THE BIOFUEL ELEMENTS ON THE BASIS OF THE NANOCARBON MATERIALS

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Abstract. **The state of studies and the new directions, which are developed recently with the creation of the biological-fuel elements of devices, based on the biological material and generating the direct generation of electrical energy with the oxidation of substrata was examined. The functioning of the microbial biological-fuel elements, which oxidizes ethanol was investigated. The bioelectrocatalyst were the intact Gluconobacter oxydans bacterial cells or their membrans fractions. The application of nanocarbonic materials at the development of the electrodes for the biological-fuel elements was considered. The cell of the biological-fuel element on the basis of thermo-expanded graphite was experimentally studied. The special features of graphene as the bases of electrodes in the biological-fuel elements at the development of electrodes was reviewed. The successful development of this subjects, which relates to the bioenergetics, possibly with the close cooperation of such areas of biotechnology as the biosensor and electrochemical studies, which are rested on the application of microelectronic technologies.**

Keywords: **biofuel elements, bioanode, direct obtaining electric energy, oxidation of substrata enzymes and microbe cells, membrane fractions, nanocarbon materials.**

PACS 82.45.Tv, 87.85 M-

Biblography - 99 references *Recieved 21.11.2014*

RENSIT, 2014, 6(2):187-208 **DOI: 10.17725/RENSITe.0006.201412d.0187**

CONTENTS

1. INTRODUCTION

Development of methods for the electricity generation using biological material as one of the main components in this process, gained breadth in recent years. The reasons of keen interest in this subject are connected with common problems of mankind - search for new power sources, as well as with the environmental problem, associated with the use of fossil energy sources - release a significant amount of carbon dioxide when they are burnt for use. The biggest challenge that humanity throws down to the natural environment consists in maintenance of electric energy production while reducing carbon dioxide emissions. It is necessary to develop an essentially new platform that will enable to produce a sufficient amount of energy while reducing the evolved CO_2 quantity. Development of microbial fuel cells technology is the newest alternative approach to this method of electricity generation [1]. Systems or fuel elements, in which is generally used a biological material, oxidizing inorganic materials (gaseous hydrogen) or, as in most cases, organics, and the generation of electric potential is produced, are called biofuel (BFC). The BFC materials are widely represented in the scientific literature [2-6]. Apparently, the first publication on this topic belongs to 1911 [7]. In the early 1990s, there was a new wave of interest in describing microbial biofuel cells (MFC) on the basis of mediators. The subsequent dush of researches refers to 1999, when the possibility of non-mediated electron transport was shown [8].

In the microbial cell the oxidation energy of organic substrates turns into two components - the electrical part, providing membrane potentials, and chemical, in the form of ATF. The BFC is an essentially cell model, simulating the electric potential generation. The electrodes can be closed by load resistance, and it is possible to obtain data on the BFC electric power by measuring there the voltage and current. In the classical microbial BFC the anode and cathode compartments, separated by a proton-permeable membrane. Microbial cells are located in the anode compartment, making oxidation of substrate and releasing electrons that are transported to the anode, and protons in the surrounding solution. The cathode compartment is sated with air, from which is used an oxygen that is reduced to water by the electrons, which flew to the cathode. The BFC releasing electric power (P) is determined by the formula $P = I \times V$, where I – the current, flowing through an external load, V – the voltage on it. Theoretically, the voltage V is determined by the difference in the formal potentials of the oxidizer E_{oxidizer} and oxidized substrate $E_{\text{substrate}}$, i.e. $V = E_{\text{oxidizer}} - E_{\text{substrate}} - \mu$. At the same time, there are irreversible losses μ, reducing the real value of the effective potential. Losses are caused by the ohmic resistance of the electrolyte, presence of the electrolyte's concentration gradient, kinetic limitations of electron transfer reactions on an electrode, internal resistance of BFC. The formal potential E is defined in terms of the Gibbs free energy change ΔG, connected with oxidation/restoration reactions of the substance $E = -\Delta G/nF$, where n – number of transferable electrons, F – Faraday's constant.

In the work [2] is proposed the general classification, including almost all types of existing fuel elements and cells and briefly describing their features. Schematically, such structure in the modified form shown in **Fig. 1** and **2**.

According to [2], the electrochemical fuel systems include those, which provide direct reception of electric energy from chemical and photochemical reactions. These include batteries, fuel elements/cells and solar cells. It is typical for batteries that anode and cathode fuels remains in the system and can't be replaced. For sources of this type are used inorganic chemical solutions. The situation changes for fuel elements/cells, where anode and cathode fuels (oxidizable substrates) are remained out of cells and can be replaced. The BFC belong to this group of current sources. Their conversion element – biological catalyst – may be both enzymes and whole microbial cells. Membrane enzymes, localized intracellularly, are involved in bioelectrocatalysis in cellular microorganisms.

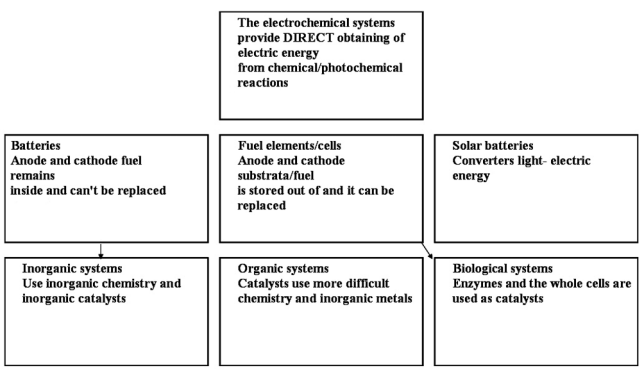

Fig. 1. *Classification of the electrochemical fuel systems.*

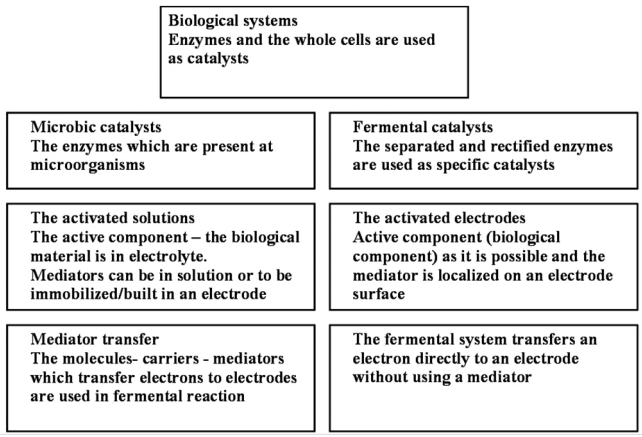

Fig. 2. *Classification of biofuel systems.*

The important characteristic of the current sources is their power characteristics (**Fig. 3**). Thus, batteries and solar cells have, depending on a structure, a wide range of developed powers, comprising in the range of from 10^{-3} to $10^{7}W$. The power level from 10^{0} to $10^{7}W$ is filled with fuel cells, related to the batteries, accumulators – to the elements, working in the redox reactions. Next is the so-called "empty segment" – 10^{0}-$5\cdot10^{-3}W$. The range, relevant to biosensors and biofuel cells, amounts from 10^{-10} to 10^{-5} and 10^{-7} to $5\cdot10^{-3}W$.

As the main point of the process in BFC can be described by the scheme of electron transfer chain "organic substrate – enzyme/cell – mediator – electrode (anode) – external chain – electrode (cathode)", having added oxygen restoration processes in the anode compartment, so it is possible on the base of this example to investigate the main directions of conducted researches on BFC in the world at the present day.

The first most significant results in the development of the alcohol dehydrogenase-based BFC were achieved by Finnish researchers, led by prof. Aarne Halme [9]. Interests of this group were concentrated on the study of various types of fuel cells and modeling of electricity generating processes [10]. For BFC, described in the work [9],

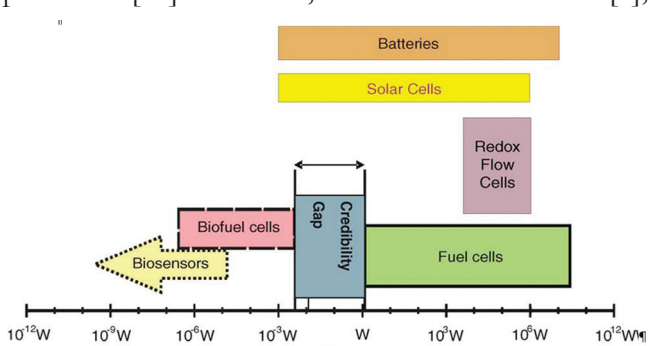

Fig. 3. *The power characteristics of various current sources.*

the authors have provided detailed information on device parameters. It was shown that from methanol oxidation of 1 gram is theoretically possible to receive about 5A·hour of electricity.

Considering the publications of the last 3-5 years, it is possible to specify the main directions of development. Today is intensively developed the application of new materials in electrodes' creating. Generally, the attention is directed to the use of carbon nanomaterials. In the work [11] is considered the option of glucose oxides immobilization onto platinized carbon nanotubes, providing the facilitated transfer of electrons on an electrode. The use of carbon nanotubes is also described in works [11, 12]. The search for new types of mediators [14] and their embedding into the electrode assemblies of BFC is performed [15]. In this case, the attention is paid not only to the anode, but also to cathodic mediators [16].

The use of osmium-based polymers allows to form structures, which are both holding elements and mediators at the same time. In the work [17] is represented the new method for the synthesis of osmium polymer, intended for use in BFC. As fuel for BFC is proposed an ethanol. This option of BFC is described in [18].

