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Original article

EFFECT OF PLANTS MORPHOLOGICAL PARAMETERS ON PLANT-MICROBIAL FUEL CELL EFFICIENCY

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Background. Plant-microbial fuel cell (PMFC) is an innovative biotechnology for the environmentally safe bioelectricity generation. The widespread use of bioelectrical systems (biosystems) is hindered by their insufficient efficiency due to limiting knowings of the relationship between bioelectricity generation and features of their biotechnological components.
Objective. The purpose of this study was to analyze the role of the plants morphological parameters and structure features of biomodules on generation of bioelectricity.
Methods. Biometric, biogravimetric, voltammetric, and statistical analysis methods were used to assess the relationship between plant`s accumulated mass of leaf and roots, multielectrode design of biosystem and bioelectricity generation.
Results. PMFC based on sedge *C. hirta* with the largest accumulated total dry leaf/stem and root mass and also the rhizome-like and developed fibrous root system were characterized by the highest power output compared to other biosystems. The power density was 970 ± 22 mW m^{-2} PGA. The parallel stacking of biomodules leads to obtain current output about 108.7 mA. That is why the developed biotechnological systems can be recommended as a foundation for the development of power supply for WiFi microcontrollers that consume 100 mA or for charging batteries.
Conclusions. Sedge *C. hirta* were appeared as the more suitable plants for biological component of biosystem of bioelectricity generation. Power density of *C. hirta* based PMFC exceeded the one of based on other plants in 9.3–37.9%.The type and level of development of the root system and of the above-ground photosynthetic surface of plant are an important prognostic factors of the PMFC perfomance. A 10-fold increase of the electrode surface of one biomodule results in 3.95 times increase of power density at 200 Ω. The multielectrodeity biomodule reveals as another lever for increasing the efficiency of biosystems which allows obtaining significantly increase power density and current density in the range of electrical resistance from 50 to 500 Ω.

Keywords: vascular plant; biosystems efficiency; plant-microbial fuel cell; bioelectricity; bioelectrical system.

Introduction

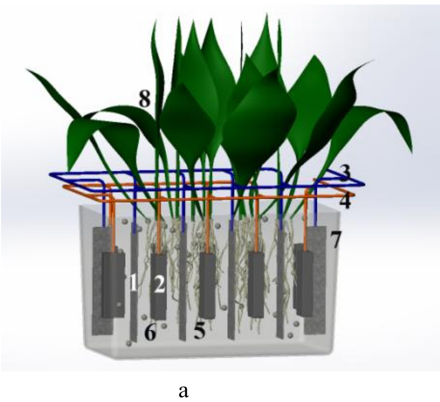
The polluted environment and the extreme climatic situation as a result of the exploitation of non-renewable energy sources lead to the need to find new ecological energy-efficient technologies. As part of the transition to a carbon-neutral strategy, an important role is given to alternative energy sources and greening, as an important way to overcome both the causes and consequences of global climate change, as well as an important resource-saving factor. Existing cities and agglomerations are subject to green modernization, and ecological principles are laid down in city development plans with the introduction of green buildings, in particular, with green roofs. Therefore, the development of energy-efficient biotechnologies based on plants is urgent and represents an important challenge for society.

The study of plant-based bioelectrical systems was started in 2008 in rice fields with *Oryza sativa* plants and with marsh grass *Glyceria maxima* [1–3] and continued both on green roofs [4–6] and *in situ* [7–9], as well as in laboratory conditions [10–12]. Plant-microbial fuel cells (PMFC) considers as an innovative ecological way of obtaining bioelectricity by introducing electrode systems into the substrate of plant growth and collecting bioelectricity produced by microorganisms in the rhyzosherical zone. Plants reveals as an important factor that allows the existence and supports the existence of microbial bioelectric systems in the soil for an unlimited time. Since without plants that results in absence of releasing of products of photosynthesis through the root system and lack of plant residues in the soil, sources for the existence of electricity-generating microorganisms in the soil are exhausted [13].

