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Investigation of the influence of microalgae on the sensory properties of carbon materials

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Blue-green microalgae/cyanobacterium Arthrospira platensis is a renewable resource, a source of food and feed protein and other valuable compounds (carbohydrates, lipids, vitamins), and can also serve as a material for studying human health monitoring technologies. A suspension was made from microalgae biomass and applied to a solid (SiO_2/Si dielectric plate) or flexible substrate (polyethylene terephthalate, PET) using 2D printing technologies. It was found that cyanobacterial films formed at atmospheric pressure react to exhaled air by significantly increasing their conductivity. A layer based on multigraphene and carbon fibers with a cyanobacterial film was formed and tested as a sensor structure. The texture and conductivity of the resulting structures were studied. High sensitivity of their electrical conductivity to human respiration, as well as to mechanical effects such as pressure on the sensor, was shown. It was found that the obtained conductive layer on the carbon fiber surface retains its conductivity under tensile deformations created by bending up to a radius of ~ 2 mm. The mechanism of interaction of synthetic carbon materials with a natural component was discussed.

Keywords: microalgae biomass, graphene, carbon fibers, sensor, breath analysis.

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Introduction

In current ecosystems the cyanobacteria (blue-green algae) prevail among various microorganisms [1]. Adaptability of cyanobacteria to the environmental conditions was due to some physiological features: ability to perform oxygen photosynthesis, to fix molecular nitrogen, to form the primary production of the organic substance [2–6]. The source of carbon for most of these organisms is carbon dioxide. The reduced compounds of sulphur, hydrogen, certain sugars, organic acids in cyanobacteria serve as exogenous donors of electrons. Due to ease of cultivation and high speed of growth the cyanobacteria are widely used to solve fundamental and practical scientific tasks. Biomass of some cyanobacteria types is grown to produce vitamin or fodder additives. They are used to treat waste water [7,8]; having cytotoxic activity, they make it possible to improve the treatment efficacy [9]; they demonstrated marked antibacterial properties [10]. Various types of biofuel may be produced from microalgae biomass [11,12]. Recently the technologies of pyrolysis and hydrothermal liquefaction are being actively promoted to produce a liquid energy carrier [12]. Cyanobacteria may be used for synthesis of nanoparticles. Thread cyanobacterium *Plectonema boryanum* UTEX 485 was used for biosynthesis of gold, silver and palladium nanoparticles [13–19].

Combination of microalgae with different materials causes development of cheap composites with various functional properties. Metal nanoparticles in a combination with cyanobacterial photosynthetic molecular complexes have high efficacy in the generation of energy and are seen as the basis to develop the energy-converting devices and sensors. Paper [20] shows that the hybrid photosystem that includes a photosynthetic reaction center connected to gold and silver nanocrystals, has higher speed of generation of excited electrons inside the reaction center as a result of plasmon resonance and fast separation of electron-hole pairs.

Use of cyanobacteria as a non-traditional material with the controlled properties is due to the following advantages:

- growth and development of microalgae require sunlight, CO_2, water with a small quantity of mineral salts;
- crop land is not required to grow algae — plantations may be arranged on the surface of water reservoirs or lands that are not suitable for agriculture;
- algae require much less water compared to cereals — they may be grown in salty water and in waste water,

weakening the anthropogenic pressure at the pure water resources;

• microalgae cells are small in size, therefore require no additional crushing, as other types of carbon materials.

Recently monitoring of breathing using portable devices is considered to be promising for many clinical applications, including monitoring of sleep, testing of some diseases, study of the breathing nature and determination of the breathing frequency [21–28]. The breathing nature may be affected by various factors, including the breathing route (for example, exhaling through the mouth or nose), exhaling speed, pressure in airways, holding breath, body frame position etc. Breathing analysis is also a universal diagnostic method of clinical use. Volatile organic compounds released from various parts of the body as a result of various metabolic processes change the composition of the exhaled air [27]. The most informative analysis may be provided by a combination of various types of sensors in a single device with account of the operation feature of every sensor type [28].

It is known that the exhaled air consists of nitrogen, oxygen, carbon dioxide, water, inert gases and more than 870 other gaseous compounds, usually in concentrations from parts per trillion (ppt) to parts per million (ppm) [29]. From the above compounds, the increase in concentration of the hydrogen peroxide is dangerous, because it is related to the functioning of the heart mitochondria respiratory chain. Hydrogen may be detected in the exhaled air, which is absorbed well in blood and is released through lungs and may therefore reflect on the one hand the composition and the metabolic activity of intestinal microbiota, and on the other hand — indicate the violation of the natural decomposition and absorption of some nutrients. Carbon dioxide is not well-absorbed from the intestine into blood and may be the cause for meteorism, pain and discomfort.

It is evident that to reduce the number of factors that impact the analysis result, sensors of simple design with high sensitivity and stability are required for the operation period, which provide for the reproducibility of the breathing analysis. Therefore, there is a huge demand for development of simple and reliable sensors.

Multiple sensors based on graphene were reported, which were used to monitor human health, including portable sensors, and implanted devices, which may measure in real time the body temperature, the cardiac rate, pulse oxygenation, breathing frequency, blood pressure, blood glucose level, electrocardiogram signal, electromyogram signal, electroencephalograph signal etc. [30]. As a 2D material, graphene has many advantages, such as a large coupled structure, large specific surface area, high conductance, synthesis simplicity, sensitivity to gas molecules. There are many factors that impact the sensors based on graphene, including the synthesis method, chemical structure, interlayer structure, test environment and properties of the substrate surface [31]. Due to Van der Waals forces and large specific surface, the 2D graphene nano-composites are prone to agglomeration of nanoparticles. To avoid this and improve the selectivity of the developed materials, usually engineering of defects and management of the layer structure are necessary for the targeted modification of their properties.

