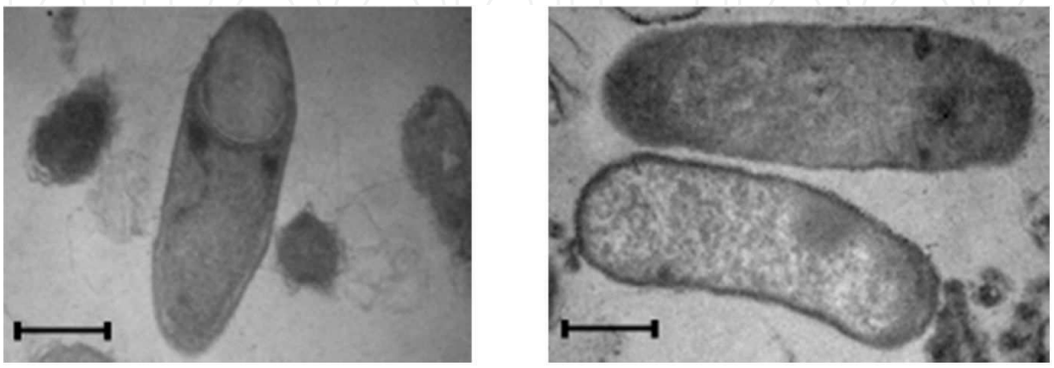

Figure 4. Cross-sections of the cells of *D. acetoxidans* IMV B-7384, harvested under normal growth conditions (third day) (electron micrograph (TEM), ×10000 times; bar, 0.5 μm)

http://dx.doi.org/10.5772/58347

Bacterial growth usually is characterized by increasing of cell quantity or cell size. Thus, analyses of cell size distribution and their relative content allow to obtain more detailed data of cell growth and division processes under various cultivation conditions in comparison with standard turbidimetric method of biomass measurement. Proposed method of cell light scattering determination allows to calculate possible changes of cell division on the basis of cumulative analyses of three histogram parameters: cell size distribution maximum, cell relative content and half-width of cell size distribution. It can be the basis for inventing of new methodologies for obtaining of synchronous cell cultures and also for development of new effective cytometers with low self-cost and high productivity.

Cell size distribution maximum equaled 0.55±0.01 μm on the third day of *D. acetoxidans* IMV B-7384 growth (period of maximal biomass accumulation). Cell relative content increased from 0.275 to 0.420 relative units under normal growth conditions on the third day of bacterial cultivation (fig. 5).

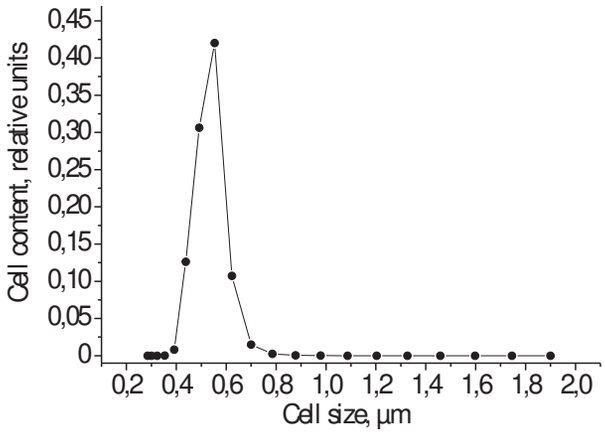

Figure 5. Medial values of *D. acetoxidans* IMV B-7384 cell relative content and size distribution during the third day of growth (five repeats, $P \leq 0.05$)

From the first to the fifth day of *D. acetoxidans* IMV B-7384 growth the maximum of cell size distribution was 0.49-0.55±0.01 μm and cell relative content with the maximum of size distribution changed from 0.275±0.011 to 0.398±0.011 relative units (table 1). Half-width of cell size distribution curves decreased from 0.23±0.01 to 0.14±0.01 μm with the increase of bacterial cultivation time from the first to the third day.

It indicated the decrease of cell size variations with the increase of cultivation time. Obviously, it is caused by intensive bacterial division on the third-fourth days of their cultivation. As a result cell's relative content with lower size distribution maximum (0.49±0.01 μm) increased in comparison with its initial value with higher maximum (0.55±0.01 μm). Possible inhibition of cell division on the fifth day of bacterial growth caused increase of cell relative content with higher size distribution maximum, which equaled 0.55±0.02 μm.

Time of cultivation, day	Cell size distribution maximum, μm	Cell content with size distribution maximum, relative units	Half-width of cell size distribution curves, μm
1	0.55±0.01	0.275±0.011	0.23±0.01
2	0.55±0.02	0.268±0.009	0.23±0.03
3	0.55±0.01	0.420±0.022	0.14±0.01
4	0.49±0.01	0.383±0.14	0.14±0.02
5	0.55±0.02	0.398±0.011	0.16±0.01

Table 1. Changes of cell size distribution maximum, cell relative content with size distribution maximum and half-width of curves of cell size distribution of *D. acetoxidans* IMV B-7384 during five days of cultivation (five repeats, $P \leq 0.05$)

Thus, it was shown that the maximal biomass accumulation is observed during third-fourth day of bacterial growth. High value of cell relative content with size distribution maximum and intensive decrease of half-width of cell size distribution indicates on significant increase of cell quantity with lower size distribution maximum. It is a possible result of intensive cell division during this time. Overall analysis of mentioned above parameters allow to assume that *D. acetoxidans* IMV B-7384 are in the middle of exponential phase of growth during the third day of their cultivation under normal growth conditions. Thus, the most intensive biosynthesis processes and power generation in microbial fuel cell possibly overlap this period of bacterial cultivation or immediately afterwards.

