# Carbon superfine materials as a promising material for *Gluconobacter oxydans* based microbial fuel cells

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**Abstract**. We have investigated the properties of a several bioelectrodes based on the immobilization of *Gluconobacter oxydans* bacterial cells on carbon superfine materials (CFMs). We use three types of CFMs (as adopted by the working classification CFM 1-3). All bioelectrodes was formed by covering the surface of the CFM via suspension of bacteria in a chitosan gel. The properties of samples are evaluated by measuring the physiological state of the bacteria immobilized: (a) recording the intensity of cellular respiration, (b) for measuring the charge transport characteristics of electrode (bioelectrocatalysis), and (c) by measuring the electrode impedance. Measurements (b) and (c) are made on two and three-electrode circuits in the oxidation of ethanol in the presence of 2,6-dichlorophenol electron transport mediator. For CFMs 1 and 2 the electron transport by the oxidation of the substrate is not registered, while for CFM 3 the current generation occurs. The resistance of CFM 3 bioelectrode is below the resistance of CFMs 1 and 2 both before (39.6 kΩ/cm$^2$ for CFM 3, 630 Ω/cm$^2$ for CFM 2, and 1329 Ω/cm$^2$ for CFM 1) and after the addition of the substrate (2.9 kΩ/cm$^2$ for CFM 3, 45 kΩ/cm$^2$ for CFM 2, and 58 kΩ/cm$^2$ for CFM 1). The bioelectrode made of CFM 3 has a capacitance of 196 μF/cm$^2$—greater than two orders of magnitude of the bioelectrode capacity of CFMs 1 and 2 (0.51 and 0.58 μF/cm$^2$, respectively). It is important to further study the properties of the CFM class of materials, which are promising as the basis of mechanically flexible electrodes with controlled parameters.

## 1. Introduction

Considerable interest has been paid to carbon fibre materials (CFMs) due to the potential ability to use them as a basis for creating of electrodes for fuel and biofuel cells. This interest is determined by characteristics such as a high specific surface of obtained materials and their unique electro-physical properties [1-7]. The promising for creating electrodes are fibrous carbon materials including both nanotubes and nanofibers [8]. The simplest and the most effective way to receive carbon nanofibrous materials is the electrospinning, which allows one to create fibers from 10 nm to 20 um and more in diameter [9-11]. This method allows to obtain carbon nanofibers based on application of different precursors, mostly polyacrylonitrile (PAN) [12, 13]. For obtaining fibrous carbon material it is

necessary to provide its oxidative stabilization under temperatures at 250-300°C; after then this procedure is completed it necessary to provide process in an atmosphere of inert gas at a temperature from 700 to 1500°C [14]. The main criterion for selecting electrode materials is their electroconductivity. Electric conductivity over 800 S/cm can be achieved by varying the ending temperature of treatment and degree of orientation of fibers in material [15, 16].

The latest interest in the development of biofuel cells and biofuel cells based biosensors is growing, and considerable attention is focused on the development of microbial fuel cells (MFC) [17, 18]. MFC can find various applications from waste water treatment to energy harvesting for remote sensors power supply. The expected practical application of MFCs is connected with features of microorganisms, whose usage significantly decreases the cost of investigation and real practical application of biofuel cells [19]. It was shown that microorganisms from *Gluconobacter* genus are classified as the most often used in the composition of MFCs [20, 21]. These microorganisms are capable to provide the high-effective charge transport to anode in the presence of electronic transport mediators, high catalytic speeds of reaction, and a wide range of oxidative substrates [22]. Moreover, due to the mostly membrane-localized enzymes of cells of this genus, electronic transport in system became greatly simplified. As an addition to the catalytic qualities of biological material, the main parameter of MFC is the type of the electrode material [23]. CFMs are materials that allow regulating electric conductivity. This opportunity can be useful for bioelectrodes creating. CFMs and the range of other graphitic materials can be biologically neutral. Moreover, a CFM has a higher mechanical properties in comparison with materials such as spectral graphite (the technical name is "spectral graphitic electrode"), which is important for MFC construction. In this respect, electrodes from CFMs are prospective.