Another promising state-of-the-art material is a carbon fiber. According to [32], sensors from carbon fiber have the potential to develop reliable and highly sensitive sensors. In carbon fibers the atom layers are basic graphene structures (planes), curvature and defect rate of which depends on the conditions of fiber production. The main structural unit of most carbon fibers is a crystallite of turbostratic structure, which consists of a package of layers of basic graphene planes shifted relative to the ideal position of the graphite crystal. Crystallites in their turn form such elements of the structure as microfibrils and fibrils of carbon fiber. The pores in the structure are long, thin, with preferable orientation along the fiber axis. Carbon fibers demonstrate extremely high values of the modulus of elasticity and strength, chemical and thermal resistance, low coefficient of linear thermal expansion, specific tribological properties, higher (compared to other fibers) heat and electroconductivity etc. [33].

The purpose of this paper was development of a simple and cheap design of the sensor to monitor the breathing structure based on natural and artificial components. For this purpose we used various carbon materials (layers and nanoflakes of multigraphene, carbon fiber) and cyanobacteria *Arhtrospira platensis*. The suspensions developed on the basis of cyanobacteria made it possible to form an active layer on the surface of multigraphene and carbon fiber with high sensitivity to the quantity of exhaled air, breathing frequency and some media that may be present in the human breath. Having the sorption properties, the biomass may accumulate these compounds. Besides, it was found that drops of cyanobacteria applied on a flexible material from carbon fiber are sensitive to mechanical action (pressing).

1. Materials and research methods

The experiments used nanoparticles of multigraphene synthesized by us in a plasma-chemical reactor based on a DC plasma generator [34], and graphene layers grown by method of chemical vapor deposition (CVD) [35]. Multigraphene is produced by variation of plasma generator parameters, pressure in the reactor, carbon precursor type, working gas type and their ratio in a plasma flow. The highest yield of graphene scales was observed when helium was used as a plasma-supporting gas. The advantage of this gas is also determined by its thermophysical properties: helium is rather common, it is easy to heat to temperature of 16 000 K, it must not be separated from the synthesis products. The disadvantage of helium is its relatively high cost. The source of carbon was a domestic gas mix of propane with butane at the ratio of 65:35 vol.%. In process of this mixture pyrolysis in the helium plasma

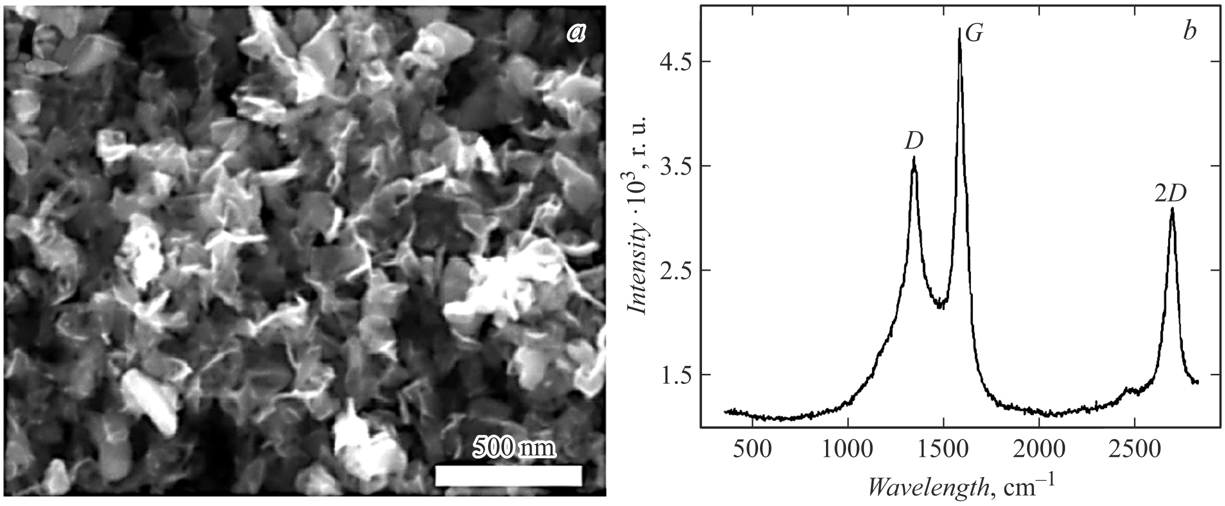

Figure 1. SEM image (*a*) and RS spectrum (*b*) of multi-graphene nanoparticles synthesized during conversion of mixture $C_3H_8-C_4H_{10}$/He with the ratio of 0.05/0.75 g/s accordingly. Current — 400 A, pressure — 500 Torr.

Elemental composition (dry ash free; oxygen content was determined by subtraction), humidity, biochemical composition (mass%) cyanobacteria/microalgae *A. platensis*

Elemental composition, mass%					Moisture, mass%	Biochemical composition at concentration CO_2 1.0 Vol.%, mass%		
C	H	N	S	O		Proteins	Lipids	Carbohydrates
61.3	6.4	8.8	1.0	22.5	3.0	70.0 ± 0.6	5.7 ± 0.6	17.1 ± 0.7

at pressure of 350–710 Torr graphene is synthesized in the volume of the plasma jet with the morphology in the form of separate scales. Morphology, number of layers, chemical impurities and topology of defects in nanostructures were found by methods of physical and chemical analysis [36]. The particle size was 100–200 nm, thickness — 1.5–2 nm. Oxygen content is within 4–7 wt.%, other impurities were not found. The advantage of the used synthesis method to be highlighted is the absence of the substrate and catalyst particles. If you compare the properties of graphene grown on the substrate using the method of plasma-enhanced CVD (PECVD), the graphene properties depend on the quality of the substrate and to a lesser degree on the plasma parameters [37]; but in both cases it is rather difficult to control the number of layers.