Currently, plant-microbial fuel cells are being developed for powering low-energy devices [14–18], but they are not widely used. The main problem interfering the development and wide application of biotechnology for obtaining bioelectricity from plant-microbial systems is its insufficient efficiency [19–21]. The task of improwing the efficiency of bioelectrical systems is complex, as it depends on a number of factors: biological (development of the root system, photosynthetic activity, plants rhizodeposition, electrogenesis of microorganisms and other bionts of the rhyzosphere zone), technical (composition and structure of the substrate, materials of electrodes, schemes of electrode arrangement and connections), as well as the influence of external factors (temperature, humidity, lighting) [13, 22, 23]. For present time, more than 90 different species of plants of various groups are known in the creation of PMFC, such as traditional wetland plants, plants that were used in the first bioelectric systems, hydrophytes, as well as drought-resistant plants and mesophytes [13, 22, 24]. In a number of cases, progress in increasing the generation of bioelectricity was made only by changing the type of plants with the same configuration of electrodes or the electrode design using the same plant [25–27]. These results indicate the importance of plant species selection and optimal electrode configuration in PMFC performance. In this regard, the purpose of this work was to compare the bioelectricity generation of PMFC based on 5 types of plants when using a two- and twenty-electrode biosystem to determine the most suitable plant among them and to study the effect of multielectrodeity of one biomodule on bioelectrical parameters. *Alisma plantago-aquatica*, *Festuca arundinacea*, *Carex hirta*, *Ocimum basilicum* plant and *Polytrichum commune* moss were chosen as plants that showed promising results in terms of bioelectricity generation in our previous experiments. The study of the relationship between the generation of electricity and morphological features of the plant, such as the length of the stems, the number of leaves and defoliation, the length and number of roots, the diameter of the stems is only beginning to be studied [25, 28, 29]. Denser developed root system and high plant biomass considered as advantageous to power production and signs of highly efficient PMFC [27, 30]. Therefore, we set ourselves the task of evaluating the relationship between the accumulated dry mass of leaf/stems and roots of plants and bioelectricity generation. Since dependence of bioelectricity generation on the surface area of electrodes was insufficiently investigated, and also the use of parallel-serial connection of biomodules faced the problems of power loss and voltage reversal [31–33], we also set the task to investigate these effects of electrodes surface area and the connection of multielectrodes inside of one biomodule under different resistance, as well as stacking biomodules for the generation of bioelectricity.

Materials and Methods

To design bioelectrical systems graphite cathodes and galvanized steel anodes were used [34]. Perforation of the anodes was applied to increase the working area of the electrode. As wires, copper wires with polyvinyl chloride insulation were used, which were connected to the electrodes mechanically by soldering with tin alloy or with the help of additional fastening elements to avoid loss of conductivity. Two-electrode and twenty-electrode biosystems with plants were constructed on their basis (Fig. 1). For the construction of multi-electrode biosystems, several graphite cathodes were connected in parallel with copper wires in the



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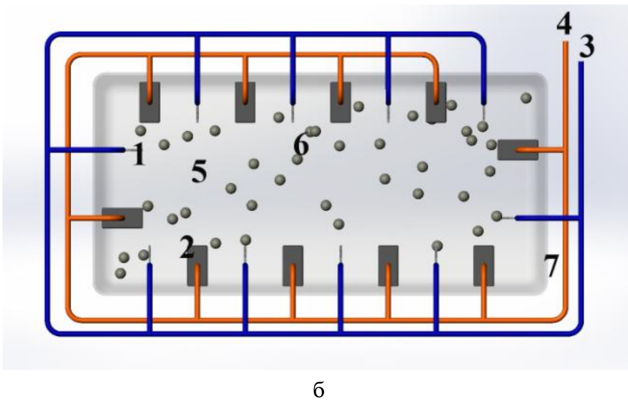

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Figure 1: Schematic representation of the biosystem (a) and, the twenty-electrode system of cathodes and anodes (b): 1 – anode; 2 – cathode; 3 – output of the wire connecting the system of 10 anodes; 4 – output of the wire connecting the system of 10 cathodes; 5 – substrate; 6 – rhizosphere microorganisms; 7 – container; 8 – plant

cathode system and several galvanized steel anodes with perforations in the anode system.

