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Properties of bioelectrochemical systems based on electrogenic processes in the root environment of lettuce during their scaling

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The work is devoted to the study of the bioelectrochemical systems (BES) properties when they are scaled by connecting in series. The lettuce variety Typhoon was chosen as the plant object, and the cultivation technology was panoponics. The resulting average voltage of one cell was 102 mV, three and twenty series-connected 197 mV and 1782 mV, respectively, which is 36% and 13% lower than the expected values. Analysis of the potential difference created in each cell in a chain of several series-connected BES showed significant unevenness between the indicators and even the presence of negative polarity. A decrease in the total power when creating batteries from BES has been noted by many researchers and is associated with the heterogeneity of the elements included in the circuit and the presence of reverse voltages. The most effective way to increase the power characteristics of BES is to accumulate the resulting bioenergy using ionistors.

Keywords: plant-microbial fuel cell, series connection, green energy, panoponics, ionistors.

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Introduction

Currently, the problem of exhaustion of the main types of resources such as energy and food is especially acute. Renewable energy is considered as one of the ways to alleviate the fuel needs of the future, overcome the global warming crisis and reduce carbon dioxide emissions, and the use of hybrid energy sources for combined use makes it possible to effectively combine production processes.

The current prospect of alternative energy is the development of natural energy resources, which include bioelectrochemical systems (BES) being devices for generating electrical energy due to the course of chemical reactions accompanying the vital activity of living organisms. BES can include biological systems such as microorganisms [1], algae [2], plants [3] and their combinations.

The principle of operation of the classical BES such as a microbial fuel cell (MFC) consists in the oxidation of organic compounds (acetate, glucose, cellulose, wastewater components) by exoelectrogenic bacteria to form electrons, protons and carbon dioxide [4]. Various designs are used for a charge separation in MFC, including both the standard scheme of a two-chamber fuel cell consisting of an anode sector with microorganisms and a cathode compartment with water separated by a proton exchange membrane [1], and various modifications — simpler single-chamber devices [5], easily integrated tube models [6] and reducing the internal resistance of a membrane-free device [7].

The availability of MFC technology has been expanded by using organic substances synthesized by plants during photosynthetic reactions and partially released into the root environment in the form of rhizodeposites as a substrate for microorganisms [3]. Such a BES, called „plant-microbial fuel cell" (PMFC), can be a source of long-term and sustainable production of renewable and environmentally friendly bioenergy, since the electricity generated throughout the growing season does not require additional external input of a substrate to maintain the vital activity of electroactive microorganisms [8]. PMFC work like solar photovoltaic cells, generating electricity in the process of photosynthetic reactions with the formation of organic compounds, the chemical energy of which is converted by microorganisms into electrical energy. A BES based on electroactive plant-microbial interactions is a promising bioenergy resource, since it allows combining the production of electricity and plant products, while not competing for agricultural territories [9].

The weak side of electrochemical systems based on bioelectrogenesis is the low electrical output parameters. One of the main disadvantages of BES is the high and different internal resistance for single cells, as a result of which the energy obtained by using living organisms can only be considered as a power source for low-power devices [10]. Moreover, the output of electricity is affected by ohmic losses, activation losses, metabolic processes and concentration effects [11]. The ohmic losses caused by high internal resistance of the system can be reduced by decreasing the distance between the electrodes or removing the ion-selective membrane [12], however, the concentration effects of ion distribution may decrease in conditions of close proximity of the electrodes and the cathode can be contaminated by microorganisms which, in turn, results in a decrease of the output power of BES [10].