New BFC structures, including membraneless, are developed [19]. Different methods for an immobilization of glucose oxidase enzyme onto the surface of carbon nanotubes, providing the facilitated non-mediated electron transfer, are considered [20]. It is shown that a rather efficient structure of enzymatic BFC is a flowing non-membranous system [21]. The new type of BFC on the basis of two enzymes – glucose oxidase and urease – is presented in [22]. In this BFC the electromotive force was created by the enzymatic activity and generation of a difference in *pH* in the anode and cathode compartments, wherein the element power was in the range of tens microwatts.

Testing of new strains of bacteria for their efficiency [23] is made and new types of catalysts [24] are developed for use in BFC. The BFC on the basis of organelles are developed. In the work [25] are represented the system characteristics on the basis of mitochondrions. In the review [26] the advantages, resulting from the use of enzymes in BFC, are considered. The important direction of development is connected with the use of BFC as

source of electric energy for the implanted devices in an organism and possibility of its mode control [27]. Approaches to a non-mediated bioelectrocatalysis are covered in [28].

In the work [29] were studied the peculiarities of charge transfer in the system "oxidizable substrate-bacterial cell-mediator-electrode" for bacterial cells of the genus *Gluconobacter*. The election of a water-soluble electron transport mediator, interacting with membrane-localized enzymes of bacterial cells. It is shown that the most efficient mediator is 2,6-dichlorophenolindophenol in comparison with 1,4-benzoquinone and potassium hexacyanoferrate (III). The effective type of a biocatalyst was chosen and evaluation of biocatalytic oxidation of substrates by genus *Gluconobacter* cells was carried out. It was found that the maximum capacity of the generated potential is reached for *Gluconobacter oxydans sb strain. sp. industrius* (VKM B-1280), using glucose as a substrate oxidation. The influence of substrate concentration, mediator, *pH* buffer solution and geometric sizes of the electrodes on the sizes of the generated potential and BFC averaged parameters were the following: the developed voltage about 6 mV at current value of 0.5 μA and loading value of 10 kOhm for the case when the internal resistance value was 90 kOhm. It is shown that the oxygen, dissolved in the anodic compartment, has no significant impact on operation of a biofuel element. In the range from 10 to 40°C, the influence of the temperature on size of the generated EMF is investigated and possibility of using waste fermentation productions as fuel is shown [30].

Application of microbial cells in BFC has its own advantages and disadvantages in comparison with enzymes. So, as an advantage of microbial BFC may be noted:

- substrate specificity of microorganisms is very diverse, in this connection, they can serve as biocatalysts for a wide range of substrates,
- the cost of biocatalysts production on the basis of micro-organisms is low in comparison with the cost of the enzyme release,
- at several stages of the electrochemical oxidation of the substrate, the electrochemical signal of whole cells will be higher, than in case of the isolated enzyme,
- some potentially suitable for BFC enzymes are unstable,
- enzymes in microorganisms are provided with the best way of protection against interfering solutes,
- many microorganisms are in detail characterized genetically,
- reasonable use of mutations can further increase the activity, selectivity and specificity of microbial BFC.
- The disadvantages of microbial BFC include the following:
- high adaptability and variability of microorganisms' properties that can change the parameters of BFC uncontrollably,
- the problem of microbial activity maintaining unchanged for long periods of time,
- the electron transfer using mediators may interfere with atmospheric oxygen in recovery reactions of microorganisms,
- microbial catalysts have a larger volume than the enzyme.

The listed features testify that in the presence of a specific practical task it is necessary to be guided by these data for search of the optimum decision.

2. MODERN DEVELOPMENT DIRECTIONS FOR BFC

Considering the possible directions of researches on BFC, in particular, on the BFC on the basis of microbial cells, we can point out several major problems.

The major task is an improvement of BFC parameters, in particular, energy efficiency increasing. This question is related to the structure of electrodes, their type, material and fuel. As the analysis of the literature data shows, a rather great value is attached to development of new types of electrodes. The challenge is to find the conditions of maximum energy output of a single BFC by finding of optimal conditions for charge transfer. For finding of optimal conditions, it is necessary to increase the electrode surface area, available for the biomaterial immobilization, and also to test the used material for creation of electrodes with the smallest resistance.

Among the actual directions of micropower is the development of the hybrid device model for power supply of micropowerful radio-electronic devices, in which the BFC is interfaced to an electronic accumulative element in the form of the high-capacity supercapacitor (ionistor) for transfer

and storage of electric energy, and also system of contactless supply of energy from the ambient electromagnetic field.

An important practical problem is to reduce the BFC sizes. The researches on miniaturization of BFC, global tendency of creation of machines and devices with a high density of functional elements have long been developing in the world. So, in 1999, were published materials of Japanese researchers, where was offered a multi-channel version of BFC, executed by means of microelectronic technology. The multichannel BFC represented a device, containing 25 pairs of BFC-cells in series and each pair contained an anode and cathodic compartment, separated by an ion - permeable membrane. As an operating enzyme was used glucose oxidase. The estimated capacity of such battery should be several watts. Its use was planned in the robotized systems [31].

In 2012, a series of works on creation of hybrid systems such as "BFC-living organism" was published [32, 33]. The research subject is not new: about a decade was discussed the possibility of oxidized substrates receiving for BFC, implanted into an organism, from living being organic resources. However, earlier the works raised the question purely theoretically and there are still only single examples of implementation in practice. In fact, in the work [32] was realized for the first time the practical option, which presents the BFC of membraneless type, implanted in freely moving snail. For the BFC formation was used a *PQQ*-dependent glucose dehydrogenase and in the cathode compartment - laccase. Electrodes represented the carbon nanotubes, providing non-mediated electron transport. The implanted electrode system provides long-term registration of current generation. So, after two weeks of BFC functioning the current level was almost equal to the initial.

The next step in the development was an attempt to show that the electric power, generated by a low-power BFC, can efficiently be accumulated by means of an ionistor – high-capacity condenser [34]. For the BFC model was used a supercondenser with a capacity of about 1 *F*. For its charging were used three BFC cells, connected in parallel. During about 60 minutes, the capacitor voltage increases to 240 *mV* at a total cumulative energy of about 28 *mJ*. The accumulated energy was enough to turn a minielectric motor rotor by 90°. Apparently, the further developing will allow to use such systems for energy provision of biomicrodevices.

The second direction of researches provides an approach to the creation of the BFC new type. We are talking about the kind of bacterial cells *Desulfobulbus*, forming a filament structure and living on the border with a high gradient of dissolved oxygen [35]. Bacteria of this type are anaerobes (oxidation of organic substances - hydrogen sulfide - occurs in anoxic conditions) and can be found in benthonic layers of reservoirs. The community of bacteria represents threads, positioned vertically. The approximate length of a single thread is about 1-2 cm with an average thread diameter of about 1-5 microns. The thread part that is above lives in excess of oxygen. They have a little or not at all oxidizable substrate – hydrogen sulfide. The thread part that faces downward is in reciprocal conditions – the environment has a high concentration of oxidizable substrate – hydrogen sulfide, but little or no oxygen for oxidation. As a result the bacteria, in the combined threads, represent electrical cables, where electrons are continuously transported in direction "bottom-up". The electrons are generated at the bottom of the thread during oxidation of hydrogen sulphide. They are transported upwards in each bacterial chain, where they participate in oxygen restoration reaction.

Analyzing this situation, it is possible to note that this structure type of the combined bacterial cells represents the BFC perfect type, created by the nature:

- bacteria are combined in the structure, performing the BFC functions – chemical energy is converted into electrical energy,
- there are no problems, associated with the immobilization of cells on electrodes for the charge transfer,
- there are no problems of using mediators
- there is no need for ion-selective membranes for separation of charges,
- thread structure must have a high degree of mechanical strength,
- conditions for preservation of electric isolation of such "electric cable" are created.

The practically important task is a performance of modeling of conditions and development of technology for receiving such threads by artificial

means or use of natural structures to form the BFC, based on the combined type of bacterial cells *Desulfobulbus.*

3. DEVELOPMENT OF THE BIOANODE OF A FUEL ELEMENT ON THE BASIS OF THERMOEXPANDED GRAPHITE

The work purpose is to develop the bioanode of a fuel element, based on bacterial membrane fractions of *G. oxydans*, immobilized on the electrode surface from thermoexpanded graphite (TRG), while oxidation of ethanol and study of its load characteristics.

The direct transformation of chemical energy compounds into electrical energy is carried out in the biofuel elements. The electrode material choice is important for development of BFC on the basis of the immobilized microorganisms or enzymes. The electrodes for BFC have to possess a number of unique properties. They must provide good electrical conductivity, chemical resistance, biocompatibility, high specific surface and processability, including the reinforcement and injection to composite materials. In this regard, the nanocarbon graphene materials, for example, thermoexpanded graphite, are rather perspective.

Original crystalline graphite is oxidized in the production of TRG. The oxidation is reduced to injection of molecules and ions of sulfuric or nitric acid in the presence of an oxidizer (hydrogen peroxide, potassium permanganate, etc.) between the layers of the graphite crystal lattice. The received oxidized graphite is washed and dried. Then the oxidized graphite is exposed to very specific heat treatment – high-speed heating at a rate of 400-600°*C/s*. Due to the extremely high rate of heating, there is an extreme allocation of gaseous products of decomposition of the embedded intercalates from the graphite crystal lattice. As a result, the interlayer distance increases sharply, and a small flake of graphite becomes fiber. Due to the fibrous structure, the thermally expanded graphite is well pressed, formed, rolled and reinforced with different additives for products receiving.