## 2. Materials and methods

### *2.1. Obtaining CFMs and its mechanical properties and elemental analysis of materials investigation*

The CFM 1 consists of microscopic fibers with 15.2 μm diameter and was provided by LLC “Niagara”, Moscow. Fibrous materials with high specific surface were obtained by the electrospinning from PAN solutions with $M_w$ = 370 kDa molecular mass. For structural transformation of PAN fibrous materials to CFM 2 and 3, we used a modified SNOL-3/11 furnace. The morphology of samples was studied using a scanning electron microscop (SEM) (Helios 600i, FEI, United States). Tensile properties of samples of nonwoven fibrous materials was determined using the Instron-5965 Universal Testing System at 15 mm/min rate of deformation; and operational length and wide of a sample of 20 and 5 mm, respectively.

### *2.2. Obtaining microorganisms, bioreceptors and recording their activity via oxygen electrode*

For the formation of a microbial biosensor, *Gluconobacter oxydans* VKM-1280 strain was used (All Russian Collection of Microorganisms from Skryabin IBPM, RAS). The suspension of microorganisms for measurement was obtained by the method described at [24]. Immobilization of cells was performed by physical sorption on filters from GF/A chromatography fiber paper (Whatman, United Kingdom) and CFM 1, 2, and 3 samples. The biochemical activity of microorganisms via oxygen electrode was measured by the method described in [25]. The oxidative substrate was ethanol with 0.125 mM concentration. Amplitude of the signal was changed as the difference of current intensity before and after the dosing of the substrate.

### *2.3. Obtaining bioreceptors and changing the electrical chemical activity of cells*

A fragment of CFM 1 was saturated by a mixture of suspension of cells (1 mg of wet weight/μL) with a 2% solution of low-molecular chitosan (Sigma-Aldrich, Co, United States) diluted in 1% acetic acid in a 2:1 ratio [26]. The electrode was a porous rectangular layer with a size of10 x10 $mm^2$ and thickness of 3 mm. The total amount of applied solution was 10.6 μL; the solution was dried at room temperature for 30-45 min. A total of 17.6 μl/$cm^2$ of the same mixture of suspension of cells with

chitosan solution was covered on each arm of CFM 2 and 3 (6 x 6 $mm^2$ at 0.5 mm of thickness) and was dried at room temperature. Changes were performed in 25 mM of potassium- phosphate buffer solution (pH 6.5) with 10 mM of sodium chloride. Volume of measurement cell was 5 mL. Concentration of substrate (ethanol) was 5 mM in working solution. We used 2.6- dichlorophenolin-dophenol (2.6-DCPIP) in concentration of 42 μM as reduction-mediator. Cycle volt-amper characteristic (CVA) was recorded via three-electrode and two-electrode circuits with speeds of scanning of potential of 3 mV/s. During the measurement in the three-electrode circuit mode, the comparison electrode was saturated silver chloride electrode, an additional electrode was a platinum layer with an area of 1.8 $cm^2$. Chronopotentiometric investigations were performed by measuring the stationary potential of working electrode from time, and by countering to the comparison electrode. Chronopotentiometric investigations were performed during a permanent potential that was chosen from voltammetric characteristics and was 200 mV. During measurement in two-electrode circuit mode, the comparison electrode was an electrode from spectral graphite with an area of 97 $mm^2$; in the anode field we used a 2,6-DCPIP (42 μM) mediator and in the cathode field we used potassium hexacyanoferrate (III) (HCF, 4 mM). The measurement of voltammetric characteristics was performed by VersaSTAT 4 galvanopotentiostat (Ametek Inc., United States). Spectral impedance characteristics were measured by a two-electrode circuit in the range of from 40 kHz to 0.01 Hz with amplitude of 10 mV.