An increase of the output power due to an increase of the size of the anode chamber also does not result in the desired proportional increase of the output electrical characteristics [13]. On the contrary, a reduction of the specific power from 420 to 89 mW/m^2 is observed in some cases with a change of the volume from 4 to 20 m*l* [14], as well as the absence of a proportional increase of power in case of expansion of the volume from 100 m*l* to 5 *l* [15]. Doubling of the cathode size resulted in an increase of power by only 62%, and doubling of the anode size resulted only in 12% power increase when using domestic wastewater in MFC [16]. It has been shown that an increase of the volume to a value exceeding 2000 m*l* results in an increase of the activity of methanogenic bacteria that compete with electrogenic microorganisms, reducing their activity jcite13,17.

Upscaling is another possible way to increase the power characteristics of a BES, i.e. an electrical connection of several single cells into a battery. However, the results of upscaling of the PMFC with green beans demonstrated that a series connection of too many cells results in voltage losses due to the possibility of a reversible voltage [18]. The received power from one, three and nine series-connected cells with an anode area of 25.8, 77.4 and 232.2 cm^2 was 0.39, 1.00 and 1.37 μW, respectively [18].

In general, it is noted that the combination of parallel and serial connection of cells in case of their upscaling significantly affects the output characteristics of batteries from PMFC [18]. When creating complex electrical circuits, it was optimal to connect no more than three BES cells in series — for example, the parallel connection of three elements from three series-connected PMFC resulted in an increase of specific power to 161 $\mu W/m^2$, which corresponded to the increase of the output characteristics of a single cell by 9.7 times and was the largest value of the studied variants for bean plants [18].

By now, the problem with the layout and upscaling of the BES is becoming more and more obvious, since the expected summation of the power obtained from one cell, like in classical galvanic cells, does not occur in case of batteries creation. Any changing of the parameters of a single cell, which are primarily related to the condition of plants and the microorganisms surrounding them, entails a decrease of the total voltage in the BES battery [19]. In addition, the greatest problem is caused by the observed changes of the polarity of the elements in the circuit, which results in overcharging of the cell from stably operating systems and possible changes of the internal impedance [20]. For this reason studies of the parameters of plant bioenergetic devices when they are connected to batteries would be relevant and novel.

The purpose of this paper was to study the properties of electrical circuits composed of BES based on the electrogenic processes of the root environment of plants and to find effective ways to scale bioenergetic devices.

1. BES design

The leaf lettuce (*Lactuca sativa* L.) of the Typhoon variety produced by LLC „Sortsemovosch“ (Russia) was selected as a phytotest object as it has a developed rod-type root system with many lateral branches. Typhoon leaf lettuce has a rich vitamin and mineral composition among the broad variety of cultivars and is characterized by rapid growth, a large open rosette of leaves and the ability to produce a stable harvest.

Artificial environments based on nutrient solutions are the best objects for the study of electrical phenomena occurring in a root environment are the best objects for the study of electrical phenomena occurring in a root environment due to fewer influencing external factors, primarily humidity, and greater controllability of the parameters and composition of the habitat. In this regard, the plants were grown using thin-layer panoponics technology [21] under controlled conditions of intensive light culture on the agrobiopolygon of Agrophysical Research Institute in a vegetation installation with HPS-400 lamps used as light sources. The irradiation was $75 \pm 5\,W/m^2$ in the field of photosynthetically active radiation (PAR), light period was 14 hours per day, air temperature was $+20-22^\circ C$ day and $+18-20^\circ C$ at night, relative humidity was 65−70%. A Knop's solution containing $Ca(NO_3)_2 \times 4H_2O$, KNO_3, KCl, KH_2PO_4, $MgSO_4 \times 7H_2O$ and microelements was used as a nutrient solution [22].

The experimental BES comprised a cell with an area of 187×137 mm and a height of 72 mm, in which sprouted lettuce seeds were placed and grown (Fig. 1). The measured characteristic reflecting the bioelectric activity of the root system and associated microorganisms and the course of metabolic processes in the root environment was the potential difference, measured in our case between the root neck and the bottom of the container for cultivation. The voltage generated in the root environment was recorded by using corrosion-resistant biocompatible electrodes made of 5 mm thick graphite felt with a large specific surface area ensuring a surface electrical contact with the root system installed in the cultivation systems. The lower electrode with a size of 50×70 mm was located at the bottom of the growing container (plant), the upper electrode with an area of 50×30 mm was placed on a platform covered with a moisture-conducting element that provides plants with nutrition due to the capillary effect. The distance between the electrodes was 50 mm, the amount of the Knop solution was kept constant at 25 mm. In most cases, the upper electrode is charged electronegatively with respect to the lower electrode.