3.2. Response of *D. acetoxidans* IMV B-7384 cells to the influence of heavy metals

The activity of antioxidant defense system of *D. acetoxidans* IMV B-7384 has been revealed under the influence of external stress factors, such as Ferric iron, Ferrous iron, Nickel, Cobalt and Copper salts. It includes:

- biosynthesis of reduced glutathione (GSH), a tripeptide that serves as universal electron donor detoxifying reactive oxygen species [38];
- activity of catalase and superoxide dismutase, a basic enzymes of antioxidant defense system [36, 39].

Reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, hydroxyl radical etc are produced as a result of prolonged influence of oxygen or toxic xenobiotics, such as heavy metal ions, on the living cell. Glutathione (γ-L-glutamyl-L-cysteinylglycine) is the most abundant intracellular thiol-dependent antioxidant, which protects living cells against oxidative stress. It has low redox-potential (E'_0=-240 mV under pH=7.0) and it is constantly maintained in reduced state because of NADF-glutathione reductase functioning. Therefore it serves as cellular redox-buffer [40]. Superoxide dismutase catalyzes disproportionation of superoxides with oxygen and hydrogen peroxide formation. Catalase causes decomposition of produced H_2O_2 with neutralization of its toxicity. H_2O and O_2 are final products of this

http://dx.doi.org/10.5772/58347

reaction [41]. Thus, aforementioned components of antioxidant defense system protect the cell against oxidative stress, which may be caused by reactive oxygen species.

It was revealed that under the influence of various concentrations of $FeSO_4$, $FeCl_3$, $MnCl_2$, $NiCl_2$, $CoCl_2$ and $CuCl_2$ on the cells of *D. acetoxidans* IMV B-7384 activity of antioxidant defense system appears in spite of their obligate anaerobic metabolism. At fig. 6 is presented the complete scheme of the dependences between the highest values of catalase and superoxide dismutase activity and glutathione content, which were observed in the cells of investigated bacteria during four days of their cultivation.

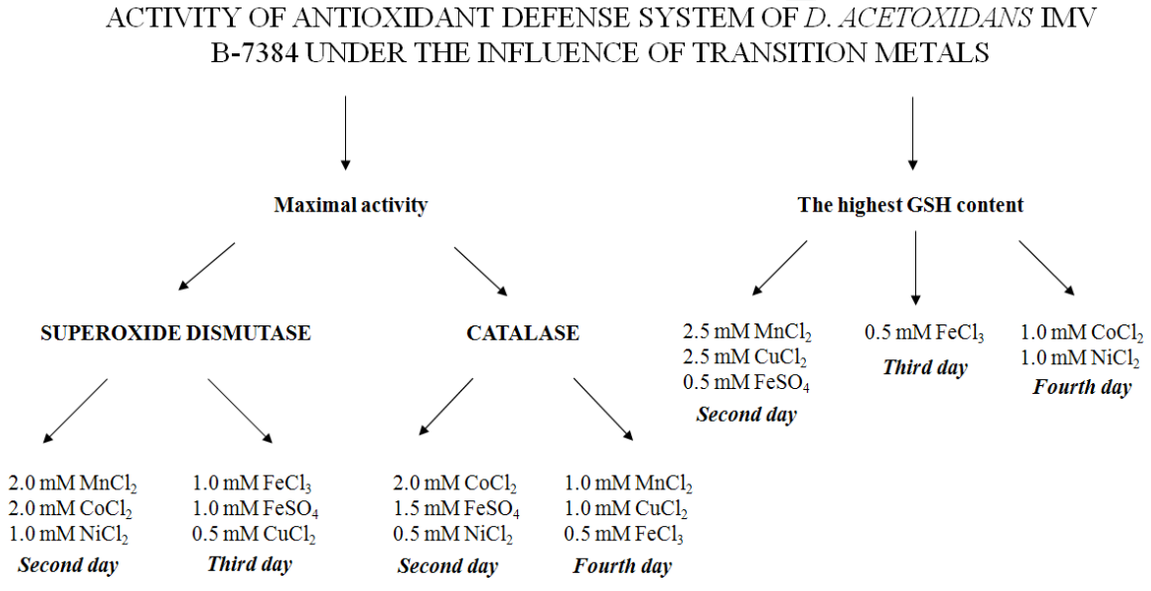

Figure 6. Maximal observed values of reduced glutathione content and superoxide dismutase, and catalase activity in the cell-free extract of *D. acetoxidans* IMV B-7384 under the influence of 0.5-2.5 mM of $FeSO_4$, $FeCl_3$, $MnCl_2$, $NiCl_2$, $CoCl_2$ and $CuCl_2$ during four days of bacterial cultivation [36, 38, 39]

Obtained results show that investigated bacteria possess specific mechanisms of rapid defense against toxic influence of external factors, such as high concentrations of various metal ions. It allows to assume their tolerance according to detrimental xenobiotics, which wastewaters are enriched with. This shows the prospect of *D. acetoxidans* IMV B-7384 application into wastewater treatment with simultaneous power generation in MFC. Also specific activity of antioxidant defense system enzymes, such as catalase and superoxide dismutase could help to prevent harmful influence of reactive oxygen species on the integrity of proton-exchange membranes in microbial fuel cells. Since catalase causes two-electron decomposition of H_2O_2 with O_2 and H_2O production, this enzyme could prevent Fenton reaction: $M^{n+}(=Cu^{+}, Fe^{2+}, Ti^{3+}, Co^{2+})+H_2O_2 \rightarrow M^{(n+1)+}(=Cu^{+}, Fe^{2+}, Ti^{3+}, Co^{2+})+{}^{\bullet}OH+OH^{-}$[42]. Neutralization of products of this reaction could significantly increase longevity of expensive proton-exchange membranes in microbial fuel cell, such as Nafion, with the next increasing of reliability and durability of power generation in MFC.

