New Designs of Biofuel Cells and Their Work Testing

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Abstract. The developed designs and modifications of biofuel elements (BFC) are presented. The approbation of their work using strains and consortia of microorganisms is given. The proposed designs made it possible to solve a number of problems that arise when working with BFC: 1) gain access to the contents of the anode BFC space without disturbing its sterility and anaerobic environment; 2) take samples from the anode space for chemical and microbiological analysis without interrupting the BFC operation; 3) conduct continuous monitoring of electrochemical processes directly in the anode space (Ox-Red media, electrode charge, concentration of hydrogen and other ions by means of potentiometry).

1. Introduction

The technology of biofuel cells (BFC) is a promising direction of alternative energy [1-3]. It allows to combine the generation of electrical energy with the utilization of various wastes [4-8]. Thus, at once two acute today's problems are being solved. However, the development of this scientific direction is only just beginning to go beyond laboratory research. This is due to the "youth" of this scientific field. In addition, today the ratio of the productivity of the currently developed BFC with their cost parameters remains unprofitable. Therefore, it is necessary to increase the efficiency of BFC operation and reduce their cost price. Partial solution of the above tasks is the development of new modifications and designs of BFC. This was the purpose of this message.

2. Materials and methods of research

Biofuel cells. The main BFC in the work was the bioelectrochemical cell [9]. It consisted of 2 identical chambers. The dimensions of the chambers are 140 mm × 125 mm × 50 mm. The bioelectrochemical cell was made of plexiglass with a wall thickness and bottom of 3 mm (PLEXIGLAS, Rohm Evonik GmbH). The chamber covers had threaded holes for placing electrodes in them. Proton exchange membranes were used to separate the anode and cathode chambers. The Russian membrane MF-4SK was obtained from CJSC Plastpolymer (St. Petersburg, Russia). Membrane CMI-7000 (Membraness International) is manufactured in USA. They were reinforced in a rectangular window between the chambers. The size of the window was 40x90 mm. The main working environment in all cells was model sewage. Its composition (mg / l): Na₂CO₃ – 50.0, KH₂PO₄ – 25.0, CaCl₂ – 7.5, MgSO₄·7H₂O – 5.0, distilled water – 1 liter. The model wastewater immediately before the experiments was...
autoclaved. BFC was taken for control, microorganisms were not added to the anode and cathode cells. Carbon fabric was used as electrodes (OJSC "Svetlogorsk Khimvolokno", Belarus). Some experiments were carried out using silicon carbide electrodes (OJSC Podolskogneupor, Russia).

**Microorganisms-bioagents.** The bioagents in the testing of the developed BFC designs were: 1. Microbiological preparation "Vostok EM-1" ("Primorskiy EM-Center" Co.Ltd, Russia). 2. Cultures of microorganisms-biodestructors. Isolates 1-I and 8, isolated by us from the active sludge of the Angarsk petrochemical company (Russia). Strain 3-B is isolated from sewage of the ANHK. Cultures 3, 4, 5, 7 – were obtained from the microbiological preparation "Vostok EM-1". Identification of isolates was carried out at the Institute of Biochemistry and Physiology of Microorganisms GK Skryabin of RAS (Department "All-Russian Collection of Microorganisms", Head L I Evtushenko). The identification method used is based on the sequencing of the 16S rRNA gene when using the MALDI / TOF method of mass spectrometry [10]. As a result of the study, the strain 1-I was assigned to the species *Micrococcus luteus*, 3-B to *Bacillus subtilis*, 3 to *Bacillus cereus*, 8 to *Serratia marcescens*, 4-B to *Pseudomonas oryzihabitans*. Isolates 5, 7 on the basis of morphological-cultural and physiological-biochemical features are attributed to the genus *Bacillus*, strain 4 – to the genus *Micrococcus*.

**Measurement of electrical parameters of BFC.** The measurement of the generated voltage was carried out continuously in an automatic mode. The potential was recorded using a system designed on the basis of a microprocessor board "Arduino Mega 2560" (microcontroller ATmega 2560). It allowed simultaneous reading of data from 16 BFC every 5 minutes of the experiment. The current was measured using a DT32 DIGITAL multimeter. Measuring the voltage and current in the experiments, we found the power of the BFC (W):

\[ P = U \times I \]  \hspace{1cm} (1)

For the comparative analysis of the operation of the BFC studied, the power per unit area of the anode (W / m²) was determined. All the experiments were carried out in at least 5 independent experiments with 3 parallel measurements in each. Statistical processing of the experimental data was carried out using the Excel software package. The tables show the average values for the sample and their standard deviations. The findings are made with the probability of an error-free forecast \( P \geq 0.95 \).

**3. Results and discussion**

The basic bioelectrochemical cell described above (see Materials and methods of investigation) proved to be sufficiently reliable and effective in the work [11,12]. At the same time, it did not allow access to the contents of the anode chamber during the operation of the BFC without compromising sterility and maintaining anaerobic conditions. To solve this problem, a hole 25 mm in diameter was cut out at the bottom of the anode chamber. In it, a sealed plug was reinforced. As it used a plug of polypropylene rubber. As a result of this, it was possible to select samples with a syringe several times sterile, without violating the anaerobic conditions of the anode BFC space, for chemical and biological analysis. Such a stub could also make the necessary components in the operation of the BFC without disrupting its operation (figure 1).