Electrode systems were placed stationary in the soil during the experiment with an inter-electrode distance of 0.01 m at a depth of 0.05 m in the soil layer, in the zone of association of plant roots and rhizosphere microorganisms, where the emission of electrons and protons occurs. The substrate was the universal soil substrate Ecoflora with the following composition: high-quality mountain peat, sod land, purified river sand and nitrogen, phosphorus, potassium mineral fertilizers and trace elements, including Fe, Mn, Cu, Zn. A container measuring 0.35×0.15×0.15 m was used.

To construct biosystems, electrode systems, substrate for plant development were placed in plastic containers, and plants were planted by introducing plant seedlings. *Alisma plantago-aquatica*, *Festuca arundinacea*, *Carex hirta*, *Ocimum basilicum* and moss *Polytrichum commune* were used as plants. Experiments were conducted with 10 biosystems of each type for one month. The moisture of the substrate was maintained by watering each two days.

Plant height was determined biometrically by measuring the above-ground part of the plant to the top of the plant. To dry mass measuring of the above-ground part and the root system, stems and leaves were separated from the roots. Roots were washed free from the soil through a system of sieves (with holes of 1, 0.5 and 0.25 mm). The plant materials were dried at 80 °C for 48 h and measured. Leaf and stem (LMA, g m^{-2}) and root mass (RMA, g m^{-2}) taken per container 0.052 m^2 through calculation were normalized per m^2 as total dry mass. For each treatment, three dried samples of each species were used [35].

Measurements of biosystems bioelectrical parameters were carried out under both open-circuit conditions: short-circuit current, open-circuit voltage (OCV), and under the load of resistors with different electrical resistances. The analysis were conducted using a short-term connection of resistors from 10 Ω to 12 kΩ. Bioelectrical parameters were measured using a UT890C UNIT-T digital multimeter. The current and power density from one PMFC with an area of 0.0525 m^2 was normalized to 1 m^2 of the area of the plant growth area covered with electrodes and plants (PGA). Results are presented as the awerage mean of all replicate experiments and their standard errors ($x \pm SE$). The significance of the difference in mean values was determined by one-tailed analysis and F-test at the 95% confidence level.

Results

The bioelectrical parameters of 5 different types of PMFCs with the same electrode configuration planted with different types of cosmopolitan plants were analyzed (Table 1). As plants, perennial plants were used, which are characterized by resistance to extreme environmental conditions, such as vascular plants: 1) water plantain *A. plantago-aquatica*, which inhabits moist soils, 2) reed sedge *F. arundinacea* and 3) rough-haired sedge *C. hirta*, mesophyte grasses, as well as 4) the annual indoor basil plant *O. basilicum*, which is used as a spice plant, and 5) a non-vascular plant the cuckoo moss *P. commune*. The last species was chosen as a unique type of moss, since mosses are known for their lack of water-conducting tissue and small stems. However, the peculiarity of this species of *P. commune* moss is owning by 1) a clear differentiation of the water-conducting tissue and the formation of a hadrom and leptom, which are analogous to xylem and phloem in higher plants, as well as 2) an exceptional height of plant stems. Despite the same average parameters of the plant height of ground part, the plants were characterized by different LMA, which ranged from the smallest values in

Table 1: Performance of the twenty-electrode PMFC depending on the species characteristic of plants

Plant component of PMFC	Plant height, m	Photo-synthetic path, type	Leaf/stem mass per area, g m^{-2}	Root mass per area, g m^{-2}	Root system, type	Power density, mW m^{-2} PGA	OCV, mV
A. plantagoaquatica	0.35 ± 0.016	C3	458 ± 14	608 ± 23	Rhizome-like, fibrous	752 ± 16	1086 ± 19
F. arundinacea	0.32 ± 0.014	C3	534 ± 19	685 ± 18	Rhizome-like, fibrous	879 ± 23	1091 ± 31
P. commune	0.20 ± 0.010	C3	396 ± 8	306* ± 9	Rhizoid	602 ± 15	1010 ± 23
O. basilicum	0.33 ± 0.015	C3	382 ± 10	459 ± 12	Taproots	654 ± 17	1017 ± 20
C. hirta	0.37 ± 0.013	C4	612 ± 17	754 ± 21	Rhizome-like, fibrous	970 ± 22	1126 ± 25

* – rhizoid mass per area.