## 3. Results and discussion

### *3.1. Investigation of mechanical features and element analysis of materials based on PAN*

Investigation of the electrospinning process PAN in dependence to potential, polymer concentration, and volume flow rate was conducted in the work [13] allows one to detect the optimal parameters of solutions and obtain experimental samples of fibrous materials on the basis of PAN from fibers with a diameter from 300 to 860 nm. To obtain carbonized fibrous material, stabilization was carried out with air oxygen at temperatures between 200-275°C. The rate of heating was varied from 0.1 to 5°C/min. The final temperature of oxidative stabilization (275°C) was chose in the basis of data [27, 28]; therefore, at temperatures higher than 275°C, loss of mass significantly increased because of the oxidative destruction of fiber. Figure 1a. shows the SEM images of CFM 1. The micrographs of original and thermal-oxidative fibrous material from PAN are shown in Figures 1b and 1c. The fiber diameter of CFM 2 and 3 decrease in the size due to the chemical processes of condensation and destruction. The stabilized fiber materials were carbonized at a rate from 0.9 to 21°C/min up to 700-1000°C in an inert environment ($N_2$). Figure 1d show the carbonize fibrous material at 1000°C.

**Table 1.** Mechanical properties of CFM samples
Symbols: (a) average result (n = 3); (s) standard deviation

| Strength, $a \pm$ s, MPa | Tensile strain, $a \pm s$, % |
|---|---|
| 1.7 ±0.4 | 50 ± 4 |
| 11.0 ±0.1 | 2 ± 0.4 |
| 10.3 ±0.7 | 7 ± 2 |

The average diameter of carbonized fiber at 700 (CFM 2) and 1000°C (CFM 3) was 490 and 430 nm, respectively. To obtain dense fibrous material, we pressed the CFM 2 sample after the end of thermal treatment. The mechanical properties of carbonized samples is given in Table 1. The results of a quantitative element analysis of organic compounds are in Table 2. Based on the presented data, it can be expected that, among all samples, sample 3 has the lowest electricity resistance due to the lower oxygen content and lack of nitrite in the sample.

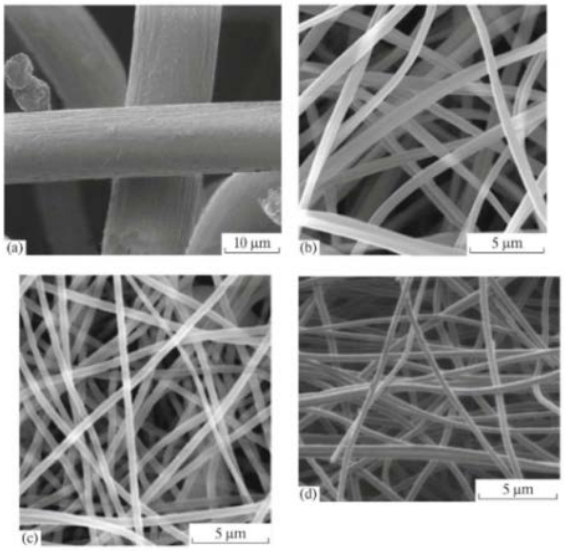


**Figure. 1.** SEM images of fibrous materials. (a) CFM 1 ($d_{aver}$ = 15.2 μm), (b) fibrous material based on PAN obtained in electrical field ($d_{aver}$ = 700 nm), (c) oxydative fibrous material based on PAN ($d_{aver}$ = 530 nm), and (d) CFM 3; carbonized at 1000°C ($d_{aver}$ = 430 nm).

*3.2. Recording of electrochemical activity of bacteria: voltammetry and chronopotentiometry*