In this work, as the electrode material was firstly used nanocarbon material – graphene-based thermally expanded graphite [36]. Technologies of thermal expansion of intercalated graphite particles and receiving of highly conductive materials with a well-developed surface belong to nanotechnological processes. The **Fig. 4** shows that the graphite particles under the influence of temperature are split practically to graphene layers.

The layered-fibrous structure of TRG allows by rolling on rollers to receive a sheet material, having a high conductivity. By sealing of a porous initial sample when changing the gap between the rolls of rollers and shift tension, generated during rotation of the rolls, the sufficient dense structure of the sheet material from TRG is formed.

We investigated experimentally the temperature dependence of the electrical conductivity of the received sheet material from TRG. The sample for the study is a plate 22 mm wide and 0.35 mm thick. The resistance measurement was performed according to the two-electrode scheme by the digital voltmeter. Maintenance of the temperature was carried out by means of the muffle MIMP-3P furnace, and the sample was placed in the measuring cell with nickel tubular electrodes on a ceramic base. It is shown that planar electrodes on the basis of TRG

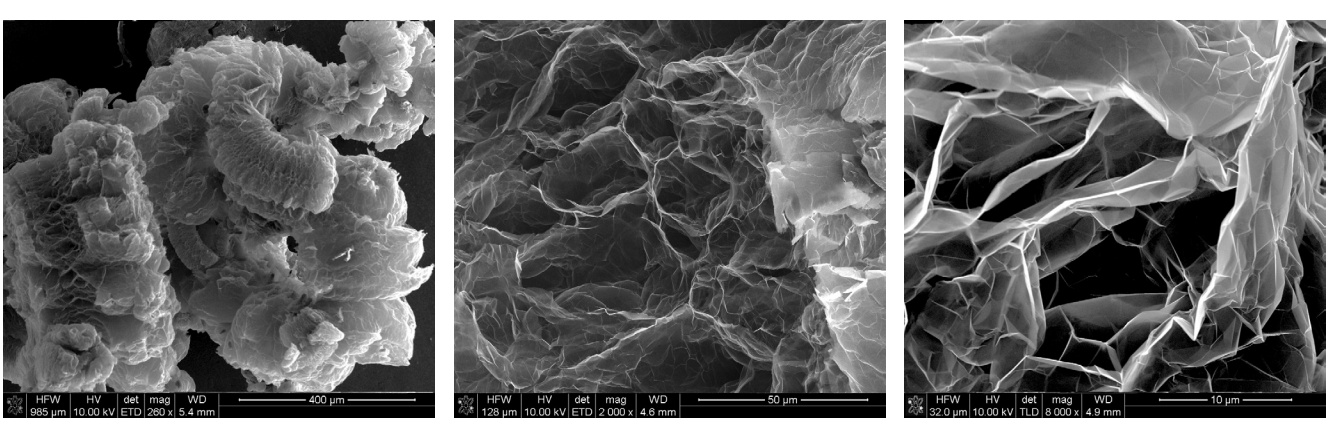

Fig. 4. *Images of TEG structure at various scales.*

possess good thermomechanical properties and rather low conductivity (**Fig. 5**).

Thus, the thermally expanded graphite is a material with good electrical conductivity, high specific surface area up to 2000 m^2/g, biocompatibility, chemical resistance in corrosive environments and can be a long time in the technical operation [37]. These qualities allow immobilization on the electrode TRG surface of the high superficial concentration of bacterial or their membrane fractions. The TRG also makes possible to form the electrodes of different shapes by a simple method of pressing. This approach is original because hitherto unknown in the literature studies, where the TRG was used in combination with biomaterial.

In microbial and enzyme BFC can be used redox mediators, which carry out electron transfer from the biocatalyst to the electrode [38]. For some enzymes [39-42] is shown non-mediated bioelectrocatalysis. Substantially non-mediated/ direct electron transfer depends on the type of enzyme immobilization method. Thus, the direct transfer of electrons has been described for *PQQ* (pyrroloquinoline) –dependent dehydrogenases as a part of enzyme biosensors and biofuel elements [43]. For the first time, the direct bioelectrocatalysis for a *PQQ*-dependent fructose dehydrogenase of *Gluconobacter* bacteria in the oxidation of fructose has been investigated in [40]. The fructose dehydrogenase enzyme was immobilized on the electrodes, made on the basis of carbon paste. The response to fructose was registered without addition of electron transport mediators. In the work [41]

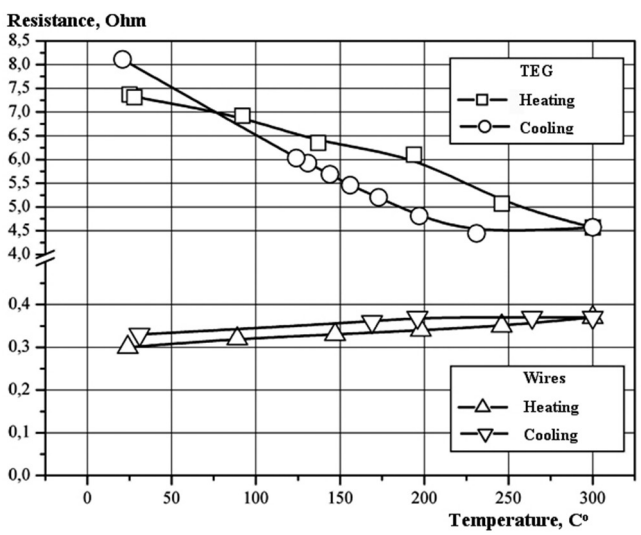

Fig. 5. *Comparative temperature conductivity dependence of the obtained sheet TEG material and material of lead wires.*

was found a direct non-mediated bioelectrocatalytic effect on a *PQQ*-dependent lactate dehydrogenase of *Gluconobacter sp. 33* on the gold electrode and electrodes, received by the dot-matrix printing. In the work [42] is represented an electrochemical cell, used as the working electrode a graphite core with an immobilized *PQQ*-dependent alcohol dehydrogenase (ADG) strain of *Gluconobacter sp. 33*. For this enzyme was observed a non-mediated electrochemical oxidation of ethyl alcohol. The ADG was immobilized on a graphite core by a cross-linking with glutaraldehyde. The maximum BFC voltage in the presence of ethanol at the opened circuit was 115 mV when using as the second electrode of a graphite core with inactive ADG. The three-electrode scheme was used for registration of the amperometric signal at +400 mV in relation to a reference electrode.

Also the non-mediated electron transfer was shown for such microorganisms as *Shewanella putrefaciens, Aeromonas hydrophila, Clostridium, Geobacter* [43]. Previously, it was found that bacteria of the genus *Geobacter* can perform the transfer of electrons to the graphite electrodes [44]. In the work [45] was demonstrated that *Geobacter sulfurreducens* can efficiently transfer electrons not only on graphite electrodes, but also when using as an anode of a gold electrode. On the anode was increased biofilm *G. sulfurreducens* and the reference electrode was the *Ag/AgCl*-electrode, where as an auxiliary electrode was used a graphite cloth. When using a gold electrode as the anode, the current density was 688 mA/m^2, and when using graphite cloth – 3147 mA/m^2.

The urgent task is to ascertain the possibility of using as an anode biocatalyst for BFC membrane fractions (MF) of microbial cells, containing a *PQQ*-dependent dehydrogenase. In a number of recent works was observed effect of direct electron transfer from a *PQQ*-dependent glucose dehydrogenase, immobilized onto multiwalled carbon nanotubes [46, 47, 48]. These data are the basis for the assumption that the use of biocomposite TRG/MF could provide a non-mediated catalysis of electrooxidation. The TRG has a high effective surface and, in this sense, is similar to nanotubes, on the other hand, the MF in fact are essentially membrane fragments, enriched with a *PQQ*-dependent dehydrogenases. As the test substrate was chosen an ethanol, which

effective oxidation of MF was shown in [47, 48]. We will note that unlike the expensive and complex techniques for receiving and purifying of enzymes, when receiving of MF is used a simpler procedure and, in this connection, the MF can be alternative to the use of enzymes in BFC.

3.1. Development and production of the bianode model

The *Gluconobacter oxydans* bacterial strain VKM B-1280 was received by the All-Russian Collection of Microorganisms of the IBPM RAS. The intact cells or their membrane fractions (MF were allocated in accordance with the procedure [47, 48] were immobilized by inclusion in gel of a chitosan on the TRG-electrode surface. For this purpose, on the electrode was applied 20 mcl of MF or intact cells, diluted twice with buffer solution. The electrode was dried at room temperature, it was applied 20 mcl of a 2% solution of chitosan in 1% acetic acid [49], and then it was dried at room temperature within 30 min.

The sinthesis of TRG was carried out on hydrosulphatic technology [36]. The process of TRG receiving included three main stages:

1) formation of intercalation connection (graphite hydrogensulphate) with stirring of low-ash graphite (GSM-1) within 30 min with a mixture of concentrated nitric and sulfuric acids at their ratio of 10:1 within thirty minutes. The graphite particles are modified with a mixture of sulfuric and nitric acids. This provided intercalation with the injection into the interlayer space of graphite particles of the intercalate molecules;

2) graphite interstitial compounds washing with water from the remains of acids to $pH \approx 6.8$ and subsequent drying of the oxidized graphite to a loose condition;

3) thermal expansion of the oxidized graphite at thermal shock of 800-900°C with receiving of TRG.

When heating of such powder, there is a destruction of the lamellar structure of graphite and transition to a layered-fibrous structure of expanded graphite. The received graphene-based TRG had a bulk density of $Pb = 16$ g/l. The elemental analysis showed that as an impurity the TRG comprises 0.5% of sulfur. By the method of raster electronic microscopy with the resolution up to 100 nanometers is observed a distinct layered-fibrous structure of the graphite material (Fig. 4).