The upscaling for increasing the power characteristics of the BES was performed by a series connection of cells ensuring contact of the lower electrode of one element with the upper electrode of another using stainless steel lead wires. The hydrogen pH in the near-electrode regions was measured using the ST20 pH meter (OHAUS, China) in aqueous extracts 1 : 50. Monitoring of changes in the

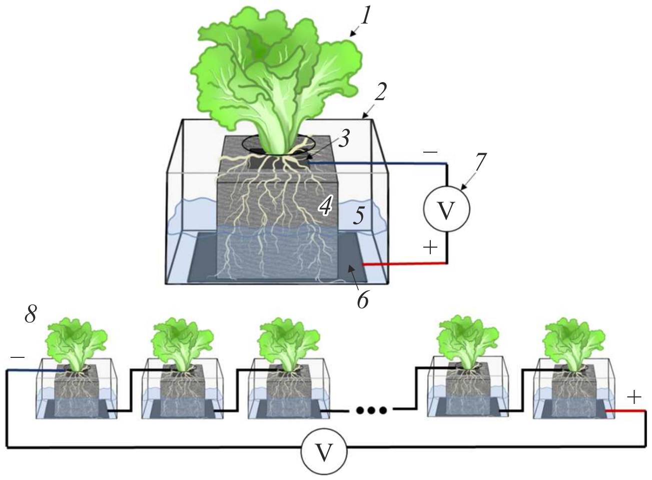

Figure 1. Schematic representation of an experimental BES, including: *1* — a plant, *2* — a container for growing, *3* — an upper electrode, *4* — a platform for plants coated with hydrophilic material, *5* — a nutrient solution, *6* — a lower electrode, *7* —a voltmeter, *8* — a serial connection of BES cells into an electrical circuit from *n* elements.

potential difference in the root environment was carried out by recording using an automated voltmeter designed based on the Arduino hardware platform every 15 min during the entire growing season [23]. The obtained experimental curves were averaged using the moving average method with a periodicity of 100 points.

2. Characteristics of one BES cell

The dynamics of the potential difference generated in the root environment of a single BES cell during the development of a lettuce Typhoon variety is shown in Fig. 2. The pH values of the solution during the experiment were within 6.2–6.4. The observed voltage in the system averaged 142 ± 20 mV in the first three days of vegetation. Then, it gradually decreased from the third to the fifth day and stabilized at the level of 82 ± 21 mV during 10 days. A slight increase of the potential difference to 108 ± 29 mV was observed starting from the fifteenth day . In general, the average voltage value was 102 ± 32 mV for the entire growing season. It can be assumed that the increase of indicators starting from the fifteenth day of lettuce development is associated with the formation of a stable biofilm and, due to this, an increase of the electroactivity of microorganisms. The resulting mass of lettuce after its cultivation in the BES was 56 ± 8 g, plant height was 17 ± 2 cm.

A certain potential difference is also observed in a cell without plants because the electrochemical system works as a concentration galvanic cell in which the nutrient solution acts as an analogue of the electrolyte [24], however, the concentration difference on the electrodes equalizes over time and the voltage drops to zero. We have previously