Fig. 1 presents the image of multigraphene nanoparticles synthesized in plasma, which was obtained on scanning electron microscope MIRA 3 TESCAN with field emission Schottky cathode in the high vacuum mode. Raman scattering (RS) spectra were recorded using spectrometer Ntegra Spectra with a DPSS-laser (green) with the wavelength of exciting radiation 532 nm. Spectra were read in the range from 300 to 3000 cm^{-1} in several points of specimen surface (fig. 1, *b*). The presence of narrow and rather intensive peak 2*D* indicates good quality of particles (low density of structural defects) and their small thickness (less than 2 nm). Presence of a considerable peak of *D*-mode in the RS spectrum is due to the total defect rate of a combination of minor nanoparticles (lateral size up to 200 nm) and first of all their edge states.

Carbon fibers joined in the form of a sheet are a current-conducting, porous, air permeable carbon-carbohydrate composite produced by wet method from hydrate cellulose threads under subsequent thermal treatments (polymerization, carbonization and graphitization) using the available technologies [38,39]. As a rule, sensors use carbon fibers in the form of lengthy threads [40]. The process of manufacturing of such sensors includes three main stages: preliminary hardening of carbon fiber, preparation of an electrical connection and incorporation of a sensor fiber into a sensor holder. When such method of carbon fiber application is used, problems arise with the reproducibility of results [31]. Use of a combination of bound, but not pressed carbon fibers in the experiments provides for their free movement and sensitivity to the loads and mechanical actions. Assuming the load is a biomass of cyanobacteria, we proceeded from its elemental composition presented in the table. These elements are usually used for functionalization of graphene and carbon nanotubes [41,42].

Clone culture *Arthrospira platensis rsemsu P (Bios)* with straight trichomes formed as a result of the natural

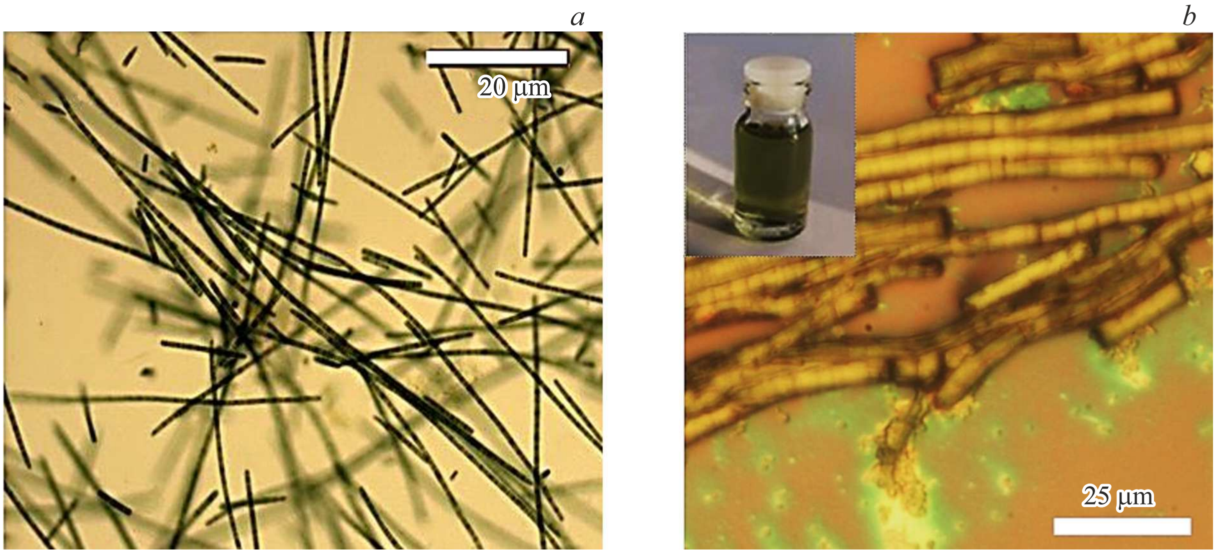

Figure 2. Photograph of cyanobacteria *A. platensis* under an optical microscope (*a*), microalgae, crushed by a disperser (*b*), insert: suspension produced from two types of elements (cellular membranes and organelles).

morphological variability, was cultivated in two stages with physiological stress in the second stage in laboratory conditions of the geographic faculty, Lomonosov Moscow State University (fig. 2, *a*).

Cultivation conditions:

• method of cultivation — semi-continuous, when a certain volume of cultural fluid is removed from the cultivator periodically to collect the biomass and is being replenished with the fresh nutrient medium;

• illumination and temperature — constant, $25 \pm 3\,\mu$E/(m^2·s) at $T = 21\,^\circ$C;

• type and volume of cultivators — planar cultivators of open type with volume of 1000 l and surface mixing device, which provides for even illumination of the microalgae cells;

• nutrient medium — classic Zarrouk medium [12].

The biomass collection process used the ability of this culture to float on the surface of the cultural fluid at rest. After collection of the floating biomass it was separated from the cultural fluid by filtration in stainless metal sieves with cell size of $150-200\,\mu$m. The concentrated biomass was dried in open air under lamps DRLF-400 and at temperature of not more than $40\,^\circ$C to the air-dry state (moisture of around 3 %).