1 – cathode chamber, 2 – anode chamber, 3 – stainless steel anode electrode clutch, 4 – cathode electrode stainless steel coupling, 5 – stainless steel cathode fitting, 6 – hermetic plug of polypropylene rubber, 7 – proton exchange membrane, 8 – stainless steel plug for anode, 9 – conductive wire for connecting measuring equipment, 10 – cathode electrode made of silicon carbide, 11 – anode electrode made of silicon carbide.
During the testing of the proposed design of BFC, the electrogenic activity of a number of strains of microorganisms-biodestructors isolated from wastewater and contaminated media was evaluated. Model wastewater with a peptone content of 0.5 g / l was used as the medium. A comparison of the efficiency of BFC with these microorganisms was carried out according to the power index, in terms of 1 m³ of anolyte (mW / m³). The experiments made it possible to identify the most promising strains among the strains studied for obtaining electricity in BFC. So, increased electrogenic activity possessed the strain *M. luteus* 1-I. The latter, when cleaning the model wastewater, generated a power of the order of 953.5 ± 113.7 mW / m³. The lower electric indexes were characterized by BFC based on strains of *S. marcescens* 8, *B. cereus* 3, *B. subtilis* 3-B, *P. oryzihabitans* 4-B – the generated power ranged from 445.9 ± 167.5 to 585.8 ± 132.5 mW / m³. The least efficient among the analyzed was the BFC based on strain 5. The power generated by it reached only 107.5 ± 16.0 mW / m³ (figure 2).

**Figure 1.** Modernized BFC with an infusion plug [13]

Thus, the strain *M. luteus* 1-I, obtained from activated sludge from treatment plants, proved to be the most promising bioagent among the isolated and selected strains of microorganisms. This strain was deposited with the All-Russian Collection of Microorganisms G.K. Scriabin RAS (registration number assigned to VKM – As-2637D, certificate of deposit No. 12310 / 02-1-4-11-49 dated June 20, 2014). Based on this strain, we proposed a bioelectrochemical fuel cell [14].

**Figure 2.** Comparison of the effectiveness of BFC on the basis of strains-biodestructors isolated from wastewater and contaminated media (medium – model wastewater, substrate – peptone 0.5 g / l, electrodes – carbon cloth). <1> – *M. luteus* 1-I; <2> – *S. marcescens* 8; <3> – *B. cereus* 3, <4> – *B. subtilis* 3-B; <5> – *P. oryzihabitans* 4-B; <6> – strain number 7; <7> – strain number 4; <8> – strain number 5.
For continuous monitoring of a large number of chemical and microbiological processes occurring in the anode BFC space, a new modification of the BFC was created (figure 3). The proposed design is characterized by the increased volume of the anode chamber (size 135 × 148 × 60 mm) and the presence of additional holes for placement of Red-Ox and pH electrodes. In addition, in the lateral part of the anode chamber there is also an opening closed by a tightly fitting rubber plug. It is necessary for sampling and depositing substrates and bioagents with a sterile syringe. The resulting structure made it possible to study the dynamics of pH, the oxidation-reduction potential of BFC, the potential of the anode and cathode electrodes. With its use it was also possible to trace the dynamics of the COD of model wastewater, changes in substrate concentrations and the increase in the number of cells of microorganisms-electrogenes (figure 3).

Approbation of the work described above by BFC (figure 3) was carried out using the microbiological preparation "Vostok EM-1" as a bioagent. The exposure time in this series of experiments was 42 hours. The main working medium in the BFC was model wastewater with the addition of 0.15 g / l of peptone as a substrate for microorganisms. The results are presented on figure 4. It can be seen that the generation of voltage and current by the microbiological preparation "Vostok EM-1" in BFC is accompanied by a significant decrease in the COD of model wastewater (from 564.2 to 302.5 mgO2 / l). The latter indicates a decrease in the substrate content under the influence of microorganisms. At the same time, the Ox-Red potential falls (from 358.5 to -426.3 mV). On the other hand, the growth of the number of microorganism cells is fixed from 4.5 ± 0.4 · 102 to 1.5 ± 0.6 · 103 cells / ml). The power generated by the BFC increased during the two-day experiment to 200 ± 25 μW (figure 4).

Figure 3. Biofuel cell, which allows to measure pH and Ox-Red anolyte potential, potential of anode and cathode electrodes. A – the general view of the working BFC, B – the BFC view from above. 1 – the hole for the anode electrode, 2 – the hole for the reference electrode, 3 – the sealed plug of polypropylene rubber, 4 – the anode chamber, 5 – the hole for the Ox-Red electrode, 6 – the hole for the pH electrode, 7 – the proton exchange membrane, 8 – hole for the cathode electrode, 9 – cathode chamber, 10 – aperture for aeration of the cathode chamber, 11 – connecting bolts for connecting the anode and cathode chambers.
Figure 4. Dynamics of various indicators of BFC with additional holes for placement of Red-Ox and pH electrodes and rubber plug in the side of the anode chamber for sampling and substrate application. A – power, B – redox potential of the contents of the anode space, C – COD content of the anode space, D – number of viable cells in the anolyte BFC (medium – model sewage, substrate – peptone 0.5 g/l, bioagent – drug "Vostok EM-1", electrodes made on the basis of silicon carbide (OJSC "Podolsknegeupor", Russia)).

The reverse dynamics of the growth of voltage, the current and the number of cells of microorganisms in relation to the curves of the decrease in the content of the substrate and the fall of Ox-Red was observed by us also with the use of other selected bioagents.

4. Conclusion

Thus, new designs and modifications of BFC have been developed and described, and their work has been tested on a number of bioagents. The proposed BFC provide solutions to a number of problems that arise when working with BFC. They allowed access to the contents of the anode BFC space without compromising its sterility and anaerobic properties. Thanks to this, it became possible to take samples from the anode space for chemical and microbiological analyzes without interrupting the operation of the BFC. In addition, the developed designs make it possible to conduct continuous monitoring of electrochemical processes directly in the anode space (Ox-Red media, electrode charge, concentration of hydrogen and other ions by means of potentiometry).

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