

Influence of humic acids on voltage generation in plant bioelectrochemical system

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The possibility of increasing the electrogenic properties of the root environment through the use of potential electron carriers — humic acids (HA) was investigated. In the experiment with lettuce (*Lactuca sativa* L.) variety Typhoon, it was determined that increasing the concentration of HA in the root environment by 2 times resulted in increase the voltage by 7–16% from the control variant, depending on the place of their addition. The best result — more stable and higher generation of potential difference already from the early periods of the plant incubation, was observed in the variant with addition of HA to the area of the upper electrode - the average voltage value was 418 pm 29 mV and the specific power was 0.2 mW/m². A number of physicochemical parameters of near-electrode areas in plant bioelectrochemical systems have been studied: electrical conductivity, pH, HA concentration at the end of the plant incubation. The potential electroactivity of microorganisms in the root environment of lettuce was revealed. It has been shown that the ability of HA to play the role of a redox mediator in a bioelectrochemical system largely depended on the location of their concentration.

Keywords: plant-microbial fuel cell, redox-mediator, root environment, electrode region.

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Introduction

Alternative renewable natural resources, such as the sun, wind, water, and biological objects, are considered promising energy sources that contribute to sustainable development and allow for environmentally friendly electricity, reduce greenhouse gas emissions into the atmosphere and reduce the effects of climate change. Such energy resources include bioelectrochemical systems (BES) — devices for obtaining electrical energy through chemical reactions associated with the metabolic activity of living organisms. BES include microbial fuel cells (MFC) [1], biophotovoltaic systems or photoMFC [2] and plant-microbial fuel cells (PMFC) [3].

The principle of operation of plant BES is based on electrogenic processes occurring in the root environment — redox reactions and ion diffusion accompanying the development of the root system, and the oxidation of rhizodeposits by microorganisms with the formation of carbon dioxide, protons and electrons [4]. The efficiency of the BES depends on a combination of a number of factors, including both the electroactivity of plants and microorganisms, and the impact of external parameters such as temperature, humidity, composition and structure of the root environment, characteristics of the light environment associated with the intensity of photosynthesis [5].

One of the most significant reasons for the low performance of the BES is the high resistance of the root environment (in the range of hundreds of kΩ [6]), that

acts as an equivalent of the electrolyte in electrochemical devices. Possible solutions for this problem include:

- 1) selection of electrode systems with a high specific surface area (carbon materials are used as such — graphite felt, fabric, granules, rod, paper [7]);
- 2) change of the distance between the electrodes [8];
- 3) introduction of a chemical mediator — a redox mediator that increases the efficiency of electron transport [9].

Redox mediators have already been shown to be effective in enhancing electron transfer from microorganisms to the anode — their use, according to various studies, increased voltage by 1.2–10 times and specific power by 1.2–38 times compared to BES variants without mediators [10,11]. Neutral red, anthraquinone-2,6-disulfonate, thionine, p-benzoquinone, 2-hydroxy-1,4-naphthoquinone, 2,6-dichlorophenol indophenol, potassium ferricyanide and viologene dye are described as intermediaries in electron transport [1]. However, the introduction of external mediators, some of which are toxic compounds, can affect the appearance and nutritional value of plants, and also increases the cost of BES [12].

Organic substances of soils and soil substrates used for plant cultivation have the ability to both receive and give away electrons [13–17], therefore they can also serve as mediators in the transfer of electrons to electrodes in PMFC. Humic acids (HA) possess such properties due to the presence in them of groups exhibiting donor-acceptor electronic properties of the quinone type [18,19]. Quinoid compounds are capable of accepting electrons, are responsible for the formation of reactive oxygen species

and can be reduced to semi-quinones, which are stabilized by aromatic rings and further reduced to more stable hydroquinones [20]. It has been shown that HA served as a non-toxic electron carrier between anaerobic bacteria and Fe (III) or the electrode [21]. It has been shown that the presence of HA could lead to an increase of specific power in MFC by 67.4% and Coulomb efficiency by 92.6% [22]. When added to an MFC with an aerated cathode with a HA concentration of 5 g/l, the power density was 77 mW/m² [21], and the addition of 2 g/l into a two-chamber MFC resulted in the generation of 52 mW/m² [23].

However, the possibility of HA usage as electron mediators in soil BES during plant cultivation remains not fully understood at the moment, which emphasizes the need for a comprehensive assessment of the HA effectiveness as intermediaries in electron transfer and studying their applicability for energy production using PMFC.