The dried biomass of cyanobacteria was crushed with a disperser (10000 rpm, 10 min) and left to settle for separation into fractions of cellular membranes (larger fragments) and organelles (finer elements: chloroplasts, kernels and plastids) (fig. 2, *b*). Sometimes cellular membranes would settle and be removed, as a result the suspension of the organelle clusters was produced. Aqueous-alcoholic solution was added to biomass to make a suspension, where water and ethanol were taken at the ratio of 30:70.

To study the morphology and local features of sensors with high spatial resolution, atomic-force microscope (AFM), and also scanning microscope Solver PRONT-MDT were used. Measurements were performed in contact and semi-contact modes. The morphology of the surface of developed films were also studied on optical microscope Altami. Resistance of the active layer of sensors was studied using a four-probe head JANDEL and measuring unit HM21 at room temperature (Jandel Engineering Limited, Limslade, UK). Current–voltage curves were measured with a Keithley 6485 picoammeter for samples fitted with two contacts made of conducting silver paste.

The produced suspension of cyanobacteria was applied on the base by two methods: by application of drops or with the help of printing with a jet printer. Films were formed on various types of hard and flexible substrates: dielectric substrate SiO_2/Si, on the surface of CVD-graphene, on the surface of carbon fiber and on the surface of flexible polymer substrates made of polyethylene terephthalate (PET). Some structures were printed using jet printer Dimatix FUJIFILM DMP-2831. Ink was used for printing, which includes 10 % ethylene glycol for viscosity. Printing made it possible to apply even a thin layer of nanoparticles. The number of printed layers varied from 1 to 3. The size of drops applied with the printer was $\sim 50\,\mu$m, and the size of printed structures — fractions of millimeter. To reduce the spread, polymer Nafion (50 % in water-alcohol solution) was added in the amount of 10 wt% of the total ink mass into the cyanobacteria suspension.

2. Carbon material testing results

2.1. Study of cyanobacteria film properties

Fig. 3 shows AFM images for layers obtained either by single (fig. 3, *a*) or triple (fig. 3, *b*) application of the

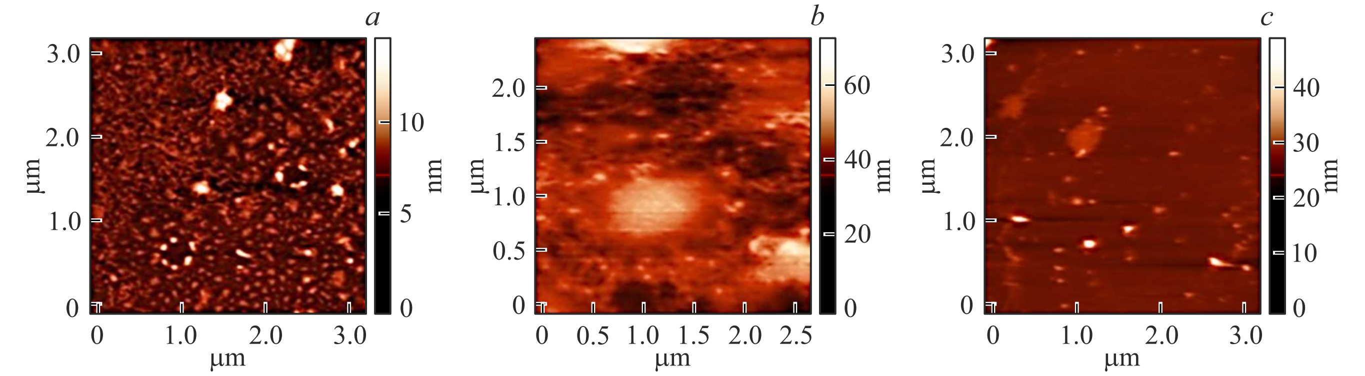

Figure 3. Films from single-component suspension from cyanobacteria: *a* — single application of suspension, *b* — triple application of suspension, *c* — printed film of four layers.

suspension, or by printing on the surface of the substrate SiO_2/Si. You can see that the solid phase of the suspension is formed by clusters. The cluster size is around 500 μm at thickness of 5−6 nm. The size of organelles in the cluster is around 30 nm at their thickness of 1.0−1.5 nm.

The developed film from cyanobacteria was studied for the component composition by method of synchronous thermal analysis (thermal analyzer STA 409PC Luxx, NETZSCH) with mass spectroscopy (quadrupole mass-spectrometer QMS 403 C Aeolos, NETZSCH). The analysis was done in the atmosphere of argon in dynamic mode when heated to 400 °C. From fig. 4 you can see that in the film the concentration N varies slightly. As temperature increases, H_2 and CO_2 are released. Therefore, the active centers in the biomass are formed with the participation of oxygen-containing groups, nitrogen and hydrogen, the presence of which is due to the physiological features of cyanobacteria.

2.2. Study of cyanobacteria impact at graphene response

A polymer PET substrate was used to study the sensitivity of the film from cyanobacteria to the nature of breathing.

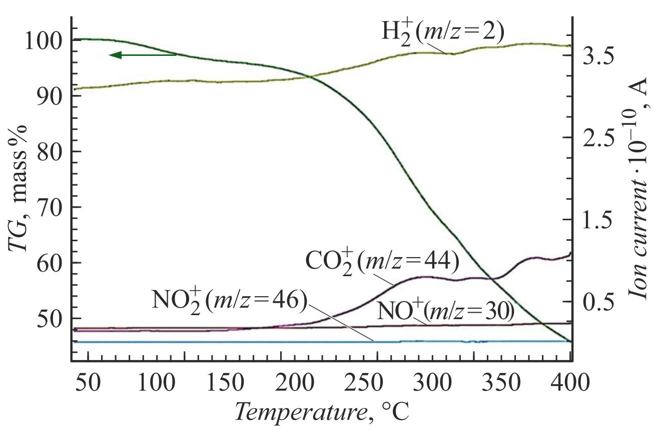

Figure 4. Thermal analysis of film from cyanobacteria.