3.3.Power generation by *D. acetoxidans* IMV B-7384 in constructed microbial-anode fuel cell

3.3.1. The influence of external load resistance on power generation by D. acetoxidans IMV B-7384 as the anode biocatalyst in constructed MFC

External load resistance in microbial fuel cell is one of the crucial factors, which influence the electricity generation. Correlation between value of external load resistance and power generation in constructed MFC was determined. The influence of changes of external load resistance on volt-ampere characteristic of constructed MFC was investigated during *D. acetoxidans* IMV B-7384 cultivation in Postgaite C medium under normal growth conditions on the third-beginning of the fourth day, when the highest power generation was observed (fig. 7).

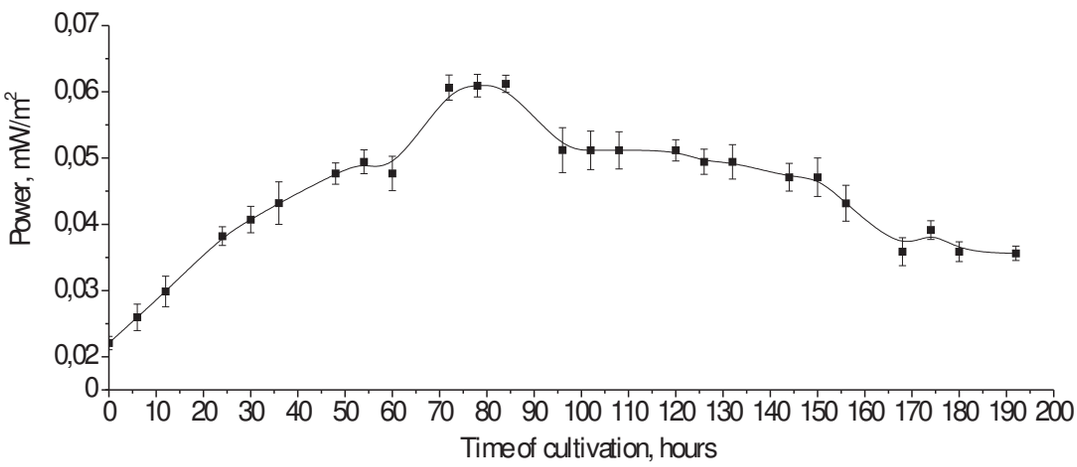

Figure 7. Power generation by *D. acetoxidans* IMV B-7384 during eight days under normal cultivation conditions in MFC, and application of 0.2 kΩ external load resistor

The maximal value of power density equaled 4.7 mW/m^2 on 84 hour of bacterial cultivation with addition of lactate (6 g/l) as electron donor and elemental sulfur (10 g/l) as electron acceptor (normal growth conditions), and application of an external load resistor of 0.2 kΩ. With the increase of cultivation time till 192 hour (eighth day of growth) generated power decreased by 42% in comparison with its maximal value.

With the aim to determine the most optimal load in term of electricity generation by *D. acetoxidans* IMV B-7384 in constructed MFC, external load resistors with values of 0.45-8.15 kΩ were applied in this investigation. The highest current strength and voltage were observed in constructed MFC under application of external load resistor with value of 2.2 kΩ (fig. 8). The highest power density, which was obtained in MFC under these conditions, equaled 5.8 mW/m^2. Increasing of external load resistor value caused decrease of generated power.

http://dx.doi.org/10.5772/58347

Therefore, all further experiments on MFC development were carried out with application of such external load resistance (2.2 kΩ), which was shown to be the most effective in term of electric power generation by *D. acetoxidans* IMV B-7384 in constructed MFC.

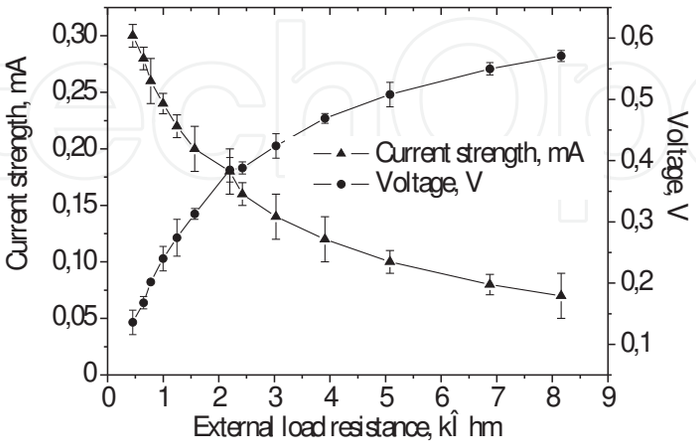

Figure 8. Volt-ampere characteristic of constructed MFC under the influence of various external load resistances on the third-beginning of the fourth days of *D. acetoxidans* IMV B-7384 growth under normal cultivation conditions

Accurate selection of external load resistance plays an important role in effective MFC development, because it significantly influences the value of generated power by bacteria, which are cultivated under different conditions in constructed MFC.

3.3.2. Power output in MFC under usage of various electron donors by D. acetoxidans IMV B-7384 in MFC

3.3.2.1. Lactic acid and power output in MFC

In previous researches it was shown that maximal power output equaled 4.3 mW/m^2 under addition the external electron acceptors, such as sulfur and Ferric iron in concentrations, which are favorable for *D. acetoxidans* IMV B-7384 metabolism [43]. Excluding of these additional electron acceptors and external load resistance optimization caused increasing of power output in constructed MFC.