The bulk density of expanded graphite powder can vary quite widely, depending on the mode of thermal expansion. The bulk density of TRG, which was determined by weighing of 15 ml after heating at temperatures of 200, 220, 250 ($\pm 5°C$) within 10 minutes, was experimentally investigated. It was shown that with increase of heating temperatures of graphite particles, the values of bulk density of TRG decreased, and, according to heating temperatures, equaled to 116-113, 46-42, 36-33 g/l [36.]

Operating electrodes from TRG formed TRG by compacting of the powder at a pressure of 150 bars. The diameter of the measuring electrode was 12 mm, thickness – 0.2 mm. The cyclic voltamperograms were recorded using three-electrode circuit at a potential scanning rate of 3 mV/s. The reference electrode was a standard chlorine-silver electrode and the auxiliary electrode was a platinum plate of 1.8 cm^2. The chronopotencio metric measurements were performed by measuring of the potential of a stationary working electrode with respect to time of the reference electrode. Measurements were performed in the 30 mM solution of potassium-sodium phosphatic buffer (*pH* 6.0). The substrates were ethanol, glucose or acetaldehyde (DiaEm, Russia). The only one concentration of substrates was used in the operating electrolyte of 10 mM (if it isn't specified especially). In some experiments, as redox mediator was used 2,6-dichlorophenolindophenol at a concentration of 8 μM (DCPIP, Sigma-Aldrich). The measurements were performed on the galvanopotentsiostata VersaSTAT 4 (Ametek Inc.).

In the ethanol oxidation by an amperometric method was determined the MF respiratory activity. The MF immobilization was carried out by physical sorption onto glass fiber filters (GF/A type, Whatman, UK). For this purpose the MF suspension of 5 μl, containing biomass in concentration of 100 mg of crude weight/ml, were deposited on a filter and dried at room temperature within 20 min. The membrane with a bioreceptor of 3×3 mm^2 was fixed on the measuring surface of an oxygen electrode like Clark (Ingold, Germany). The respiratory activity of MF was caused by areas of the respiratory chain of the cellular membrane,

conjugated with clusters of *PQQ*-dependent dehydrogenases. The amperometric measurements were carried out in an open container of 2 ml. The Galvanostat-potentiostat IPC2L were used (LLC "Kronas", Russia), interfaced with a personal computer. For measurements in the measuring cell, containing buffer solution and oxygen electrode, the ethanol samples of 100 mcl were brought, containing various concentrations of the substrate. The recorded parameter was the maximum rate of a signal change (nA/s).

For receiving of images with a scanning electron microscope (SEM) on an electrode surface with cells and MF, a thin layer of gold was deposited. The deposition was carried out in a vacuum sputtering installation JFC-1100 (JEOL, Japan). The electron microscopic analysis of the samples was measured by SEM JSM-6510 LV (JEOL, Japan).

3.2. Electrophysical researches

During formation of the operating BFC electrode, it is necessary to carry out an immobilization of cellular material onto a surface of the volume electrode and as much as possible to fill its porous part. The view of whole cells of *G. oxydans* and MF, immobilized onto TRG, is given in **Fig. 6.1** and **6.2**, respectively; the images were received by means of SEM. The concentration of cells for immobilization was chosen such that they formed a monolayer with insignificant gleams. The scaly structure of TRG, pressed as a result of an electrode formation is visible in places of gleams (Fig. 6.1). The MF, being significantly smaller structures compared with cells, formed almost complete covering of the TRG-electrode (Fig. 6.2).

Since the bacterial membrane fractions of *G. oxydans* represent a fragment of the respiratory chain, for the characterization of their catalytic activity in the oxidation of ethanol was used the

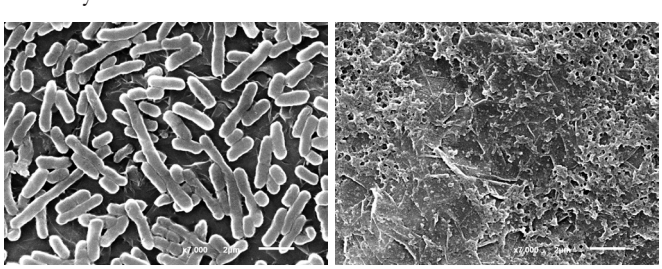

Fig. 6. *Whole cells of G. oxydans (6.1) and their MF (6.2) obtained by the scanning electronic microscopy. Immobilized biomaterial is covered with a layer of a hitozan.*

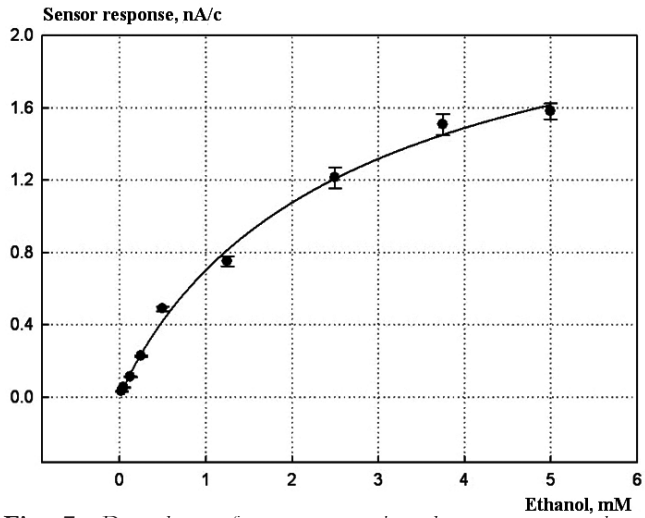

Fig. 7. *Dependence of an oxygen electrode response on ethanol concentration in presence of membrane fractions of G. oxydans bacteria.*

Clark-type oxygen electrode. From the dependence of the oxidation rate on the concentration of ethanol (**Fig. 7**) in the reactionary solution were calculated apparent K_M^{eff} (Michaelis's constant) and V^{eff}_m (the maximum reaction rate) values that were 2.8±0.7 mM and 2.5±0.3 nA/s, respectively.

The **Fig. 8** shows the dependences of potential on time, registered for the bioanode with immobilized MF by adding to the working electrolyte of ethanol, acetaldehyde or glucose. It is visible that after applying an ethanol in media, containing the measuring electrode, the stationary potential of the bioanode shifted to negative values of the potential. 1200 seconds later after the addition of ethanol the working electrode potential variation from a reference value was about 50 mV. This effect is an indication that the MF of *G. oxydans* bacteria contains the enzyme (a *PQQ*-dependent alcohol dehydrogenase), which

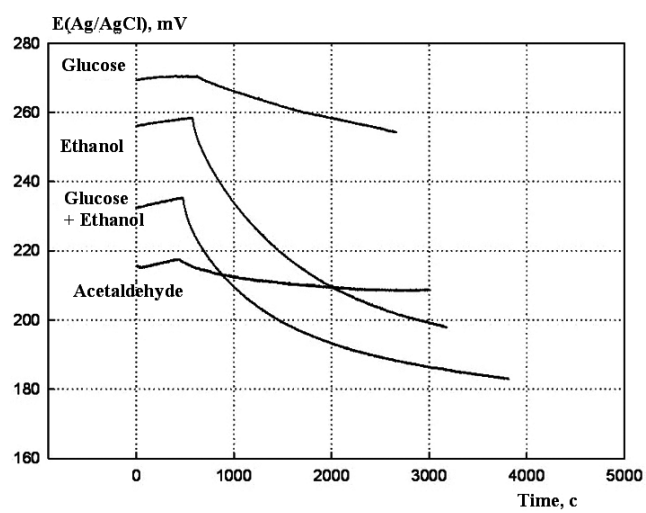

Fig. 8. *Dependences of stationary potentials from time for TEG-electrodes with immobilized by MF at addition in electrolyte of ethanol, acetaldehyde, glucose.*

catalyzes the electrooxidation of ethanol on the mechanism of non-mediated bioelectrocatalysis. After applying of acetaldehyde or glucose occurred only minor changes in the potential. The **Fig. 9** shows the dependence of the stationary potential of MF/TRG-electrode according to different concentrations of ethanol. The maximum value of the measured potential is achieved at an ethanol concentration of 10 mM in the operating electrolyte. The further increase in concentration didn't lead to an increase in potential change.

The cyclic voltamperogramma (CVA), registered by a measuring electrode with immobilized MF, are given in **Fig. 10**. It is seen that in the presence of ethanol, the anode current is increased compared with the control in the absence of a mediator. The observed increase in the anode current was not too considerable that may be associated with a relatively low concentration of electrocatalytically active points on the electrode surface. This may also be due to the low efficiency of electron transport between the electrode and biocatalyst.