PET provides a response by current to breathing at the level of 10^{-11} A. Use of multiple frequent exhalations made it possible to visualize the response better, having brought it to the current values of 10^{-10} A (fig. 5, *a*). The film from cyanobacteria applied on the PET substrate responds to breathing with amplitude of peaks $\sim 7 \cdot 10^{-8}$ A (fig. 5, *a*, lower part). Fig. 5, *b* shows the result of the breathing analysis for the structure from CVD-multigraphene on PET with the applied one or two layers of cyanobacteria ($G + 1L$ and $G + 2L$, on PET). Fig. 5, *b* differs from fig. 5, *a* by increase of the current to the value of $\sim 2 \cdot 10^{-7}$ A for one layer ($G + 1L$, on PET) and $\sim 8 \cdot 10^{-7}$ A for two layers ($G + 2L$, on PET). Besides, these drawings also contain longer responses to a series of 10 quick inhalations (similarly to fig. 5, *a*), which caused a long response. You can see that the combination of the sorption capacity of graphene, biomass and PET substrate provides a response by current with amplitude of 4−5 orders. Application of the second layer from cyanobacteria on graphene ($G + 2L$) increased the current and the sensitivity of the structure three times. The main components of the exhaled air flows are oxygen, CO_2, water vapors and practically unchanged nitrogen. During exhalation, air from the respiratory organs, the airways, is added to the alveolar air. The main participants of gas exchange in lungs and tissues were water, oxygen and carbon dioxide. We suggest that the main contribution is from water vapors that increase the conductance of the active layer in the structure. It seems that the interaction of multigraphene with microalgae promotes the transfer of electrons between the reagents and multigraphene. As a result of increased content of reagents, the current and duration of response improve.

When a film of CVD-graphene was applied on the substrate SiO_2/Si it was found that the response to breathing (conductance) is practically absent, but the conductance itself is relatively high (fig. 6, *a*). From fig. 6, *b* you can see that after application of a layer of cyanobacteria on the graphene film, noticeable sensitivity of the produced structure appears in respect to the exhalation, but its conductance decreases. Probably, this effect and the

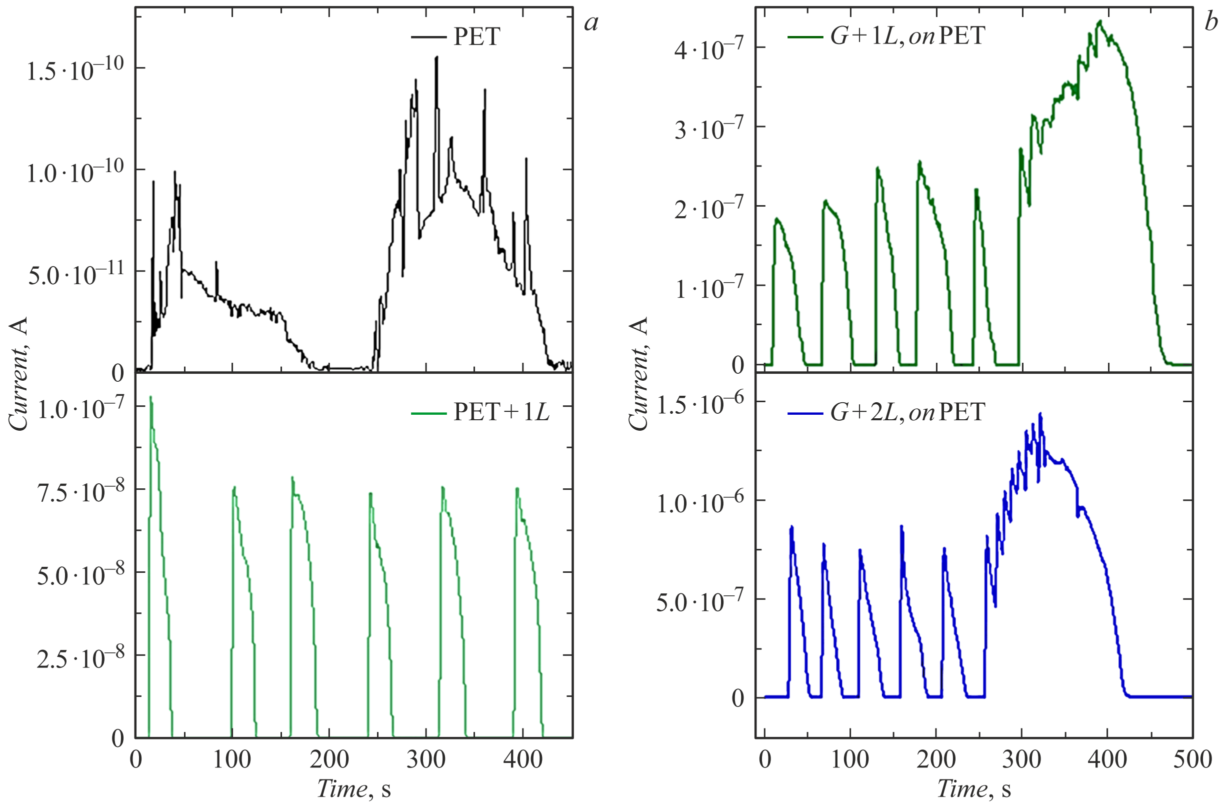

Figure 5. Responses to breathing (*a*) for PET (upper part of the drawing, PET) and layer of cyanobacteria on PET (lower part of the drawing, PET+1*L*) (*b*) for the layer of cyanobacteria on the structure of CVD-graphene/PET (upper part of the drawing, *G* + 1*L*, on PET) and two layers of cyanobacteria on the structure of CVD-graphene/PET (lower part of the drawing, *G* + 2*L*, on PET). Voltage applied to the structure when recording the characteristics was 1 V.