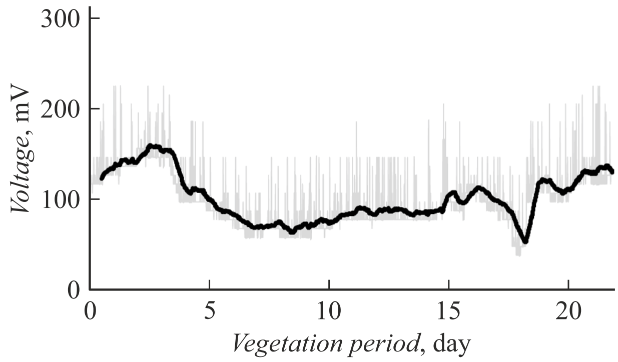

Figure 2. Dynamics of the formation of a potential difference in one BES cell when growing a lettuce plant.

shown that the root system of plants plays the role of EMF in BES, enhancing diffusion processes and ion transport during its vital activity [25].

3. Serial connection of three BES

According to the theory of electrical circuits, it is expected that when several elements are connected in series, the voltage of the system will be proportional to the number of cells included in it. So, the expected voltage increase is 3 times for a three-cell battery. However, experimental data show that the output characteristics are lower than expected.

Figure 3 shows the dynamics of the formation of a potential difference in three series-connected cells of the BES. In general, the voltage change was similar to that in a single cell in a three-cell circuit. The generation of a potential difference in the root environment was observed at the level of 276 ± 30 mV in the first three days. Then the values dropped to 130 ± 24 mV by the fifth day of the growing season and were stationary at this level until the fifteenth day of lettuce development. An increase of the voltage to 350 mV was observed after that. The average value of the potential difference throughout the growing season was 197 mV. The average weight of lettuce plants was 46 ± 7, 73 ± 13, 67 ± 11 g for the first, second

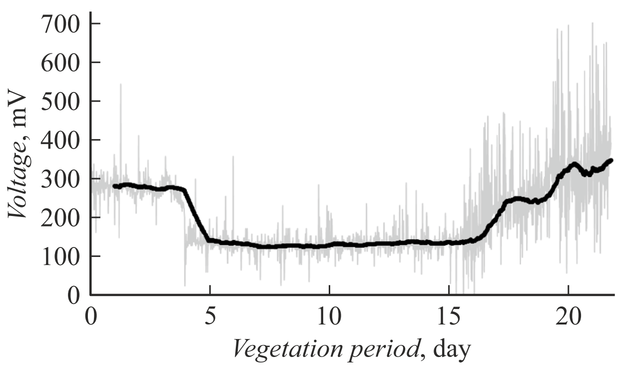

Figure 3. Dynamics of potential difference formation in three BES cells connected in series during cultivation of lettuce plants.

and third cells and height was 18 ± 2, 21 ± 3, 17 ± 3 cm, respectively.

Thus, the resulting increase of voltage when three cells were connected in series was 1.9 times in the first three days of vegetation, 1.6 times on the fifth to fifteenth days of development and 2.4 times after the fifteenth day. The data obtained are consistent with the results of other studies. For instance, when green beans were used in a BES, the average voltage of three series-connected PMFCs was also only 2.4 times higher than the voltage of a single cell [18].

4. Serial connection of twenty BES

The same effect of reduction of the total voltage when creating circuits from BES is observed in case of connection of a larger number of cells. Fig. 4 shows the measured dynamics of the potential difference created when growing lettuce in twenty BES connected in series. The generated voltage is at the level of 2057 ± 101 mV in the first three days. Then the potential difference drops to 1281 ± 171 mV in the root environment, like in case of a single cell, and remains stationary for about 10 days. The voltage increases again to 2306 ± 200 mV on the fourteenth-fifteenth day. The value of the potential difference for a circuit of twenty BES was on average about 1782 mV, which is lower than the expected 2040 mV by 13%.

It was found that it was possible to achieve only a 14-fold increase of voltage in the first days when twenty cells were connected in series. Then, this increase was 16-fold from the fifth to the twelfth days, and the voltage increase was proportional to the number of cells connected in the circuit only after the fifteenth day of the growing season.