Another pairing option of biochemical and electrochemical reactions is the mediator way. As the redox-mediator was used DCPIP, which is an effective electron acceptor in various biochemical processes. Furthermore, the standard redox-potential of the couple $DCPIP_{reduct}/DCPIP_{ox}$ is +0.217 V that allows to carry out the bio-electrochemical oxidation reaction of ethanol with relatively low values of the electrode potential under aerobic conditions. After adding of modified MF to the buffer solution of DCPIP and ethanol for the bioanode, the cathode

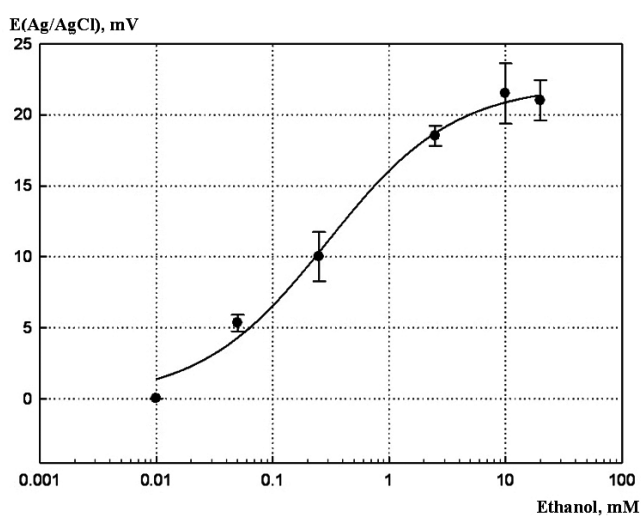

Fig. 9. *Change of stationary potential MF/TEG-electrode from concentration of ethanol in working buffer solution.*

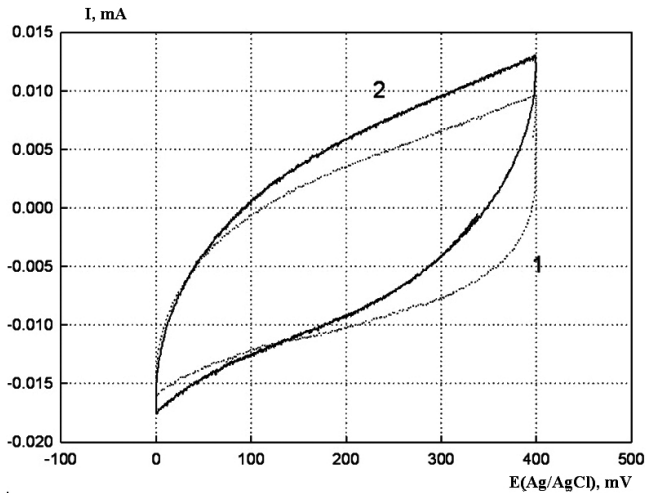

Fig. 10. *VAC are written down for TEG-electrode with immobilized MF in 30 mM potassium-sodium-phosphatic buffer solution, pH 6.0 (1) and in presence of 10 mM of ethanol (2).*

stationary potential change in the absence of substrate was 120 mV.

The **Fig. 11** shows the CVA for the bioanode with immobilized MF in buffer solution (control condition) and in a solution, containing ethanol and DCPIP. It is seen that in the presence of a redox mediator, on the cyclic voltammograms, starting with zero potential, there is a significant increase in the anode current in comparison with a control state, indicating on the mediated bioelectrocatalytic mechanism of the ethanol oxidation.

In the work was also used the bianode option on the basis of TRG with immobilized intact cells of *G. oxydans*. In the preliminary experiments, it was shown that in the absence of a mediator after adding of ethanol to the electrolyte solution there was no any changes of stationary potential of the bioanode.

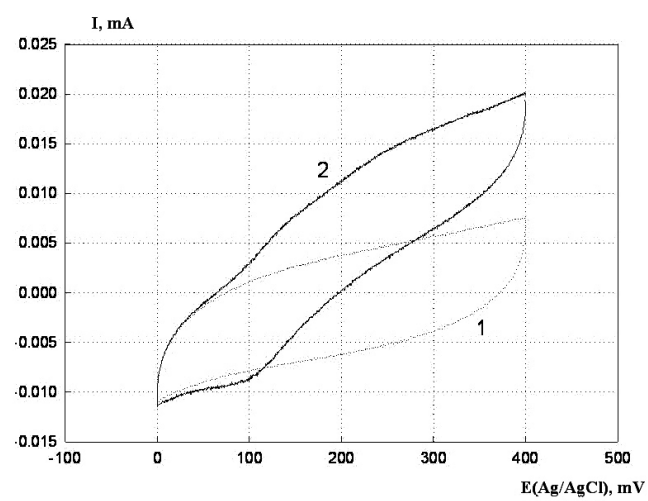

Fig. 11. *Voltamperometric characteristics (VAC) are written down for TEG-electrode with immobilized MF in 30 mM potassium-sodium-phosphatic buffer solution, pH 6.0 (1) and in presence of DHFIF (8 μM) and ethanol (10 mM) (2).*

This was due to the absence of a non-mediated electron transport from a donor-substrate (ethanol) on the electrode with immobilized intact cells. So, for enhancing of bioelectrochemical reaction of oxidation of ethanol, a redox mediator DCPIP was injected into the reaction system. After adding of redox mediator and ethanol into the reaction electrolyte, the change of the electrode potential was 160 mV from a reference value. The CVA, registered for bioanoda of TRG with immobilized intact cells in buffer solution and in the presence of a redox mediator and ethanol, are shown in **Fig. 12**.

Thus, the possibility of forming of the fuel cell's bioanode, based on thermoexpanded graphite and immobilized membrane fractions of *G. oxydans* bacteria, which are bioelectrocatalysts of oxidation reaction of ethanol, was demonstrated for a first time. The membrane fractions were immobilized onto the TRG-electrode, allow to perform non-mediated bioelectrocatalytic ethanol oxidation on the electrode, however, the process rate was low. The redox mediator 2,6-dihlorofenolindofenol significantly increases the rate of bio-electrochemical reactions, involving immobilized MF, and also allows an electrooxidation of ethanol using immobilized intact cells.

4. APPLICATION OF A GRAPHENE IN BIOFUEL ELEMENTS

Currently, a graphene (Gr) is the object of a great interest in various fields of science, including biology and biotechnology. The Gr is a two-dimensional nanomaterial, having high electrical conductivity and mechanical strength and a number of other

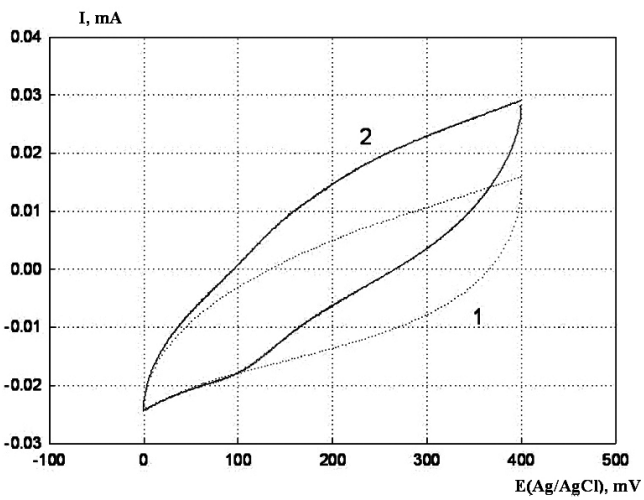

Fig. 12. *VAC registered for the TEG-electrode with immobilized intact cells in 30 mM potassium-sodium-phosphatic buffer solution, pH 6.0) - (1) and in presence of DHFIF (8 μM) and ethanol (10 mM) – (2).*

properties, valuable in designing of electronic devices. It is found that Gr is well compatible with biomaterial that allows to use it in forming of biosensors and biofuel cells - devices, where the biocatalyst converts the energy of oxidation of organic substrates/biofuel into electric energy. The Gr can be used for the preparation of the high-structured electrode surfaces, which due to the high ratio of surface area to volume can produce an immobilization of a significant amount of the biocatalyst, that is, in principle, can result in a high output electric power of BFC. Taking into account that both subjects of Gr and of the BFC are intensively developed directions of researches now, in this work we consider the current state of the Gr use in BFC, where enzymes and microbial cells are used as a biomaterial. Actually, the question, on which researchers of various groups would like to get the answer, may be the following – whether is it really necessary to use Gr instead of the well-known and positively proved nanotubes or metalnanoparticles.

The graphene is the single layer of sp^2-carbon, consisting of the condensed six-membered rings. The material, consisting of two such layers, rather strongly differs in physical characteristics from a single-layer graphene, not to mention the bigger number of layers. An even greater difference is observed in "thick" scales.

For convenience, we use the following notations: Gr – graphene (without specifying the number of layers); Gra – graphite; HOPG – highly oriented pyrolytic graphite; nGra – nanosized graphite; PGra – penographite, ICGra – graphite's intercalation compounds, GraO – graphite oxide; 1sGraO – single-layered graphite oxide, mlGrO – multilayered graphite oxide, redGraO (RGO) – reduced graphite oxide (1 single-layer or multilayered); 1slGr – single-layered graphene, mlGr – multi-layered graphene (graphite plates with thickness of a few graphene layers), CMG – chemically modified graphene (graphene, containing substituents (*H, F* et al., and/or functional groups).

Physical properties of graphite

The free carbon in nature meets in two main types: diamond and graphite, and among synthetic types should be noted carbine, fullerenes, nanotubes, pyrolytic graphite, etc. A variety of modifications is caused by ability of carbon atom to accept tetrahedral sp^3 – (diamond), trigonal sp^2 – (graphite, graphene,

fullerenes, nanotubes) or linear *sp* – (carbyne) hybridization [50, 51].

The usual graphite is a tabular mass with a lot of metallic lusters, having various degrees of crystallinity and orderliness; individual particles can look almost as perfect crystals. The most frequently used in the laboratory graphite is a pyrolytic, which is received by decomposition of hydrocarbons on a graphite substrate at a temperature over 2000°*C*. To improve the regularity of the crystal is used the recrystallization, implying hot uniaxial pressing under a pressure of 300-500 kg/cm^2 at 3000°*C*. In this way, we receive samples of more than 10 mm thick and density of 2.27 g/cm^3, which is 99.95% of the theoretical density of graphite. The subsequent annealing of material at 3400-3500°C results in a highly oriented pyrolytic graphite.