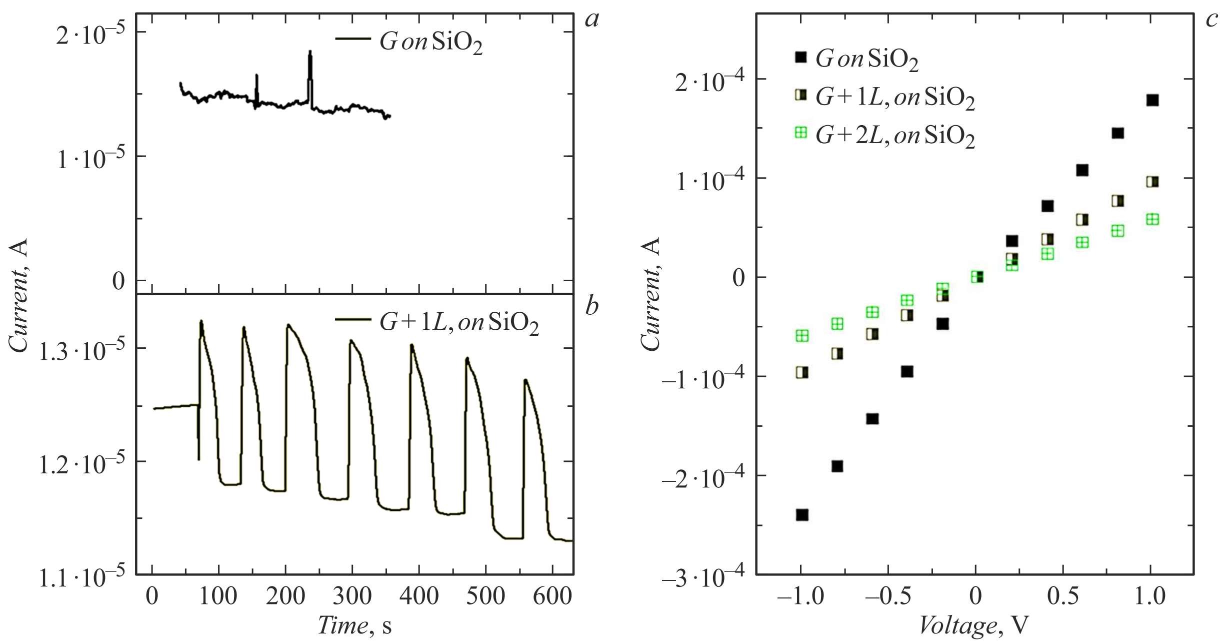

Figure 6. Responses to breathing (*a*) for CVD-graphene, applied on SiO_2/Si, and response of layer (1*L*) of cyanobacteria, applied on CVD-graphene/SiO_2/Si (*b*). Voltage applied to the structure when recording the characteristics was 0.1 V. Current-voltage curves for a film of CVD-graphene on the surface SiO_2/Si with different number of applied suspension layers: without cyanobacteria (*G* on SiO_2), with one layer of cyanobacteria (*G* + 1*L*, on SiO_2) and two layers of cyanobacteria (*G* + 2*L*, on SiO_2) (*c*).

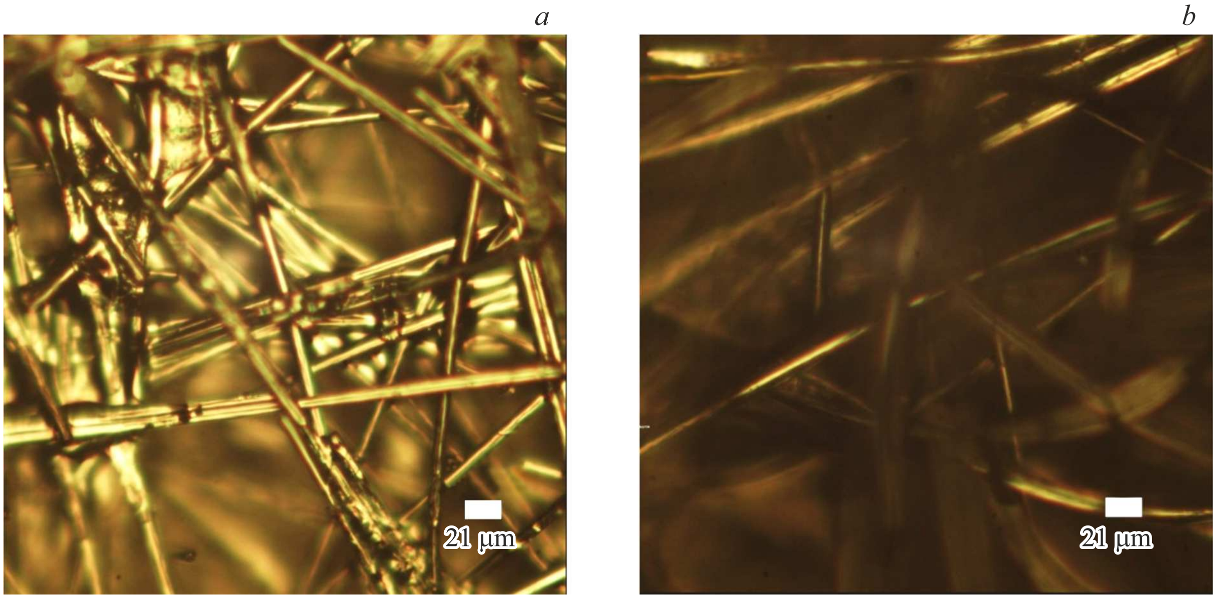

Figure 7. Optical image of carbon fibers without (*a*) and with addition of suspension from cyanobacteria (*b*).

observed jump in fig. 5 are caused by the interaction of breathing products with biomass components (water, hydrogen, nitrogen).