Lactic acid was applied as the sole organic electron donor in concentrations 6 and 9 g/l during *D. acetoxidans* IMV B-7384 cultivation in the anode chamber of constructed microbial fuel cell. The highest power output equaled 5.74±0.29 mW/m^2 on the 64 hour (third day) of *D. acetoxidans* IMV B-7384 cultivation under addition of 6 g/l of $C_3H_6O_3$ (fig. 9). Its value decreased by 41% in comparison with the maximal power, obtained in this investigation by 250 hour of bacterial cultivation (tenth day). Power output equaled 1.33±0.18 mW/m^2 on the 480 hour of bacterial cultivation (twentieth day), which was lower by 77% in comparison with its maximal value.

Thus, gradual lactate oxidation and the following diminishing of its quantity in the anode chamber because of bacterial growth in MFC caused gradual decrease of produced power with the increase of duration of cultivation time. Thus, application of lactic acid as the single electron donor in the aforementioned concentration in constructed MFC can't be used for sustainable long-term electricity generation.

Concentration of lactic acid in the anode chamber has been increased up to 9 g/l. Under these cultivation conditions the highest power output equaled 5.90±0.21 mW/m^2 on the 64 hour (third day) of *D. acetoxidans* IMV B-7384 cultivation (fig. 10). After ten days it decreased by 37% in comparison with its highest measured value. By the 480 hour (the twentieth day) of *D. acetoxidans* IMV B-7384 cultivation power production in constructed MFC decreased by 33% in comparison with its maximal measured value.

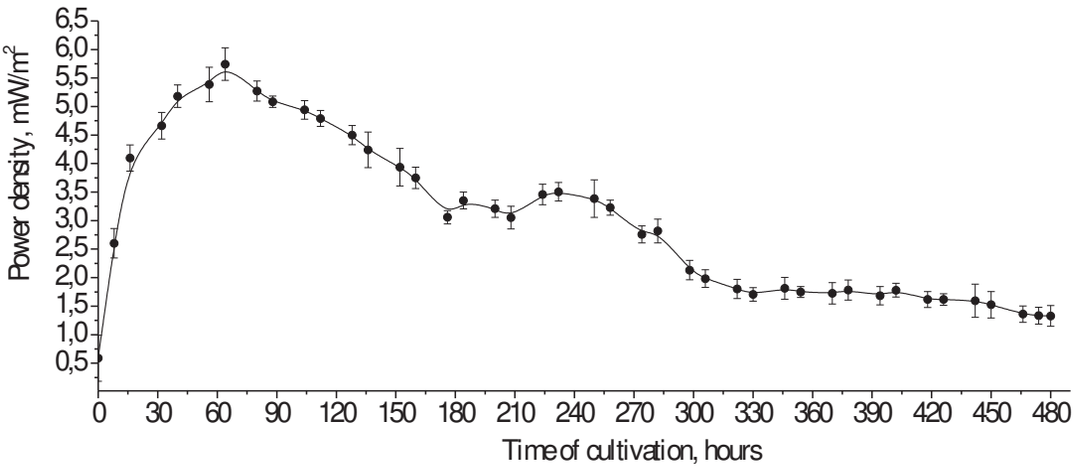

Figure 9. Power density in MFC during twenty days under addition of 6 g/l of lactic acid into the growth medium of *D. acetoxidans* IMV B-7384

Increase of lactic acid content in the anode chamber caused enhance of stability of power generation in constructed MFC, but such manipulation did not boost its value significantly in comparison with application of lower concentration of lactic acid.

Thus, lactic acid as the sole electron donor in high concentrations supports durability of constructed MFC with application of *D. acetoxidans* IMV B-7384 as the anode biocatalyst. However, its effectiveness does not change significantly under the application of lower and higher lactic acid concentrations.

3.3.2.2. Fumaric acid and power output in MFC

With the aim to determine the most optimal electron donor in term of electricity generation lactic acid has been substituted by fumaric acid in the anode chamber of constructed MFC. The highest value of generated power equaled 5.69±0.29 mW/m^2 on the 56 hour (the third day) of bacterial cultivation under usage of 6 g/l of fumaric acid as the sole electron donor (fig. 11). It's

http://dx.doi.org/10.5772/58347

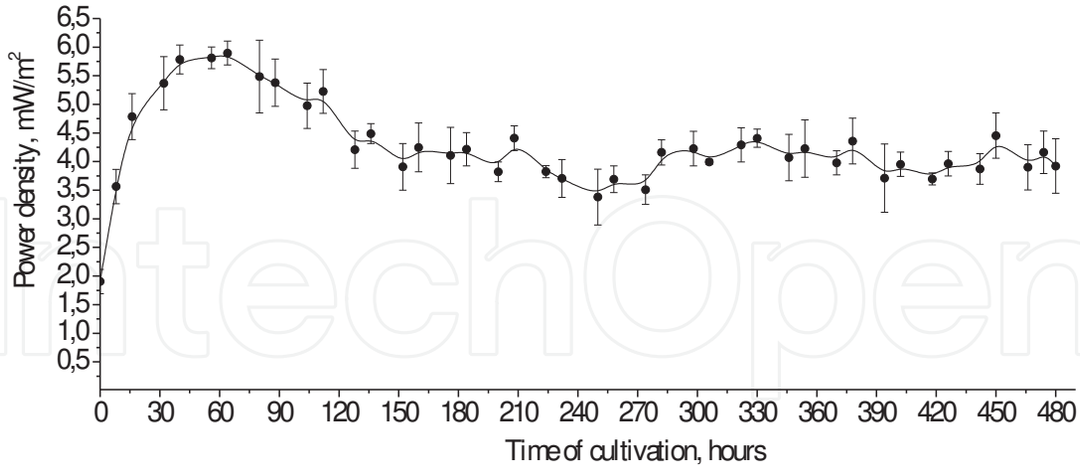

Figure 10. Power density in MFC during twenty days of *D. acetoxidans* IMV B-7384 cultivation under application of 9 g/l of lactic acid

value decreased by 38% on the 250 hour (the tenth day) and by 43% on the 480 hour (the twentieth day) of *D. acetoxidans* IMV B-7384 cultivation. The medial power density, which was observed under these cultivation conditions equaled 3.58±0.20 mW/m^2.