Similar results were observed for twenty MFC cells connected in series and parallel to an electrical circuit — such a BES battery could generate 2.3 V and 0.5 mA [26].

5. Properties of BES cells when they are connected in series

It can be seen based on the data obtained that the summation of the output characteristics measured for single

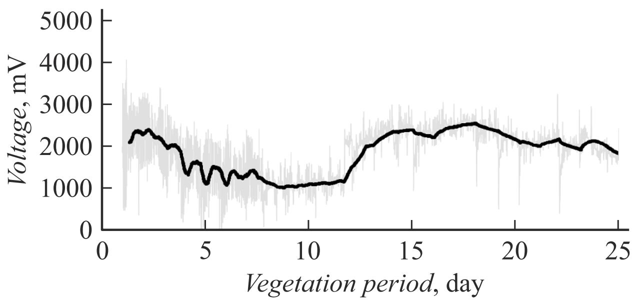

Figure 4. Dynamics of the formation of a potential difference in twenty BES cells connected in series during growing of lettuce plants.

Figure 5. General appearance and numbering of twenty BES connected in series during growing of Typhoon lettuce at the end of the growing season.

cells of the BES, which is expected according to the second Kirchhoff's voltage law, does not occur. An analysis of the potential difference generated in each cell in a circuit of several BES connected in series was performed (Fig. 5), which showed a significant unevenness between the indicators and even the presence of a negative polarity.

The maximum voltage obtained of 173 ± 23 mV was characteristic of cell № 14, and the minimum values of 18 ± 13 and 21 ± 13 mV were characteristic of cells № 3 and 20, respectively (see table). It is interesting to note that negative polarity and reverse voltage were observed in BES located in the middle of an electric circuit of twenty elements: -33 ± 8 mV in cell № 11 and -62 ± 18 mV in cell № 13. At the same time, they were between the BES with the largest potential differences — 128 ± 13 mV for cell № 10, 151 ± 27 mV for cell № 12 and 173 ± 23 mV for cell № 14.

Probably, such variations of the generated parameters of cells when they are connected in series and, as a result, the reduced output characteristics of upscaled BES are associated both with the effect of plant organisms on the physiological state because the flow of current through the connected cells affects the electrogenic properties of the system and with nonuniform charging of heterogeneous elements incorporated in the battery because the elements with a higher capacity are under-discharged when connected to the circuit and the elements with lower capacity are over-discharged.

No pronounced relationship between the morphophysiological and electrical parameters of the root environment was found. There is only a weak negative correlation of the average potential difference with plant weight (correlation coefficient $r = -0.17$) and a moderate negative relationship with the height of lettuce ($r = -0.44$) at the end of the growing season at the stage of technical maturity of the lettuce.

The pH of the upper electrode was higher than that of the lower one in almost all cells. This corresponds to the polarity observed in most cases — the upper electrode is electronegative and has a more alkaline pH and lower hydrogen cation activity. However, the values of the

Characteristics of the BES cells that are part of an electrical circuit of twenty elements connected in series