O. basilicum to highest in *C. hirta samples*, depending on the number of leaf and the minimum density of the plant growth per unit area of each plant species. Conversely, mosses with smaller plant height due to high density of leaf and the plant growth were characterized by LMA at the level of higher plants. The total dry mass of the root systems was the highest in *C. hirta*, which is characterized by a well-developed rhizome and fibrous system, and the total dry rhizoid mass *P. commune* was in 1.5–2.5 smaller than the root mass of the other samples.

Increasing the number of electrodes within one PMFC biomodule of the same size and plants had a different effect on the bioelectrical parameters of biosystems depending on the electrical resistance of the resistors. This action on the power of biosystems was most effective in the range from 50 to 500 Ω, and from 200 to 12,000 Ω for voltage and in the range of 10–500 Ω for current density (Fig. 2, a, b). Thus, the highest recorded power of a twenty-electrode PMFC, 0.711 W m^{-2} PGA at 200 Ω, was 3.95 times higher than the maximum power of a two-electrode biosystem, which was 0.180 W m^{-2} PGA at the same resistance. The current density of the multi-electrode PMFC was 2.01 times higher at 10 Ω than the two-electrode of the same area. The voltage was the highest at the highest applied electrical resistance of 12,000 Ω.

The developed biomodule, thanks to its multi-electrodity, can reach an average of about 52.3 mA at 10 Ω compared to 2.5 mA of a two-electrode one (Fig. 3). As we have shown, parallel stacking of biomodules leads to an almost proportional increase in current [34, 36], allowing to obtain about 108.7 mA from two biomodules.

A month-long experiment with 5 different types of PMFCs showed the highest values of bioelectrical indicators in *C. hirta* based PMFCs. The average voltage of the open circuit of these biosystems was 1126 ± 25 mV and was 1.03–1.11 times higher than the parameters of biosystems with other types of plants. The power density measured when using an electrical resistance of 200 Ω exceeded the power of other biosystems by 9.3–37.9% and was 970 ± 22 mW m^{-2} PGA (see Table 1). The lowest values of bioelectricity were characteristic of PMFC with *P. commune* and *O. basilicum* 602 ± 15 and 654 ± 17 mW m^{-2} PGA, and 1010 ± 23 and 1010 ± 23 mV, respectively.

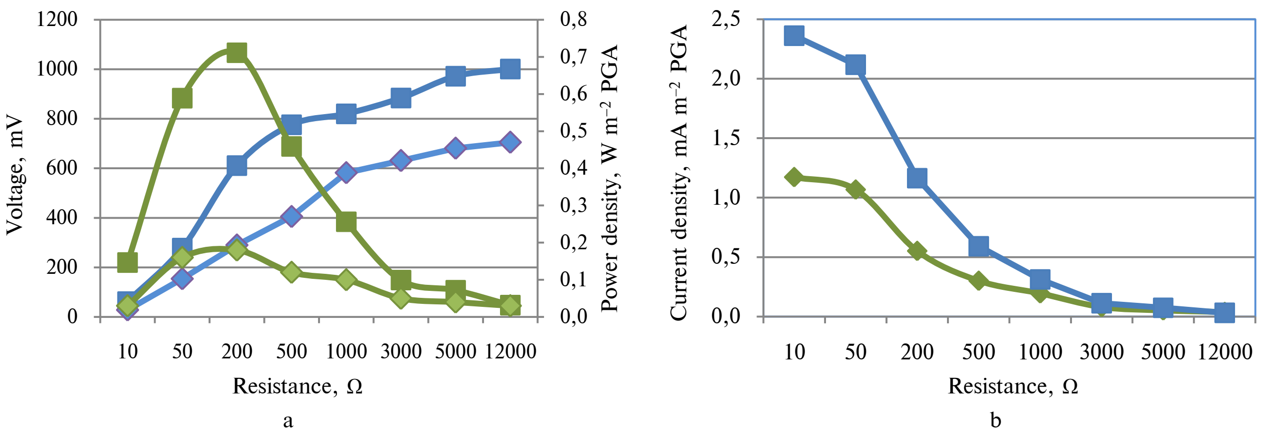

Figure 2: Bioelectrical parameters of multi-electrode (10K/10A) (1) and two-electrode (1K/1A) (2) biosystem, (a): U1 (—■—) and U2 (—◆—) – voltage of biosystems (1) and (2); P1 (—■—) and P2 (—◆—) – power density of biosystems (1) and (2); (b): J1 (—■—) and J2 (—◆—) – current density of biosystems