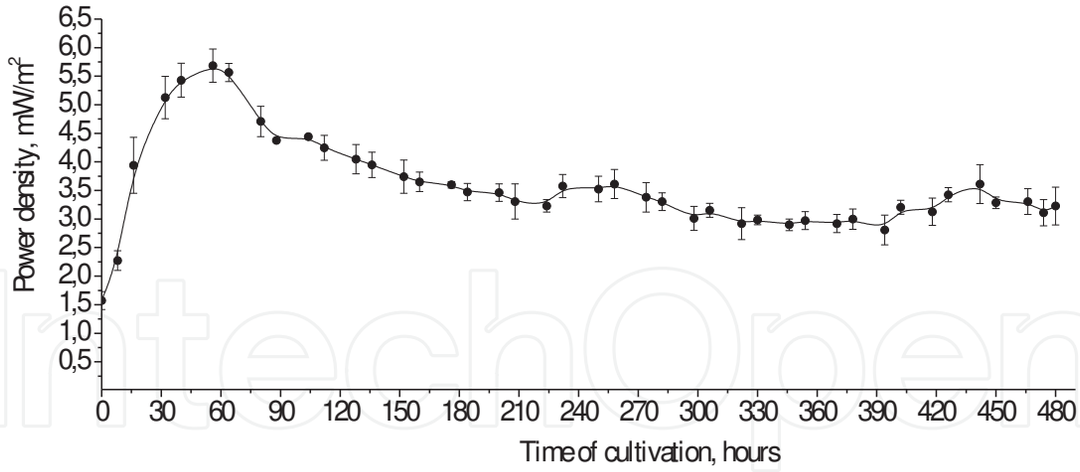

Figure 11. Power density in MFC during twenty days under addition of 6 g/l of fumaric acid into the growth medium of *D. acetoxidans* IMV B-7384

Thus, application of fumaric acid (6 g/l) causes higher stability of power generation by investigated bacteria in constructed MFC in comparison with the lactic acid in the same concentration. Therefore, fumaric acid is more preferable electron donor in term of power generation by *D. acetoxidans* IMV B-7384 in comparison with application of lactic acid.

It was shown that increase of fumaric acid concentration up to 9 g/l caused maximal power generation (2.38±0.10 mW/m^2) at 56 hour (fig. 12).

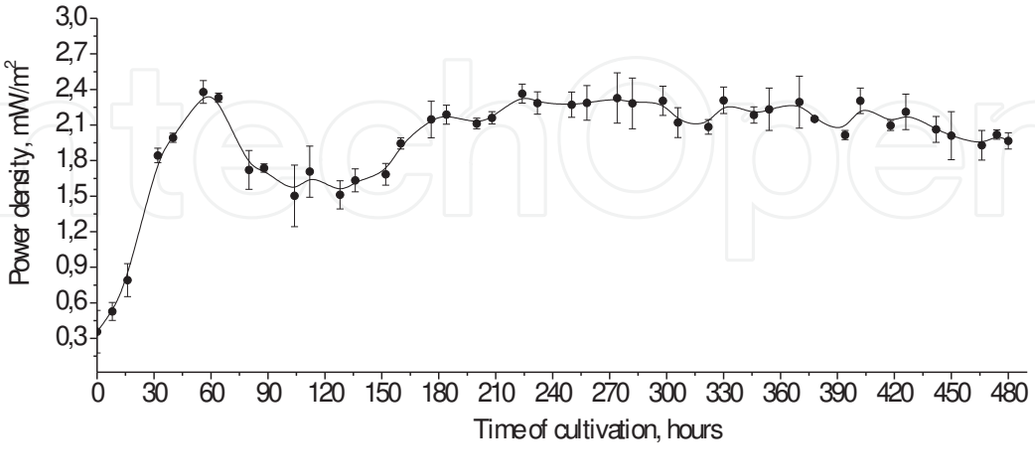

Figure 12. Power density in MFC during twenty days under addition of 9 g/l of fumaric acid into the growth medium of *D. acetoxidans* IMV B-7384

It was less by 2.4 times in comparison with the highest power density value, which was observed under bacterial cultivation with application of less concentration of fumaric acid (6 g/l). The minimal observed power density value equaled 1.95±0.047 mW/m^2 on the 160 hour of bacterial cultivation under these conditions. Increasing of cultivation time caused insignificant enhance of power production. On the 480 hour it value equaled 1.97±0.07 mW/m^2, which was less by 17% in comparison with its maximal measured value in this investigation.

Thus, increase of fumaric acid concentration from 6 to 9 g/l in the anode chamber of constructed MFC reduced its productivity but enhanced its stability in comparison to the application of lower concentration of investigated electron donor.

3.3.2.3. Acetic acid and power output in MFC

Acetic acid was applied as the separate electron donor in constructed MFC. It was shown that the maximal power value equaled 5.78±0.24 mW/m^2 on the 40 hour (second day) of bacterial cultivation under addition of 6 g/l of CH_3COOH (fig. 13).

Its value decreased by 42% on the 232 hour (10 day) of *D. acetoxidans* IMV B-7384 growth. Generated power insignificantly enhanced with the increase of cultivation time. It equaled 3.1±0.12 mW/m^2 on the 480 hour of bacterial cultivation. Thus, application of acetic acid as well as fumaric acid in low concentrations caused high stability of electricity generation in constructed fuel cell apart from application of lactic acid.