2.3. Study of cyanobacteria impact at carbon fiber response

Optical images of carbon fibers before and after application of the active layer from the dispersion based on cyanobacteria (algae) is presented in fig. 7. Addition of cyanobacteria to volume material complicated generation of contrast images in connection with formation of an additional layer on the surface of individual threads. Conductance of the manufactured carbon paper with thickness of $500\,\mu$m made around 3–5 mA at voltage of 0.1 V (20–30 Ω). When a four-probe head was used, the layer resistance was 2.0–2.3 Ω/sq. Study of the breathing nature impact at this material was shown in fig. 8. Air exhalation onto the material caused a complex reaction: first increase of current through the structure and then decrease of current through the structure relatively to the initial value (fig. 8, *a*), besides, the presence of the lighting (daylight) had no impact on the material response. Application of the suspension onto the material changed the contact between individual fibers, and the material response changed radically: exhalation reduced the current at the level of 1 mA with the applied voltage of 0.1 V (fig. 8, *b*). The pulse relaxation time was several seconds. Besides, daylight substantially reduced the pulse related to breathing. According to [31], a small change in the load on the carbon fiber or increase of the load causes drastic change in the number of conducting elements.

To restrict water access to the carbon fiber, Nafion was added to the suspension of cyanobacteria. This polymer membrane material makes it possible to form thin porous coatings, which may be used for mechanical protection and for limitation of the quantity of water fibers and gaseous reagents, such as hydrogen and oxygen, which penetrate the sensor layers. The produced composite was applied into the carbon fiber. Application of one drop of composite somewhat reduced the total conductance of material (from ~ 6.3 to ~ 2.5 mA) and made it possible to observe weaker dependence of the current in the layer on breathing (fig. 8, *c*): peak amplitude reduced from ~ 1 to ~ 0.3 mA. It seems that the addition of polymer increased the threshold of the load on the fibers and, since the surface of the carbon fibers in the paper consists of graphene layers, the area of contact between them reduced. This matches the results of the following experiment.

High porous carbon material with addition of cyanobacteria and Nafion composite demonstrated a response to mechanical actions (pressing on the material). Fig. 9 shows the change of current under such pressing. You can se that the current through the structure changes (decreases) approximately by two orders. Use of the carbon fiber as the base demonstrated that whenever the suspension of cyanobacteria was applied on the material surface, the nature of its conductance response to breathing changes. It is connected first of all to the relatively high thickness of this material. At the same time the high thickness of the substrate is a cause of response to pressure. Mechanical actions change the ways of current passage in the porous carbon paper, which causes quick response of design. Fig. 9, *b* shows the response of the sensors from graphene synthesized in plasma with addition of a layer of cyanobacteria with Nafion. In this case the current growth prevails as the response to pressing. In the end of measurements, longer pressing was used, which caused