Chemical properties of graphite

Graphite is quite inert under normal conditions. It is oxidized by atmospheric oxygen to *CO* at a temperature over 400°*C*, and to CO_2 - at temperature over 500°*C*. The temperature of the reaction beginning is higher, when the crystal structure of graphite is more perfect. Oxidation is accelerated in the presence of iron ions, sodium, copper and other metals, and slows in the presence of molecules of chlorine, phosphorus and boron compounds. Graphite hardly reacts with molecular nitrogen, with atomic at a usual temperature forms cyanogen C_2N_2. Halogens are being imbedded into the crystal lattice of graphite, giving interstitial compounds. With the majority of metals and their oxides graphite gives carbides. It with all alkaline metals, some halides, oxyfluorides, halogen oxides, oxides and sulfides of metals forms interstitial compounds with nitrides of metals at temperatures above 1000°*C* – solid solutions of nitrides and carbides, with borides and carbides – eutectic mixtures with melting temperatures of 1800-3200°*C*.

Covalent and intercalation compounds of graphite

Due to the layered structure, some atoms, ions and molecules can be imbedded into the interlayer space of graphite. The result are the so-called covalent compounds (CCGra) and intercalation compounds (ISGra) of graphite. The CCGra include fluoride graphite and graphite oxide – GraO. While formation of CCGra there is a partial transition of carbon atoms from sp^2- to sp^3-hybrid state and, as a result, a deformation of flat carbon grids [53].

So, the graphite oxide, which still saves developed reticular structure, is the most oxidized compound of graphite. Chemical methods of receiving of graphite oxide, based on the oxidation of graphite in concentrated acids (nitric acid, sulfuric acid) by such strong oxidants as $KMnO_4$, $KClO_3$, $(NH_4)_2S_2O_8$, MnO_2, etc. [54]. The lower rate of formation of graphite oxide in comparison with the rate of formation CCGra testifies numerous violations of the *C-C* bonds within each carbon hexagonal grid.

The carbon atoms in Gr are joined by sp^2 bonds in a hexagonal two-dimensional (2*D*) lattice. From the viewpoint of materials science, the single-layer Gr is a substance, furthermore, it is a separate molecule. From a chemical point of view, the single-layer Gr - a polymer.

Preparation of graphene and its analogs from the oxidized graphite

Due to not very successful initial attempts to receive graphene by direct dispersing of graphite, the dispersion of Gra derivatives, in which the interaction between the layers is reduced, appeared more promising. As such Gra derivatives are known GraO, CCGra and PGra. Most often the Gr is received using GraO. In turn, GraO opened in the 19th century, long before the discovery of the Gr, is received in three ways: 1)by the Brody's method [55], 2)the Shtaudenmayer's method [56] and 3)the Hammers's method [57]. All three methods include the step of processing of Gra with strong acids and oxidizers.

On the basis of GrO, which solubility (dispersibility) in water and other solvents is high, carried out the procedure of thin films coating with application potential in electronics. The oxidized Gr is an insulator, but its electronic characteristics can be controlled within certain limits by changing the degree of oxidation. Besides change of scales geometry, the properties of oxidized Gr are determined by the nature and ratio of oxygen-containing functional groups - carboxyl, hydroxyl or epoxy. The GraO, which still saves a developed grid structure, is the most oxidized compound of Gra. The lower rate of GraO, compared with the rate of CCGra formation, suggests numerous violations of the *C=C* bonds within each carbon hexagonal grid. The observations, made by scanning electron microscopy, showed that the GraO has a rough

surface with an average height of irregularities of 0.6 nm and amorphous structure due to the large amount of sp^3 *C-O* bonds [58].

Currently there is no single formula for GraO, since this compound is determined by the synthesis conditions and nature of the original graphite. The GraO often attribute a formula $C_8O_2(OH)_2$, where the oxygen is in the carboxyl, hydroxyl, ketonic, epoxy and other *O*-containing groups, which determine the acid-base properties of GraO and its hydrophilic [54]. The interlayered distance in GraO changes reversibly from 0.6 to 1.2 nm with increasing relative humidity of the product, which may indicate the formation of a grid of hydrogen bonds between the *O*-containing groups. The idealized structure of a single-layer GraO shown in **Fig. 13.**

A number of authors specify that homogeneous dispersions of GraO in water solutions and organic solvents can be received with simple processing of GraO by ultrasound (US) [60]. The hydrophilic GraO is dispersed in water with the maximum concentration of 3 mg/ml, forming brown and dark brown dispersions. The GraO dispersion in various organic solvents such as ethylene glycol, dimethylformamide (DMF), N-methylpyrrolidone, allows to receive a GraO concentration about 0.5 mg/ml [61].

The large number of functional groups, typically hydroxy and epoxy, allows to stabilize GraO skales in water. However, such functionalization destroys delocalized electrons π-system of graphene. The GraO actually becomes more insulating than a semimetal and this is its fundamental difference from graphene [62]. It is shown that under the action of powerful ultrasonic irradiation the GraO splits into fragments that subsequently formed by self-assembly fullerene and its analogs, carbon nanotubes and high-condensation products [63].

The chemical modification of the *O*-containing groups in GraO by various reagents (e.g., isocyanates) leads to the formation of the corresponding

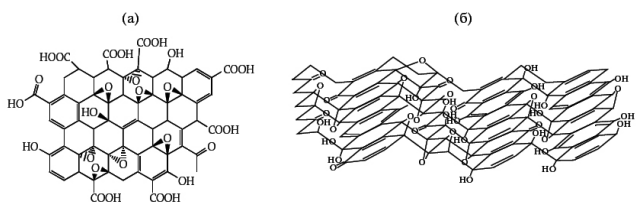

Fig. 13. *Graphite oxide structure: a) top view of idealized structure of one layer of GO, b) side view of model of single-layer GO* [59].

derivatives and increased concentrations of homogeneous dispersions in organic solvents [64].

Reduction of the oxidized graphene

For receiving of Gr from a single-layer GraO it is necessary to reduce the last – to remove the *O*-containing groups and to restore the system of *C=C* bonds. The process may be carried out at low temperature of about 180°*C* using as reducers such solvents as dimethylformamide, ethanol, butanol and others. The reaction progress is registered by a change in color of the dispersion. It is shown that the GraO can be effectively reduced by a simple long (more than 24 hours) heating at 220°*C* and even at 100°C in water or heating for 1 hour at 180°*C* in dimethylformamide [65].

To improve the solubility of reduced GraO, the product receiving is conducted in three stages: 1)reducing of oxygen group of GraO in $NaBH_4$ solution, 2)the reduced product is arylated by 2-sulfo aryldiazonium salt and 3)repeated performance of reduction with hydrazine to remove the remaining oxygen-containing groups. Such sulfonated reduced GraO is steady in water with a *pH* = 3-10 and concentration of 2 mg/ml. For the same purpose, it is possible to use, as shown in [66], some cellulose derivatives (sulfo, carboxymethyl or carboxypropyl derivatives).

In the work [67] as the reducing agent is proposed to use vitamin *C*, as well as a stabilizer – amino acids.

Experiments showed that the reduced GraO isn't equivalent to Gr; in other words, it isn't possible to fully reduce the GraO to Gr. Thus, the products, received in the reduction of GraO, contain substantial amounts of oxygen and, possibly, defects that destroy the delocalized π-system and significantly reduce the graphene electronic characteristics. From organic chemistry of aromatic compounds (to which single-layer Gr formally belongs), it is known that a number of *O*-containing groups are not reduced by the given above reducers. In confirmation of it, the calculations showed that reducing of less than 6.25% of Grao area is complicated in view of complexity of removal of hydroxyl groups. For these researches the GraO's model, containing hydroxyl and epoxy groups, was used and with nuclear relation *C/O* = 16 [68].

It would seem that thermal recovery methods are easier to execute, but they lead to the same results – the oxygen is retained in the final product [69].

At the same time, it is shown that the calcination of the samples of the reduced GraO [70] opens the possibility of a complete reducing of the grid of sp^2 bonds of six-membered rings. The GraO's reducing showed according to electron microscopy that the reduced GraO's scales consist of graphene islands, ranging in size from 1 to 6 nm, separated by a defective cluster, forming a flat area with quasiamorphous sp^2 *C-C* bonds; in addition, they contain large number of topological defects. On this basis, the following scenario of graphite redox was provided. Initially, during the oxidation locally are formed strongly oxidized areas, whereas 60% of the surface remains unchanged. After reducing the constant area remain unchanged, and the oxidized area reduced to sp^2-related grids, which, however, lose (not reduce) the original crystallinity (orderliness) of the graphene. In the structure of disordered areas are formed the so-called topological defects, so the reduced graphene is usually different from the single-layer graphene and in English literature usually denoted as RGO. The conductivity of the reduced graphene in 10 times or more lower than of the initial graphene [71].

In the work [72] by the method of atomic power microscopy was shown that the structure of reduced scales of graphene oxide differs significantly from the graphene, received by mechanical peeling. Being deposited on the smooth surface of highly oriented graphite, they have a non-flat globular morphology, indicating on the distortion of the carbon skeleton. Even more definitely the distortions of the structure were found in the work [73], where GraO's scales were reduced by three methods: aqueous hydrazine, under the action of the electron beam, or by heating at a temperature 300-600°*C*. In all cases, as the authors specify, were obtained a highly disordered graphene nanoscales. In the work [71] were studied the mechanical and electrical characteristics of the reduced scales of the suspended reduced GraO. It is shown that the mechanical properties (elasticity, flexibility) practically do not differ from single-layer graphene while electrical conductivity is significantly reduced and is at normal metallic conduction. In confirmation of it, it is shown that the local electrical properties of the reduced scales of GraO differ from properties of a single-layer graphene [74]. Thus, the presence of a large number of defects significantly reduces the electrical conductivity of these materials, however, makes them suitable as materials for stabilization of *Li* ions in supercondensers.