Number of cells	Average difference of potentials, mV	Height of plants, cm	Weight of aerial part of plant, g	Weight of roots, g	pH of top electrode	pH of bottom electrode
1	56 ± 17	21 ± 4	79 ± 7	8 ± 1	6.9 ± 0.2	6.2 ± 0.1
2	107 ± 11	24 ± 4	88 ± 6	14 ± 2	6.5 ± 0.2	6.2 ± 0.1
3	18 ± 13	21 ± 3	68 ± 6	14 ± 2	6.5 ± 0.1	6.5 ± 0.1
4	115 ± 13	21 ± 4	24 ± 6	10 ± 1	5.8 ± 0.1	5.9 ± 0.1
5	90 ± 4	20 ± 3	34 ± 5	11 ± 1	6.4 ± 0.2	6.3 ± 0.2
6	75 ± 8	19 ± 4	35 ± 6	9 ± 1	6.7 ± 0.2	6.2 ± 0.2
7	97 ± 36	27 ± 4	85 ± 7	19 ± 1	6.5 ± 0.1	6.3 ± 0.2
8	91 ± 9	28 ± 4	93 ± 7	14 ± 1	6.5 ± 0.2	6.2 ± 0.1
9	48 ± 7	22 ± 3	33 ± 6	9 ± 1	6.4 ± 0.1	5.8 ± 0.1
10	128 ± 13	22 ± 3	58 ± 6	13 ± 1	6.4 ± 0.1	6.1 ± 0.1
11	-33 ± 8	28 ± 5	68 ± 7	14 ± 1	6.7 ± 0.2	6.4 ± 0.2
12	151 ± 27	24 ± 4	53 ± 6	16 ± 1	6.5 ± 0.2	6.2 ± 0.1
13	-62 ± 18	26 ± 4	68 ± 6	14 ± 1	6.6 ± 0.1	6.3 ± 0.1
14	173 ± 23	18 ± 3	52 ± 5	12 ± 1	6.6 ± 0.1	6.4 ± 0.1
15	116 ± 15	19 ± 3	54 ± 5	8 ± 1	6.4 ± 0.1	6.0 ± 0.1
16	63 ± 14	20 ± 4	47 ± 6	16 ± 2	6.0 ± 0.1	6.2 ± 0.1
17	114 ± 28	23 ± 3	67 ± 5	20 ± 2	7.0 ± 0.1	6.6 ± 0.1
18	105 ± 25	18 ± 4	34 ± 6	8 ± 1	6.2 ± 0.1	6.3 ± 0.2
19	114 ± 36	20 ± 3	57 ± 4	17 ± 1	6.4 ± 0.2	6.2 ± 0.1
20	21 ± 13	24 ± 4	59 ± 6	13 ± 2	6.5 ± 0.2	6.3 ± 0.1

hydrogen index of the electrode systems also had only a weak negative correlation with the voltage values in the root zone — $r = -0.18$ for the upper and $r = -0.16$ for the lower electrodes.

The presence of negative polarity in an electric circuit of several BES in some cells is confirmed by other experimental data. Thus, the results of upscaling of PMFC with green beans, showed that too many cells connected in series leads to voltage losses due to the possibility of a reversible voltage [18]. It was suggested that the change of the polarity of the BES voltage may be due either to the application of an external resistance, or to a change of the state of the biofilm formed at the anode [27].

6. Accumulation of generated BES energy

It was proposed in papers [28,29] to minimize the voltage reduction in batteries from the BES either by controlling the voltage of each unit cell and connecting only the same elements to the circuit, or by maintaining the same operating conditions in the reactor by externally changing the characteristics of the element or changing its polarity.

In our opinion, it is more efficient and less labor-intensive to increase the power characteristics of a BES by equalizing the voltage parameters of single cells, for example, by using energy storage with the use of a storage device. Basically, the conservation of bioenergy generated by MFC and PMFC is realized by connecting energy accumulators, which can act as capacitors or supercapacitors [30].

An ionistor which is a supercapacitor with a capacity of 0.1 F. was used as an energy storage medium for the studied plant BES. The energy storage device was connected to the BES in parallel, and the charging speed was monitored using an automated voltmeter. Figure 6 shows the dynamics of charging an ionistor with a capacity of 0.1 F from a BES cell when growing lettuce Typhoon variety. The potential difference generated in the reactor without external load was on the order of 150 mV. The charging time of the ionistor before reaching the plateau, i.e. equalization of the voltage of the BES and the ionistor, was about 40 h. Moreover, apparently, the voltage to which the capacitor was charged did not exceed a quarter of the value generated in the cell and was on the order of 60 mV due to the high internal resistance of the BES. The accumulated energy was 0.18 mJ per cell with a volume of $0.0018\,m^3$. It is interesting to note that the system recovery was observed after disconnection of the external load from the BES.