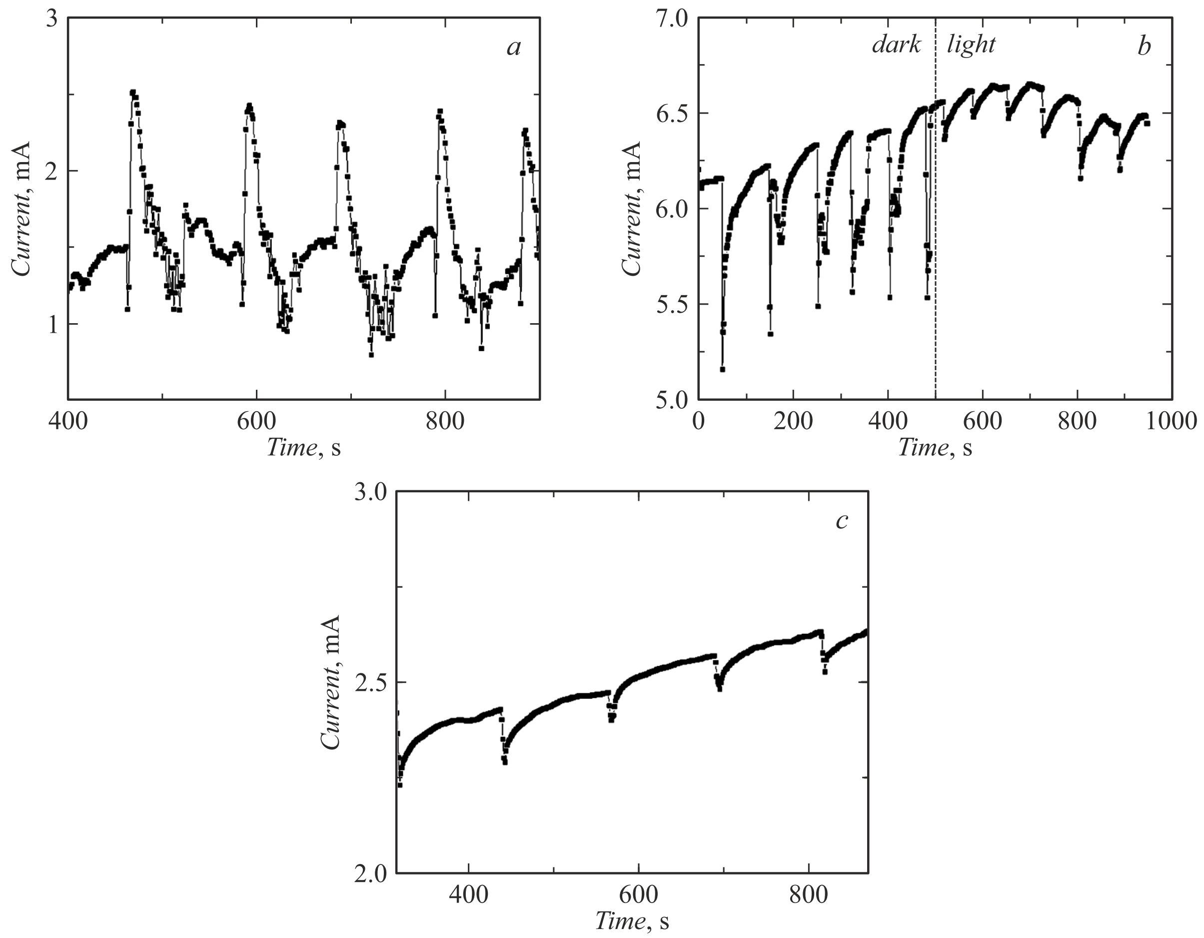

Figure 8. Response of carbon fiber to breathing (*a*). Carbon fiber response to breathing with addition of cyanobacteria with and without lighting (*b*). Carbon fiber response to breathing with addition of a layer of cyanobacteria and Nafion polymer mix (*c*). Voltage applied to contacts was 0.1 V.

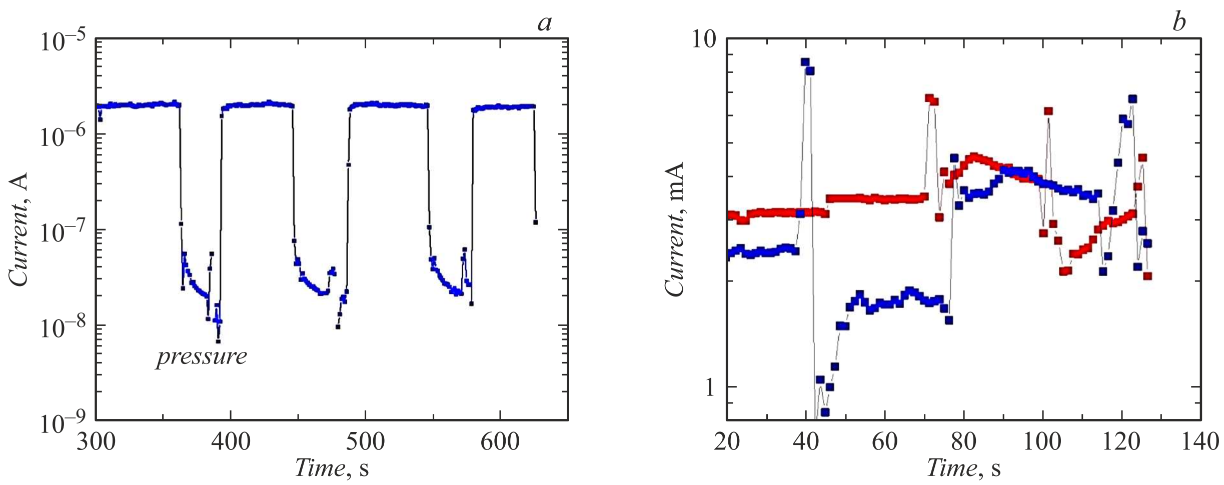

Figure 9. Change of current in time when pressed for carbon fiber with addition of cyanobacteria (*a*). Response to pressing from two sensors from multigraphene synthesized in plasma with addition of cyanobacteria and Nafion (*b*). The applied voltage was 1 V.

wider peaks. Another polarity of response to pressure is determined simply by formation of a better contact between cyanobacteria and graphene when pressing the sensor with force of 7–10 kPa. The dependence was studied between the resistance of carbon fiber with addition of cyanobacteria and stretching strain arising from sensor bend. It was found that the material does not change its characteristics when bent to radius of ~ 2 mm.

Conclusion

Sensor structures of simple design on flexible and solid substrates were made on the basis of biomass of cyanobacteria *Arthrospira platensis*. The main advantage of the produced sensors is their sensitivity to human breath. Depending on the volume and moisture (and, possibly, presence of other components) of the exhaled air, various conductance responses are observed. When the suspension of cyanobacteria is applied on the surface of multigraphene or carbon fibers, the response of this material to mechanical actions (pressing and vibration) increases. Cyanobacteria biomass has perfect adsorbing properties due to high concentration of negatively charged sugars on the surface [1–3]. Various particles or molecules may be adsorbed on these charge states. Depending on the properties of fluids or gases their sorption happens with various speed and in various volumes, which causes different conductance of the structures. Besides, the adsorption process is also impacted by the structure of used cyanobacterial elements (cellular membranes, cellular organelles), which causes a change in the time of sensor relaxation after breathing pulses.

Cyanobacteria are a renewable source with constant properties, which makes them attractive for use in development of cheap and flexible sensors.

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Author contributions

M.B. Shavelkina and I.V. Antonova — experiments, preparation and review of the manuscript, N.A. Nenbogatikova and A.I. Ivanov — measurements, data processing, S.V. Kiseleva and N.I. Chernova — biomass processing, T.B. Shatalova — measurements.

Conflict of interest

The authors declare that they have no conflict of interest.

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