For identification of Gr are often used circular scattering spectroscopy [75] and atomic power microscopy [76], more rare – scanning tunneling microscopy [77].

4.1. The application of graphene materials for BFC electrodes

The one of the main problems of the industry development today consists in minimizing of financial costs and reducing the environmental load that accompany the overall growth of investments in production of food and energy. A promising way to solve the problem in the field of energy is to learn how to receive it via biocatalysis – isolated enzymes or microbial cells, which are biocatalysts, provide power generation in BFC devices [78].

Like the standard chemical element of the electric power – batteries, accumulators, etc., the BFC includes two electrodes, anode and cathode. The biocatalyst contains in the anode compartment, where are supplied substrates or fuel. The substrate is oxidized by anode biocatalyst, the released electrons flew on the anode surface. On the external circuit, the electrons move on other electrode – electrode, which also contains the biocatalyst, providing oxygen reduction [2].

The catalytic activity of the immobilized biomaterial is the most important factor, providing the generation of electrical energy in BFC. In this case, the question of its immobilization is also essential – on what material of an electrode the immobilization is made, what type of biomaterial is used, how the charge transfer to (from) the electrode(s) happens. The transfer of electrons to the anode and cathode can be done by two ways – by a carrier, i.e., mediator and by direct transfer (*DT*). The corresponding type and fixing of biomaterial, type of material of the anode and cathode can provide both mediate and *DT* of the electron to (from) the electrode(s). The *DT* allows to receive more effective BFC as thus the element structure becomes simpler, its internal resistance decreases, the use of additional connections as mediators isn't required. This moment is especially important and it should be noted that recently the considerable number of the conductive nanomaterials by means of which implementation of *DT* became possible,

was described. For the first time the *DT* was described for the laccase enzyme in 1979 [79]. After a while, the following step on use of nanoconductors was taken and fullerene was introduced into practice – a zero-dimensional material [80] and one-dimensional material – nanotubes [81].

After the described method of Gr preparation in the laboratory of A.Geym and K.Novoselov [81], the flow research was aimed at studying the possibility to use a two-dimensional structure for immobilization of the redox-enzymes. Immediately after it was shown that the Gr has high electronic conductivity and relatively easily produced, the stream of researches directed on attempts of its application for bioelectrodes construction considerably grew [83]. The Cr could be received mechanically by removal of the graphite layers or by chemical way – vapour-deposition method; graphene materials are received by chemical synthesis. The Gr oxides have high solubility in water, because it contains in the plane sites atoms of oxygen and hydroxyl (**Fig. 14**).

The Gr reducing can be carried out thermally, chemically or electrochemically, and in addition biologically, for example, by means of microorganisms [84]; the reduced Gr (GrR) should be distinguished from the "pure" Gr, received by synthesis. This is due to the fact that the procedure is also characterized both by a recovery process, and extent of purification of material [85].

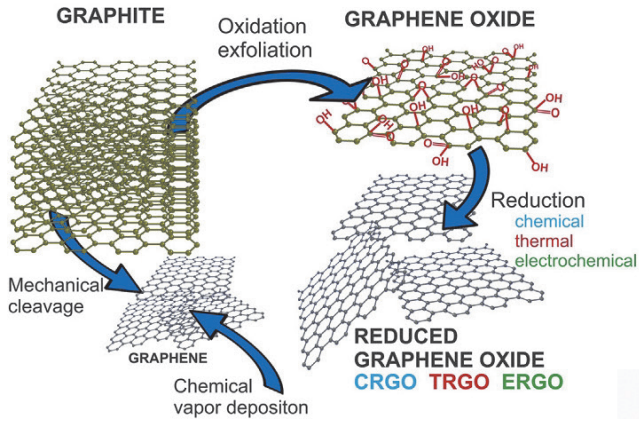

Fig. 14. *Schematic representation of methods obtaining G and GO (graphene oxide)* [84].

The first application progress was obtained by the use of Gr in the design of biosensors [86]; the data showed that Gr may be effectively used for interfacing with the biomaterial and actually opens the way to use it in the BFC. Nevertheless, it is quite surprising that were described only a little number of examples of the Gr use as a part of enzyme BFC. The joint use of microbial cells and Gr is studied more intensively; evidently, such situation is due to the fact that in general the total number of publications on microbial BFC is three times higher than on the enzymatic BFC.

Biofuel elements on the basis of enzymes

The data on BFC, based on graphene and graphene-like materials in combination with enzymes and microbial cells are summarized in the **Table**,

Table

The configuration and characteristics of some BFE on the basis of a graphen and enzymes/microbic cells (according to the review [84])

Configuration and characteristics of some BFE on the basis of enzymes						
	Anode configuration	Cathode configuration	Open-circuit voltage, mV	Maximum specific power, μW/sm^3	Voltage at the maximum power, mV	Source
1	TMOS gel + CRGO + FM + GOx on Au plate	TMOS gel + CRGO + ABTS + BOD on Au plate	580	24.3	380	[33]/43
2	GCE/graphene nanoplatellets/ GOx/Nafion	TMOS gel + CRGO + ABTS + BOD on Au plate	≈550	58.0	220	[34]/44
3	Au plate/CRGO/FM + GOx/PPy	GCE/graphene nanoplatellets/Lac + BSA/Nafion	790	78.3	500	[35]/45
4	GCE/ERGO-MWCNTs/GOx/ Nafion	GCE/CRGO + Pt nanoparticles + Nafion	400	46	≈80	[37]/47
5	GCE/ER(GO + GOx)	GCE/MWCNT-ZnO/Lac	60	0.054	50	[36]/46
6	AuE/electrodeposited(GO + Co(OH)2 in CHI) + GOx	AuE/electrodeposited(GO/ Co(OH)2/CHI)/Lac	600	517	460	[38]/48
7	G/CNTs-COOH/GOx	G/CNTs-COOH/Lac + ABTS in solution	1200	2270	500	[39]/49

TMOS–tetramethoxysilane; FM–ferrocenemethanol; ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); BOD–bilirubin oxidase; PPy–polypyrrole; GCE–glassy carbon electrode; CHI - chitosan.

Table (continued)

The configuration and characteristics of some BFE on the basis of a graphen and enzymes/microbic cells (according to the review [84])

The configuration and characteristics of some microbic BTE						
	Anode configuration; mediator; carbon source	Cathode configuration/final acceptor of electrons; membrane	Open-circuit voltage, mV	Maximum specific power, μW/sm^3	Voltage at the maximum power, mV	Source
8	SSM/CRGO-PTFE; E. coli, (HNQ); glucose	Nafion CP/[Fe(CN)6]−3/-4	---	267	530	[107]/50
9	CP/CRGO/PEDOT; E. coli, (HNQ); glucose	PEM CP/[Fe(CN)6]−3/-4	≈700	87	430	[108]/51
10	CC/ERGO; P. Aeruginosa; glucose	Nafion CC/[Fe(CN)6]−3/-4	---	5.25	460	[109]/52
11	Vacuum-stripped G-CHI composite; P. Aeruginosa; glucose	Nafion CC/[Fe(CN)6]−3/-4	---	153	550	[110]/53
12	CP/GO nanoribbons; S. oneidensis MR-1; lactate	CMI7000 CP/[Fe(CN)6]−3/-4	---	3.4	---	[111]/54
13	CVD-grown G foam/PANI; S. oneidensis MR-1; lactate	PEM CC/[Fe(CN)6]−3/-4	≈700	77	200	[112]/55
14	CP/IL-rGO; S. oneidensis MR-1; lactate	Nafion CP/IL-rGO/[Fe(CN)6]−3/-4	---	60	160	[113]/56
15	Ni foam/TRGO; S. oneidensis MR-1; lactate	CMI 7000S NA/[Fe(CN)6]−3/-4	620	≈80	250	[114]/57
16	Graphite felt/GO/PPy; S. oneidensis MR-1; lactate	PEM CF/[Fe(CN)6]−3/-4	---	133	≈420	[115]/58
17	CP/G nanoribbons/PANI; S. oneidensis MR-1; lactate	Nafion CP/[Fe(CN)6]−3/-4	---	86	≈175	[116]/59
18	CP/GO nanoribbons; MBFC microbial consorcium; acetate	CMI7000 CP/[Fe(CN)6]−3/-4	≈740	32.6	≈530	[111]/54
19	CC/crumpled rGO-Nafion; anaerobic sludge; acetate	CMI7000 carbon brush/ [Fe(CN)6]−3/-4	≈660	3.6 Вт/м^3	400	[117]60
20	CC; anaerobic sludge; acetate	Nafion CC-BRGO/dis. O_2	390	32	200	[118]/61
21	SSM/PU sponge-G; sludge-based consorcium; glucose	AMI-7001 CC-Pt nano/dis. O_2	---	157	---	[119]/62
22	CC/bacterially reduced GO; anaerobic sludge; acetate	PEM CC-Pt nano/air cathode	≈600	191	300	[120]/63
23	CC/crumpled rGO; anaerobic sludge; acetatет	PEM carbon brush/ [Fe(CN)6]−3/-4	≈800	240	520	[121]/64
24	CC/ERGO/PANI; anaerobic sludge; acetate	Nafion CF/[Fe(CN)6]−3/-4	770	139	460	[122]/65
25	CP/RGO-PEI layers; anaerobic sludge; glucose	CMI7000 CP/[Fe(CN)6]−3/-4p	≈730	37	≈370	[123]/66

AC–activated carbon; CC–carbon cloth; CF–carbon felt; CP–carbon paper; CB–carbon black; PANI–polyaniline; N-G–N-doped graphene; HNQ–hydroxynaphthoquinone; FeTsPc - iron tetrasulfophthalocyanine; BRGO–bacterially reduced GO; SSM–stainless steel mesh; dis. O_2–O_2 dissolved in aqueous solution; PTFE–polytetrafluorethylene

which shows the basic constructive and operating characteristics of BFC structures. We will consider some details of the presented systems.