7. Examples of the use of BES

BES can serve as a power source for the operation of various low-power electronic devices and sensors, for example, for online monitoring of environmental parameters, water quality, pollution detection, assessment of microbial activity [31]. MFC and PMFC can also supply power to small electronic devices used in various applications, such

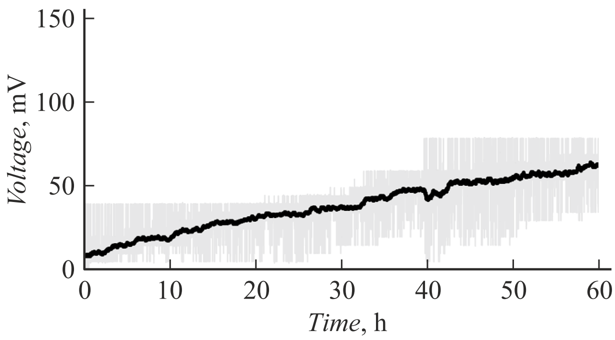

Figure 6. Dynamics of charging an ionistor with a capacity of 0.1 F from BES.

Figure 7. *1* — „ElectroPlant" power plant in phytotechnical complex conditions, *2* — blue LED power supply from energy storage module connected to thirty BES.

as photo sensors, LED light sources, pH sensors, mobile devices, etc. [32].

For example, a two-module PMFC of 24 elements connected in series with a capacity of 0.6 *l* with multi-electrode systems connected inside the BES in parallel allowed for autonomous power supply of a digital hygrometer thermometer 1.5 V, and a three-module PMFC supplied 3 V power to a digital indoor unit of the weather station or three LEDs during the three months of the experiment [33]. Partial charging of a mobile phone became possible after the accumulation of energy generated by three PMFC [34]. 12 PMFC connected in series with cacti grown in them were capable to ensure the operation of LEDs and digital clocks in real time [35].

In the conditions of the phytotechnical complex [36], we tested the operation of „ElectroPlant" power plant containing 30 BES for the cultivation of lettuce plants, the accumulated energy this power plant was sufficient for powering a blue LED 3.3 V for 3 min (Fig. 7).

Robotics is the expected promising application of BES technology [37]. For example, robots were developed that use the energy generated by MFC based on *E. coli* to charge batteries and operate various controllers [38]. Ecobot-II with a 12-day duty cycle was the first successful demonstration of a robot fully powered by 8 MFC [39]. All these examples of robots and sensors with autonomous power supply using BES can be considered energetically self-sufficient facilities that can be used both in space conditions and in remote locations without power grids.

Conclusion

Thus, the properties of BES connected in series in electrical circuits, were considered when growing lettuce plants using the panoponic method. The average voltage of a single BES cell was 102 mV, and the average voltage of three and twenty BES cells connected in series was 197 and 1782 mV, respectively, which is lower than expected values by 36 and 13%. The effect of reduction of the total power when creating batteries from BES has been noted by many researchers, and it is associated with the presence of reverse voltage and uneven charging in the presence of heterogeneous elements in the circuit, which is confirmed by the data we obtained that characterize the properties of BES that are part of an electric circuit. The most effective way of upscaling at the moment is to increase the power characteristics of the BES by equalizing the voltage parameters of single cells, for example, by accumulating energy using a storage device.

The use of BES in agriculture opens up a wide range of opportunities for the creation of low-power autonomous sources of renewable electrical energy. BES energy, on the one hand, can supply power in natural conditions for various environmental sensors, sensors of the state of plant organisms, alarms, LEDs. On the other hand, such a system can support the operation of plant life-supporting devices in protected ground conditions in emergency situations, for example, in case of a temporary shutdown of external power supply, which is necessary for the safety of cultivated plants. BES can be easily combined with the cultivation of plant products both in urban farming conditions and in the open ground. It is also possible to use it as a biosensor for monitoring the condition of plants and adjusting cultivation technologies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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