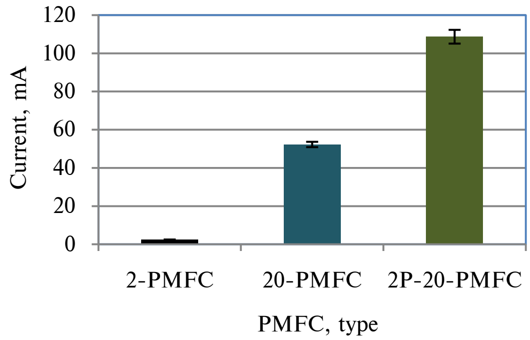

Figure 3: Average current of two-electrode (2-PMFC), twenty-electrode (20-PMFC) and parallel-stacked two multi-electrode (2P-20-PMFC) biosystems at 10 Ω

Discussion

The choice of plant for electrobiotechnology is an important factor that determines how efficiently it will work. When using the same electrode system, but with different plants, the performance of PMFC differed significantly [26, 27, 36], the difference in bioelectricity generation reached up to 10 times, as described in experiments [25]. The plant can be considered an engine of bioelectricity generation, since photosynthesis and excretion of photosynthetants and secondary metabolites, as well as the root system itself, which can be hydrolyzed to nutritious compounds, represent a significant source of power for electroactive microorganisms. As it knows, each plant species has its own genetically determined rhizodiposition, including chemoattractants, on which the development of electricity-generating microorganisms depends. Rhizodeposition can be correlated with the level of development of the root system and of above-ground biomass. In many cases, plants of highly efficient PMFC possessed a denser developed root system and correspondingly higher level carbon excretion of roots and release of oxygen compared to less electricity productive biosystems [27, 30].

PMFCs based on *P. commune* with a rhizoid system or the annual plant *O. basilicum* with a less developed ground biomass and a small taproot system were characterized by the least efficiency in bioelectricity generation in comparison with biosystems based on other plants with developed densely root systems. The biosystems with *C. hirta* and *F. arundinaceae* were characterized by the highest electricity generation, probably, due to the developed rhizome like and fibrous root systems with additional roots and their high biomass of their photosynthetic surface inherent in these plants species (see Table 1). Obviously, the development of leaf and stems and roots are not the only factors affecting the efficiency of biosystems, but they play an important role. Pamintuan and Apollon have achieved significantly higher energy power values, including using a new plant component in biosystems. PMFCs based on well-known low-growing plants, such as succulents *Opuntia* or water lettuce *Pistia stratiotes* or plants with poorly developed root systems such as water spinach *Ipomea aquatica* [17, 19], were characterized by lower power, 6.07 and 3.54–6.35 mW m^{-2}, respectively, than PMFC based on plants with more developed roots and green biomass such as *Vigna ungiculata ssp. sesquipedalis*, 160.86 mW m^{-2} [38] or *Stevia rebaudiana*, 66–132 mW m^{-2} [29, 39].

Nevertheless the absence of a clear correlation in a number of studies regarding the type of photosynthetis path and its effect on electrical productivity [13], the type of metabolic pathway of CO_2 fixation is definitely an important characteristic of plants that plays an important role in electrogenesis. The increase of both biomass production and CO_2 fixation with light intensity and CO_2 concentration in C4 is faster than that in C3, which reflected more efficient use of light and CO_2 in C4 plant. C4 plants approximately have 50% higher photosynthesis efficiency than those of C3 plants [32, 40, 41]. Apparently, the C4 pathway of *C. hirta* sedge is another advantage of PMFC based on it, which contributes to its effectiveness.