Direct and indirect with use of mediator electron transfer. There are many publications, describing features of catalytic reactions in systems on the basis of the glucose oxydas (GO), used as a model enzyme, and Gr. In 2009, were published the first works on the possibility of coupling of GO and Gr [87]; a year later appeared the publication on the use of GO and Gr, as part of BFC [88]. As the cathode catalyst mainly was used laccase. The GO contains flavin adenine dinucleotide, cofactor, surrounded by a protein and glycan structure, limiting the effectiveness of the exchange of electrons between the active point of the protein and surface of the electrode; thus, the structure of the enzyme forms a barrier for bioelectrode operating conditions. It should also be noted that even if the non-mediated electron transport is described for a biosensor, containing GO, it does not mean that the catalytic

current is associated with direct transfer between the electrode and cofactor. This effect may be the result of non-enzymatic reaction of hydrogen peroxide or oxygen, involved in the catalytic cycle of GO on the electrode surface [89]. On the other hand, the direct transfer is known for the cathode application of GO, when the current is generated in the process of FAD-cofactor reducing, which is then reoxisized by oxygen. Obviously, this case is not acceptable for the BFC functioning [84].

This situation is, of course, allows to design the biosensors, based on GO, but the principle is not applicable to formation of BFC for the following reasons: 1)GO more efficiently transfers the electrons to oxygen than the electrodes, which can be judged by the value of the constants of electron transfer; 2)the resulting from the reaction hydrogen peroxide is not suitable substrate. This reaction that competitive to oxygen, is the most important on the merits. It should be considered in a case when arises the question about the use of GO in BFC. Even in the case of direct, or more precisely, non-mediated electron transport between the electrode and GO, the mechanism can not be proved with absolute precision, therefore there is always a counterversion about reality of direct transfer of electrons. In this regard, it should be assumed that the direct anode electron exchange between GO and electrode is forbidden, and it is possible to use only mediated transfer (**Fig. 15**). Such reasonings are confirmed by an example of BFC, presented in the fifth position of the Table; the anod consisted of the GO enzyme, immobilized onto graphene oxide and glassy carbon electrode, the cathode represented by laccase, immobilized on multiwalled carbon nanotubes, modified with zinc oxide. BFC had open-circuit voltage of 60 mV,

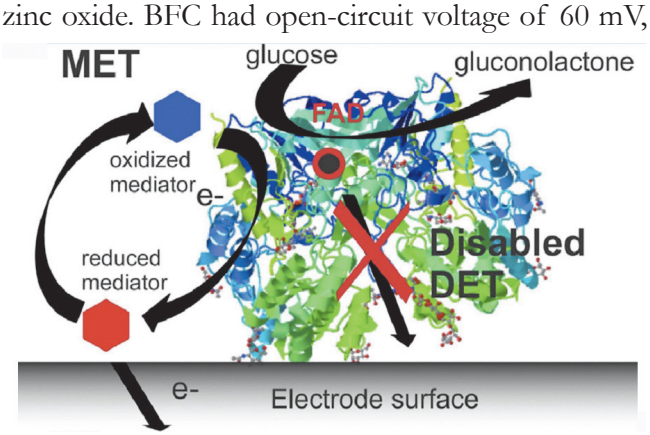

Fig. 15. *Electron transport caused by a mediator between FAD- dependent GOD and an electrode for a case when direct transfer is impossible. FAD-cofactor is designated of red and black circles* [84].

maximum specific power of 0.054 $\mu W \cdot cm^{-3}$ and voltage at the maximum power of 50 mV [83]. Such parameters in the given number of BFC are the smallest on value. However, the example of BFC, represented in the seventh position of the Table, contradicts the described scheme of the mechanism of GO functioning, since the parameters of BFC are the best of the submitted for enzyme BFC [87].

Graphene-based microbial biofuel cells

Despite the fact that the idea of electricity generation by oxidation of organic substrates with microbial biocatalyst was first formulated more than a hundred years ago [90], the decades were required in order to receive important results on microbial BFC. The task to provide effective electronic transport between an electrode surface and enzyme, localized in a microbic cell, was rather difficult. The challenge was not only to ensure the efficient transport of charge, but also to provide transport of the biocatalyst substrate; it was significantly harder for microbial cells than for an enzyme. As a result, the maximum power of BFC, based on enzymes, was higher than capacities of microbial BFC. In this regard, in the early 1980s, the main direction of research was the use of electron carriers – mediators [91]. At the same time, in two decades, there were reports about the possibility of non-mediated transport [93] and from that period, the study of microbial BFC gained new scope [94]. It was established that bacterial cells can have three main ways of electrons exchange with electrodes- via secreted mediators and with use of cytochromes and bacterial piles or nanowires (**Fig. 16**) [95-97].

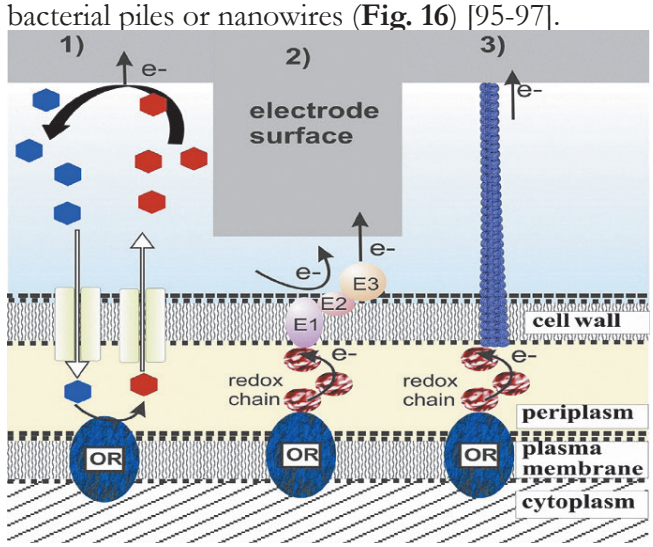

Fig. 16. *Schematic illustration of mechanism bacterial electrons exchange by means of 1) secreting mediators (at the left - an oxidized mediator, on the right - restored), 2) superficial cytochromes designated as E1-E3 and 3) bacterial pilis – nanowires or oxidoreductases* [84].

However, even in the presence of the specified assumptions, concerning mechanisms of an exchange, the further intensive studying of details was required. So, there was a question - whether is the transmission mechanism from one site of cytochrome to another or transfer is carried out on the conductivity mechanism in metals through π - π connected electrons in aromatic rings of amino acids [97]. Also it should be noted that the combination of three mechanisms of transfer for bacterial cells brings the difficulties in the unambiguous interpretation of the electron transfer process [98]. Regardless of the existing complexity of an explanation of transfer mechanisms, the discovery of *DT* effect of electrons near bacterial cells gave ample opportunities for BFC designing. These bacteria are called "ekzo-electrogen", i.e. capable independently to generate the electric power and containing nanosystems, which can be used for creation of a reagent-free microbic BFC. The BFC power on their basis reaches the power of many BFC on the basis of enzymes. However, on the base of data from the Table, it is possible to see that even use of graphene materials in microbic BFC doesn't allow to reach the best power values of BFC on the basis of enzymes. At the same time, the microbic BFC possess other positive qualities - for example, considerable higher operational stability, extraordinary wide range of substrate [99].

Now, the great attention when designing of microbic BFC, and including on the basis of ekzo-electrogen, paid to the application as a conductive nanomaterials (carbon nanotubes, carbon nanofibers and polymer, graphite particles) and conductive macrodimensional materials - carbon fabric, carbon paper, carbon felt. At the same time, despite the complex nature of the interaction with microbial cells, the frequency of Gr use in microbial BFC increases [84]. However, from the Table data can be seen that even when using the Gr in BFC, their parameters still recede, but in some cases considerably superior the BFC parameters, based on enzymes.

5. CONCLUSION

Thus, when considering various approaches, it may be noted that the current research in the field of BFC development are directed on studying of the properties of conductive nanomaterials to create electrodes, for the search of new schemes of nanomaterials use as electrodes, search for new enzymes and microbial cells, capable effectively to carry out an electron transfer using mechanism of mediated and direct bioelectrocatalysis. Graphene was among the carbon nanomaterials, both in biosensors and in BFC (biosensor technologies are precursors of BFC technologies, since the structure and function of BFC and electrochemical biosensors are very similar) [100].

The analysis of the available in the literature data on creation of BFC with new features suggests that one of tendencies is the development of small planar and volumetric BFC. For such systems will be required respectively small-sized electrodes - anode and cathode. Along with other known nanomaterials, the graphene materials have properties that enable their use in creation of BFC - so they have a high ratio "surface/volume", possess the high and controlled conductivity, high durability. The application in BFC of graphene materials extends the range of possibilities and allows us to develop a new generation of devices.

The obtained results allow to properly assess further ways of development of biofuel cells, including the creation of small BFC that can be effectively used in biorobotics, as well as in medical technology as implantable elements.

This work was partially supported by RFPF (grants 13-07-12052, 13-07-00979, 15-29-01292).

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