To check the ability of using polymeric materials as electrodes of biofuel elements, we studied the influence of these materials on the respiratory activity of bacterial cells via recording the change in oxygen level on the measurement layer of a Clark electrode containing a CFM and immobilize bacteria. For this reason, we measured the amplitude of the signal of the oxygen electrode and the first derivative of the front edge for different polymeric materials. We used GF/A material (glass-fiber filter) as the control variant. It was shown that the materials do not demonstrate an injurious effect on bacterial cells via recording the respiratory activity of cells by a Clark electrode (a comparison was performed with microbial cells immobilized on GF/A neutral material). We found that bacterial cells can be kept on a CFM surface both by sorption and immobilized agents like chitosan. Cells immobilized by sorption on CFM 3 showed the most activity by amplitude and derivative of signal of oxygen electrode (amplitude was 46.7 nA; derivative was 3.39 nA/s). To study the electrochemical activity of carbon polymeric materials, we investigated transfer of charge during bioelectrocatalysis - the transfer of electrons during potential applied on measurement electrode in the oxidation of substrate by bacteria immobilized on the electrode surface. When adding ethanol to the measuring cell in the absence of a mediator on an electrode from CFM 3, there are no useful signals. This proves that there is lack of direct transfer of electrons from bacteria to the electrode when applying the 200 mV potential to an electrode. In Figure 2 there are cycle voltammetric characteristics (CVA) of an electrode from CMS 3 received by a three-electrode circuit of measurement. A step up of the electrode current is observed during the addition of the substrate (ethanol) to the system. In Figure 3 a change in CVA measured by two-electrode circuit for an electrode of CFM 3 is shown. The immobilization of biomaterial on the electrode leads on the current step up (curve 2) to the control condition of the electrode (curve 1) (measurements were performed in the range of potentials from -500 to +500 mV). Adding a mediator and substrate (ethanol, curve 3) to the measurement solution leads to a significant current step up in the range from -300 to +500 mV. During the potential of 500 mV, the value of the current is 60 times more than the level of electrode in the control condition and 6 times more than the level of current for electrodes with biomaterial. For electrodes based on CFM 1 and 2, weak anode and cathode currents are observed in the range of potentials from -500 to +500 mV both without biomaterial and after adding a biomaterial and substrate. Thus, only CFM 3 provides high electroconductivity and electric contact with bacterial cells, which leads to the transfer of an electron from an oxidate substrate to the surface of an electrode and the generation of high-amplitude signals during the addition of a substrate to the system.

**Table 2.** Data of quantitative element analysis of organic compounds of CFM samples

| Sample | Atomic ratio of chemical elements, % | | |
|---|---|---|---|
| | C | N | O |
| 1 | 76.53 | 15.02 | 8.45 |
| 2 | 77.26 | 17.19 | 5.55 |
| 3 | 97.36 | - | 2.64 |

*3.3. Impedance measurements*

To investigate the effectiveness of application of CFMs as electrodes for MFC, we used electrochemical impedance spectroscopy. Full output capability of MFC is limited by their internal resistance; this makes an analysis of different components of internal resistance and their contribution to total value of internal resistance is necessary, as well as a search for methods to decrease the internal resistance of MFC and improve its production. In this case we investigated the contribution of material of MFC bioanode to total internal resistance of MFC and its capacity. Depending on the electrochemical properties of the electrode material, its capacity can be changed several times, and it directly effects the capacity of the biofuel cell. Due to, it was necessary to find the material that will ensure minimal internal resistance of an element in association with cells. In this work we used the two-electrode circuit for measurement, in which the working electrode was the electrode from the studied material; the electrode from spectral graphite was used as the opposing electrode. For the treatment of the impedance frequency characteristics, we used a circuit that is used for studying the impedance of biofuel elements [29]. In this circuit each electrode ("anode" from studied material and "cathode" from spectral graphite) has its own pair from capacity and resistance of charge transfer; the resistance of anolite and catholite are cooperated into a common $R_e$ element (resistance of electrolyte). Because the chemical content of solutions in cells is not changed in different experiments, resistance of electrolyte was the same and did not change, while material of electrodes was changed. In all measurements of this batch, we used the same cathode; this is why values of capacity and resistance of this electrode are not used for measurements.

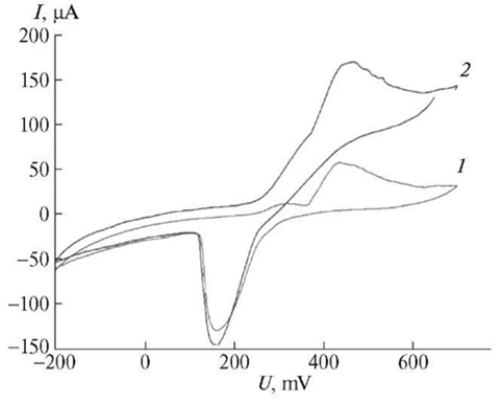


**Figure 2.** CVA characteristics for CFM 3 containing bacterial cells by three-electrode circuit. (1) Electrode with applied biomaterial with mediator presence; (2) electrode with covered biomaterial with mediator and the presence of ethanol (5 mM).