D. acetoxidans IMV B-7384 has been cultivated in MFC under addition of 9 g/l of acetic acid as the sole electron donor into the growth medium (fig. 14). Under these cultivation conditions the maximal power value equaled 3.6±0.30 mW/m^2 on the 64 hour (third day) of bacterial cultivation. It was lower by 61% in comparison with the maximal power value obtained under

http://dx.doi.org/10.5772/58347

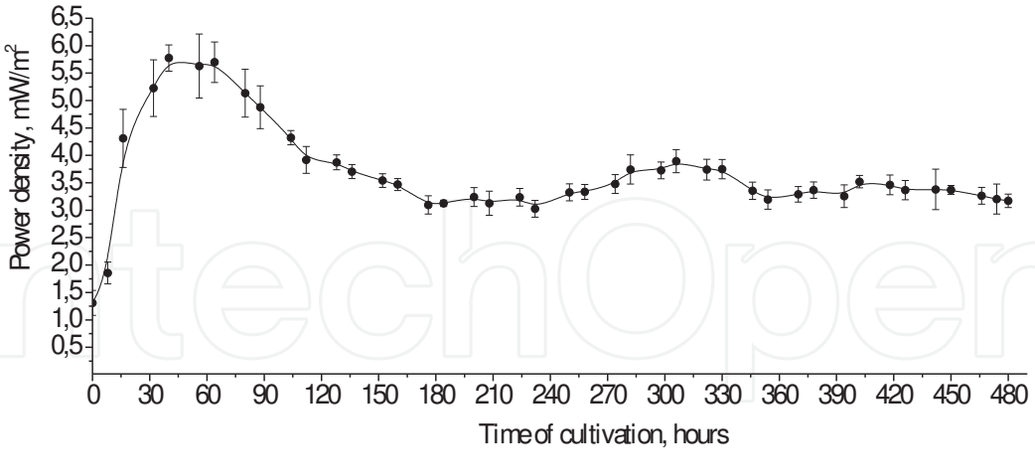

Figure 13. Power density in MFC during twenty days under addition of 6 g/l of acetic acid into the growth medium of *D. acetoxidans* IMV B-7384

usage of 6 g/l of lactic acid by bacteria in the anode chamber of MFC. Increase of cultivation time up to 480 hour caused decrease of power value till 1.60±0.11 mW/m^2.

Thus, increase of acetic acid concentration as electron donor in the anode chamber of MFC caused partial inhibition of electricity generation in comparison with application of its lower content.

It can be summarized that low concentrations of investigated organic acids caused higher stability of power generation in constructed microbial fuel cell apart from their higher concentrations.

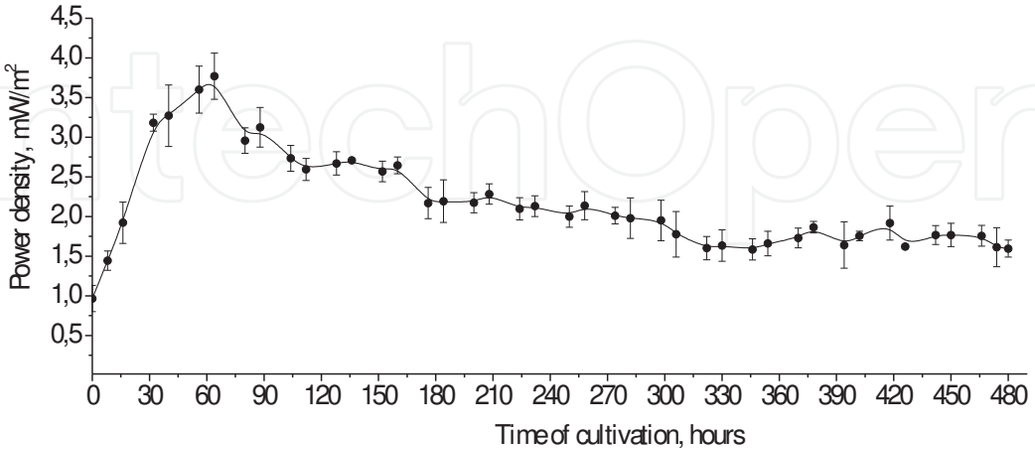

Figure 14. Power density in MFC during twenty days under addition of 9 g/l of acetic acid into the growth medium of *D. acetoxidans* IMV B-7384

Possibly, it may be explained because of raising of by-products concentrations in the growth medium under increasing of organic source concentration. It may cause negative influence according to *D. acetoxidans* IMV B-7384 metabolism and their respective ability of exoelectrogenesis.

4. Conclusions

D. acetoxidans IMV B-7384 is exoelectricigenic sulfur-reducing bacterium which influences environmental biogeochemistry by maintenance of reductive stage of sulfur cycle. Its metal-resistant strains play significant role in heavy metal ions remediation from the aquatic environments because of interaction between the final product of bacterial dissimilative sulfur-reduction – hydrogen sulfide and metal ions with their next combining in form of insoluble metal sulfide precipitates. It was shown that *D. acetoxidans* IMV B-7384 synthesizes such components of antioxidant defense system as catalase, superoxide dismutase and reduced glutathione under the influence of aggressive external factors, such as heavy metal ions. It causes its resistance against environmental pollution by these xenobiotics. Enzymatic and non-enzymatic components of antioxidant defense system found in the cells of *D. acetoxidans* IMV B-7384 are highly effective in neutralization of reactive oxygen species. Antioxidant system activity of investigated bacterial cells may be useful for increasing the durability of proton-exchange membranes in MFCs because of the creation of defensive barrier against detrimental influence of these oxidants. It shows a prospect of efficient and economic application of *D. acetoxidans* IMV B-7384 as the anode biocatalyst in microbial fuel cell with simultaneous wastewater treatment.