Since PMFCs are hybrid systems using both biological and technical components, another lever for efficiency improving can be the configuration of electrode systems. The use of multi-electrodes as part of one module of the biosystem allows obtaining significantly higher values of power density and current density in the range of electrical resistance from 50 to 500 Ω. As were shown parallel stacking of biomodules leads to obtain current output more than 100 mA. That is why the developed biotechnological systems can be recommended as a foundation for the development of power supply for WiFi microcontrollers that consume 100 mA or for charging batteries, in particular, Ni-MH, with a current of 70 mA.

Conclusions

Sedge *Carex hirta* were appeared as the most suitable plants for biological component of biosystem of bioelectricity generation. PMFC based on *C. hirta* plant, which had the largest accumulated total dry leaf and stem mass, and also dry root mass and the developed rhizome-like and fibrous root system were characterized by the highest efficiency compared to other biosystems. The maximum power density was 970 ± 22 mW m^{-2} PGA and exceeded the one of biosystems based on other plants in 9.3–37.9%. The type and level of development of the root system and plant photosynthetic surface serve as an important prognostic factors of the PMFC performance. A 10-fold increase of the electrode surface of one biomodule results in

3.95 times increase of power density at 200 Ω. The use parallel connected multielectrodeity biomodule of the biosystem allows obtaining significantly increase power density and current density in the range of electrical resistance from 50 to 500 Ω.

Interests disclosure

The authors have no conflicts of interest to declare.

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ВПЛИВ МОРФОЛОГІЧНИХ ПАРАМЕТРІВ РОСЛИН НА ЕФЕКТИВНІСТЬ РОСЛИННО-МІКРОБНОГО ПАЛИВНОГО ЕЛЕМЕНТА

Проблематика. Рослинно-мікробний паливний елемент (PMFC) є інноваційною біотехнологією екологічно безпечного отримання біоелектрики. Масштабне застосування біоелектричних систем (або біосистем) гальмується їх недостатньою ефективністю, зумовленою лімітованістю знань про взаємозв'язки між генерацією біолектрики і особливостями їх біотехнологічних компонентів.
Мета. Проаналізувати роль морфологічних параметрів рослин і структурних особливостей біомодулів на генерацію біоелектрики.
Методи реалізації. Щоб оцінити взаємозв'язок між накопиченою масою листків та коріння, а також багатоелектродною структурою біосистеми і генерацією біоелектрики, було застосовано біометричні, біогравіметричні та вольтамперометричні методи і методи статистичного аналізу.
Результати. PMFC на основі осоки *C. hirta*, яка мала найбільшу накопичену тотально суху листяно-стеблеву і кореневу масу та розвинуту мичкувату кореневу систему, характеризувалися найвищою ефективністю порівняно з іншими біосистемами. Густина потужності становила 970 ± 22 мВт·м$^{-2}$ площі, вкритої рослинами та електродами. Паралельне з'єднання двох біомодулів забезпечує отримання сили струму 108,7 мА. Відповідно, розроблені біотехнологічні системи можна рекомендувати як основу для розробки джерел енергоживлення WiFi-мікроконтролерів, що споживають 100 мА, або для заряду акумуляторів.
Висновки. Осока *C. hirta* виявилася найбільш відповідною рослиною як біологічна складова біосистеми генерації біоелектрики. Густина потужності PMFC на основі *C. hirta* перевищувала таку ж на основі інших рослин на 9,3–37,9 %. Тип і ступінь розвитку кореневої системи та наземної фотосинтетичної поверхні рослин є важливими прогностичними факторами ефективності функціонування PMFC. Збільшення поверхні електродів одного біомодуля в 10 разів призводить до збільшення потужності за 200 Ом в 3,95 разу. Багатоелектродність біомодуля виступає ще одним важелем підвищення ефективності біосистем, що дає змгу отримувати істотно вищі значення потужності та сили струму в діапазоні електричного опору від 50 до 500 Ом.

Ключові слова: судинні рослини; ефективність біосистем; рослинно-мікробний паливний елемент; біоелектрика; біоелектричні системи.