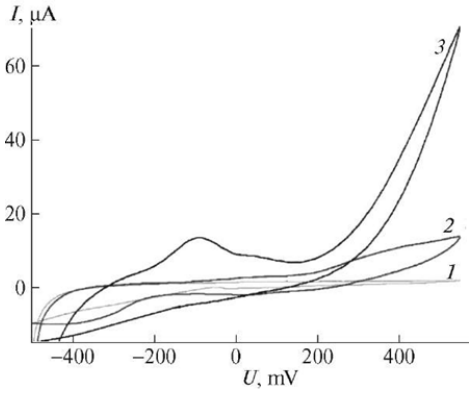


**Figure 3.** CVA characteristics of electrode by two-electrode circuit for CFM 3. (1) Unmodified electrode in buffer solution, (2) electrode covered with biomaterial in buffer solution, and (3) electrode covered with biomaterial in the presence of ethanol (5 mM) and mediator.

To investigate the electrochemical properties of materials, we chose parameters such as the value of resistance of charge transfer through the anode ($R_a$) and its electrical capacity ($C_a$). All measurements were performed in the range of frequencies from 40 kHz to 0.01 Hz with amplitude in 10 mV at zero constant potential. Impedance characteristics of electrodes were checked both before the immobilization of cells on it and after the immobilization of the *G. oxydans* cells - chitosan mixture. The data are presented in Table 3. As is seen from the data, CFMs 1 and 2 have shown high values of resistance of charge transfer. During the immobilization of cells on their surfaces, the resistance of these materials decreased several times, but was still too high for their usage as working electrodes of biosensors or anodes of biofuel elements. Before the immobilization of cells, the electrode from CFM 3 has a resistance of ~15 lower than the electrode from CFM 2 and ~35 lower than the electrode from CFM 1. During the immobilization of cells on the surface of this electrode, resistance decreased about five times and its value was several times lower than values of resistance of electrodes from CFM 1 and 2 with covered cells. During the addition of a mediator of electric transport and substrate of *G. oxydans* cells to measurement cells, the electrical resistance of the anode decreased from 39.6 to 2.9 kΩ/cm$^2$. Thus, electrodes from CFM 3 can be used as working electrodes of biosensors or anodes of biofuel cells. In an anode compartment we added DCPIP as a mediator; in a cathode one we added HCF. The maximal power output of this MFC was 9.48 μW/cm$^2$, and the total internal resistance of MFC was 1.75 kΩ/cm$^2$.

**Table 3.** Impedance properties of anodes

| Electrode | Resistance $R_a$, kD/cm$^2$ | Electrical capacity $C_a$, F/cm$^2$ |
|---|---|---|
| CFM 1 | 1329.0 | 0.072 |
| CFM 1 + cells + mediator + substrate | 44.7 | 0.51 |
| CFM 2 | 630.0 | 0.084 |
| CFM 2 + cells + mediator + substrates | 57.9 | 0.58 |
| CFM 3 | 39.6 | 2.3 |
| CFM 3 with immobilized cells | 7.3 | 137.4 |
| CFM 3 + cells + mediator + substrate | 2.9 | 195.9 |

## 4. Conclusions

It can be noted that CFMs 1 and 2 do not have the necessary electrochemical characteristics to be used as working electrodes in biofuel cells and biosensors because they have low electrical conductivity. CFM 3 can be used for the creation of anodes for biofuel cells because it shows characteristics that are correlated with characteristics of spectral graphite, which is widely used as a material of electrodes. Preference for usage of CFM 3 in comparison with spectral graphite is due to its mechanical features, particularly in elasticity with high tensile strength, as well as in the presence of high specific surface determined by its fibrous structure. Moreover, the carbonization of CFM 3 allows to decrease the resistance of material in 35 times in comparison with original sample (CFM 1) and for electrode with immobilized cells in 15 times in comparison with an electrode based on CFM 1.

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