The optimal external resistance in constructed MFC in term of power generation was determined to be 2.2 kΩ. Separate application of lactic, fumaric, and acetic acids caused differences in power generation by investigated bacterium. It was shown that addition of fumaric and acetic acids in concentration 6 g/l improved stability of generated power in constructed microbial fuel cell in comparison with application of lactic acid in the same concentration. Increase of concentration of investigated organic electron donors up to 9 g/l reduced generated electric power .

Thus, *D. acetoxidans* IMV B-7384 may be applied for effective treatment of wastewater enriched with heavy metals, acetic and fumaric acids-containing refuses with simultaneous electricity generation in the scaled-up microbial fuel cells. Additionally it can be used for treatment of highly polluted sulfur-containing aquatic environments with alterations of sulfur cycle.

4.1. Possible directions of further research on application of *D. acetoxidans* IMV B-7384 as the anode biocatalyst in MFC

Exploration of the utility of *D. acetoxidans* IMV B-7384 for development of efficient MFC through determination of optimal cultivation and fuel cell construction parameters may be highly beneficial for the progress of microbial fuel cell study with simultaneous heavy metals pollution control for cost effective environmental remediation. Further research of *D. acetoxi-*

dans IMV B-7384 as the anode biocatalyst may include detailed analyses of its molecular biochemistry with the aim of profound understanding of interconnections between the processes of organic source consumption and electric current generation. Further analyses of interrelations between specific reactions of sulfur cycle (e.g. polysulfide reductase activity), reduction of transition metals, such as iron and manganese, and processes of electrogenesis, which are conducted by the cells of *D. acetoxidans* IMV B-7384 may substantially influence the microbial fuel cell study with the aim of increasing of its productivity, reliability and durability.

Acknowledgements

We are highly grateful to Dr. Neelkanth G. Dhere, Jaroslav Ferensovych, Dr. Vasyl Getman, Dr. Dariya Fedorovych, Dr. Yurij Boretsky, Dr. Oleksandr Kulachkovskyj, and all other our coworkers for their support provided in carrying out of investigations and book chapter preparation.

Author details

Oleksandr Bilyy[1*], Oresta Vasyliv[2] and Svitlana Hnatush[2]

*Address all correspondence to: bily2011@yandex.ru

1 Ivan Franko National University of Lviv, Faculty of Electronics, Laboratory of Optical-Electronic Device, Ukraine

2 Ivan Franko National University of Lviv, Biological Faculty, Department of Microbiology, Ukraine

References

[1] Zhuwei D., Haoran L., Tingyue G. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnology Advances 2007; 25, 464-482.

[2] Logan BE. Microbial Fuel Cells. John Wiley & Sons., New Jersey; 2007.

[3] Cheng S., Dempsey BA., Logan BE. Electricity generation from synthetic acid-mine drainage (AMD) water using fuel cell technologies. Environmental Science & Technology 2007; 41, 8149-8153.

[4] He Z., Minteer SD., Angenent LT. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environmental Science & Technology 2005; 39, 5262-5267.

[5] Heilmann J., Logan BE. Production of electricity from proteins using a microbial fuel cell. Water Environment Research 2006; 78, 531-537.

[6] Logan BE., Murano C., Scott K., Gray ND., Head IM. Electricity generation from cysteine in a microbial fuel cell. Water Research 2005; 39, 942-952.

[7] Min B., Logan BE. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental Science & Technology 2004; 38, 5809-5814.

[8] Rabaey K., Lissens G., Siciliano SD., Verstraete W. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology Letters 2003; 25, 1531-1535.

[9] Rabaey K., Clauwaert P., Aelterman P., Verstraete W. Tubular microbial fuel cells for efficient electricity generation. Environmental Science & Technology 2005; 39, 8077-8082.

[10] Lovley DR. The microbe electric: conversion of organic matter to electricity. Current Opinion in Biotechnology 2008; 19, 1-8.

[11] Wineld J., Ieropoulos I., Greenman J., Dennis J. The overshoot phenomenon as a function of internal resistance in microbial fuel cells. Bioelectrochemistry 2011; 81(1), 22-27.

[12] Winkelmann G. Microbial Transport Systems. New York: WILEY–VCH; 2002.

[13] Ma Zh., Jacobsen FE., Giedroc DP. Metal transporters and metal sensors: how coordination chemistry controls bacterial metal homeostasis. Chemical Reviews 2009; 109 (10) 4644-4681.

[14] Vasyliv OM., Bilyy OI., Getman VB., Ferensovych YP., Yaremyk RY., Hnatush SO. The influence of different metal ions on light scattering properties of pattern microbial fuel cells' bacteria *Desulfuromonas acetoxidans*. in: Dhere NG., Wohlgemuth JH., Lynn KW. (eds.) Optics+Photonics / Solar Energy+Technology: Reliability of Photovoltaic Cells, Modules, Components, and Systems IV: proceedings of the International Symposium of the Society of Photo-Optical Instrumentation Engineers, 21-25 August 2011, SPIE, Bellingham, WA; 2011.

[15] Vasyliv OM., Bilyy OI., Getman VB., Kushkevych IV., Hnatush SO. The changes of spectroscopic characteristics of sulfur-reducing bacteria *Desulfuromonas acetoxidans* under the influence of different metal ions. in: Hoover RB., Davies PCW., Levin GV. (eds.) Optics+Photonics / Optical Engineering+Applications: Instruments, Methods, and Missions for Astrobiology XIV: proceedings of the International Symposium of

the Society of Photo-Optical Instrumentation Engineers, 21-25 August 2011, SPIE, Bellingham, WA; 2011.

[16] Tender LM., Gray SA., Groveman EG. The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy. Journal of Power Sources 2008; 179 (3) 571-575.

[17] Debabov VG. Electricity from Microorganisms. Microbiology 2008; 77 (2) 123-131.

[18] Garity G., Winters M., Searles D. Taxonomic outline of the procariotic genera Bergey's manual of systematic bacteriology. Second edition. Michigan. USA: Springer-Verlag; 2001.

[19] Peck JrHD., Lissolo T., Cole JA., Ferguson SJ. The Nitrogen and Sulphur Cycles. Cambridge: Cambridge University Press; 1988.

[20] Brugna M., Nitschke W., Toci R., Bruschi M., Giudici-Orticoni M.-Th. First evidence for the presence of a hydrogenase in the sulfur-reducing bacterium *Desulfuromonas acetoxidans* // Journal of Bacteriology 1999; 181 (17) 5505-5508.

[21] Roden EE., Lovley DR. Dissimilatory Fe (III) reduction by the marine microorganism *Desulfuromonas acetoxidans.* Applied and Environmental Microbiology 1993; 59 (3) 734-742.

[22] Alves AS., Paquete CM., Fonseca BM., Louro RO. Exploration of the cytochromome of *Desulfuromonas acetoxidans*, a marine bacterium capable of powering microbial fuel cells. Metalomics 2011; 3, 349-353.

[23] Haveman SA., DiDonato RJ., Villanueva L., Shelobolina ES., Postier BL., Xu B., Liuand A., Lovley DR. Genome-wide gene expression patterns and growth requirements suggest that *Pelobacter carbinolicus* reduces Fe(III) indirectly via sulfide production. Applied and Environmental Microbiology 2008; 74, 4277-4284.

[24] Shi L., Squier TC., Zachara JM., Fredrickson JK. Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multihaem *c*-type cytochromes. Molecular Microbiology 2007; 6512-6520.

[25] DOE Joint Genome Institute. *Desulfuromonas acetoxidans* DSM 684 Home: http://genome.jgi-psf.org/desac/desac.home.html (accessed 24 May 2010)

[26] Aubert C., Lojou E., Bianco P., Rousset M., Durand M.-Cl., Bruschi M., Dolla A. The *Desulfuromonas acetoxidans* triheme cytochrome c_7 produced in *Desulfovibrio desulfuricans* retains its metal reductase activity. Applied and Environmental Microbiology 1998; 64 (4) 1308-1312.

[27] Gebhardt NA, Thauer RK., Linder D., Kaulfers P.-M., Pfennig N. Mechanism of acetate oxidation to CO_2 with elemental sulfur in *Desulfuromonas acetoxidans.* Archives of Microbiology 1985; 141, 392-398.

[28] Lovley RD., Holmes DE., Nevin KP. Dissimilatory Fe (III) and Mn (IV) reduction. Advances in Microbial Physiology 2004; 49, 219-286.

[29] Bond DR., Holmes DE., Tender LM., Lovley DR. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 2002; 295, 483-485.

[30] Bond DR., Lovley DR. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Applied and Environmental Microbiology 2003; 69, 1548-1555.

[31] Bilyy OI., Getman VB., Matvijchuk JM. The method of determination of microparticles distribution under their sizes in the disperse environments. Measurement and calculation techniques in the technological processes 2001; 2, 23-26.

[32] Bilyy OI., Getman VB., Kotsyumbas IJ., Rozko MS. New optical method of the monitoring of bacteria in the liquid solutions. Biophysical visnyk 2001; 1(8), 121-126.

[33] Reynolds ES. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. The Journal of Cell Biology 1963, 17, 208-212.

[34] Goth LA. Simple method for determination of serum catalase activity and revision of reference range. Clinics Chimica Acta 1991; 196, 143-152.

[35] Bernas T., Dobrucki JW. The role of plasma membrane in bioreduction of two tetrazolium salts, MTT, and CTC. Archives of Biochemistry and Biophysics 2000; 380 (1) 108-116.

[36] Vasyliv OM., Hnatush SO. Influence of transition metal compounds on superoxide dismutase activity of sulfur-reducing *Desulfuromonas acetoxidans* bacteria. Mikrobiologichny Zhurnal 2013; 75 (2) 37-44.

[37] Owen JB., Butterfield DA. Measurement of oxidized/reduced glutathione ratio. Methods Mol Biol. 2010; 648, 269-77.

[38] Vasyliv OM., Hnatush SO. Effect of ferrum and manganese compounds on the glutathione content in cells of sulfurreducing bacteria *Desulfuromonas acetoxidans.* Studia Biologica 2011; 5 (1) 5-10.

[39] Vasyliv OM., Hnatush SO. Effect of transition metal compounds on catalase activity of sulfur-reducing bacterial *Desulfuromonas acetoxidans* cells. Visnyk of L'viv University. Biological Series 2011; 57, 207-215.

[40] Cotgreave IA., Gerdes RG. Recent trends in glutathione biochemistry – gluthatione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochemical and Biophysical Research Communication 1998; 242, 1-9.

[41] Brioukhanov AL., Thauer RK., Netrusov AI. Catalase and superoxide dismutase in the cells of strictly anaerobic microorganisms. Microbiolology 2002; 71 (3) 281-285.

[42] Hongsirikarn K., Mo X., Goodwin Jr. JG., Creager S. Effect of H_2O_2 on Nafion® properties and conductivity at fuel cell conditions. Journal of Power Sources 2001; 196, 3060-3072.

[43] Vasyliv OM., Bilyy OI., Ferensovych YP., Hnatush SO. Electric current generation by sulfur-reducing bacteria in microbial-anode fuel cell. in: Dhere NG., Wohlgemuth JH., Lynn KW. (eds.) Optics+Photonics / Solar Energy+Technology: Reliability of Photovoltaic Cells, Modules, Components, and Systems IV: proceedings of the International Symposium of the Society of Photo-Optical Instrumentation Engineers, 12-26 August 2012, SPIE, Bellingham, WA; 2012.

IntechOpen