The purpose of this work was to identify the impact of HA on the formation of potential differences in BES based on the electrogenic properties of the root environment in the cultivation of agriculturally significant plant.

1. Experimental BES and the study subject

The lettuce (*Lactuca sativa* L.) variety Typhoon was selected as a phytotest object that had a well-developed tap-root system with numerous branches. The plants were cultivated under controlled conditions of intensive light culture in a vegetative irradiation plant device with HPS-400 lamps used as light sources. The irradiation was 70–75 W/m² in the area of photosynthetically active radiation, the light period was 14 hours per day, air temperature was +20–22°C during the day and +18–20°C at night, relative humidity was 65–70%. Peat soil (Agrobalt C, LLC „Pindstrup“, Moscow region, Russia) based on high peat of a low degree of decomposition containing at least: N — 150 mg/l, P₂O₅ — 150 mg/l, K₂O — 250 mg/l, Mg — 30 mg/l, Ca — 120 mg/l was used as a root environment. Humidity of the substrate was at the level of 60–70% of the total moisture content and the amount of micro- and macronutrients necessary to obtain high-quality plant products was maintained by adding a Knop's solution [24].

The developed BES was a container for growing plants with a volume of 90 × 70 × 70 mm. The electrical characteristics were measured by placing electrodes with a size of 60 × 60 mm in a root environment of bioelectrochemical cells, which provided surface electrical contact with the root system and the root zone. The lower electrode was located at a distance of 30 mm from the bottom of the container and was made of graphite felt with a thickness of 5 mm. The upper electrode made of stainless steel mesh with a cell size of 8 × 8 mm was placed at a distance of 30 mm from the lower electrode and was electronegative with respect to it. The electrodes were positioned in such a way that the upper electrode was in contact with the root neck, and the

lower electrode was located at a distance measured from the root neck of plants and corresponded to the experimentally determined maximum potential difference. The electrodes were made of a porous biocompatible corrosion-resistant material to allow the root system to grow through them and thereby ensure surface electrical contact. The potential difference variations in the BES were monitored using Arduino hardware platform; values were recorded every 15 min during the plants cultivation time.

pH and electrical conductivity in the near-electrode regions were measured using the ST20 pH meter (OHAUS, China) and the COM80 electrical conductivity meter (HM Digital, Russia) in aqueous extracts by diluting 5 g of the root environment selected from the electrodes in 100 ml of distilled water.

2. Determination of the content of HA in the root-inhabited environment

A standard method for the extraction of HA was used for determining the content of HA in the peat substrate used for plant cultivation [25]. The content of carbon, hydroxyl and carboxyl groups in the isolated HA was determined by conventional methods [26].

The HA content in the substrate used after the experiment was estimated using the fast determination method using spectrophotometric analysis [27]. The calibration curve was plotted in Excel 2010 based on the optical density of a series of humic acid solutions with a known concentration (0–0.5 g/l) (Fig. 1). The HA content in the samples was determined based on the calibration curve data and the values obtained were recalculated per gram of dry soil.

It was determined that 1 g of the dried initial used peat substrate contained 67 ± 3 mg of HA. The concentration of carboxylic, hydroxyl and phenolic groups is listed in Table 1. A comparison of these quantitative characteristics with the literature data showed that the peat soil used for growing plants contained the least amount of carbon and carboxyl groups compared with humic acids obtained from coal,

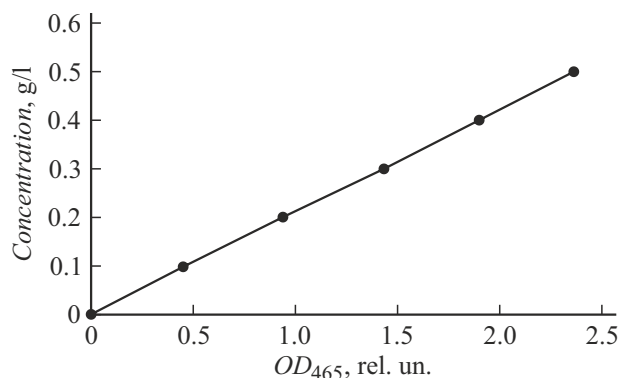


Figure 1. Calibration curve for determining the HA content in the substrate, where the absorbance OD at a wavelength of 465 nm is the average value of two measurements.

Table 1. Content of carboxyl and phenolic hydroxyl groups in HA of different origin

Source of HA	Groups		Concentration of carbon, (wt%)
	Carboxylic, mmole(s)/g of dry HA	phenolic hydroxyl, mmole(s)/g of dry HA	
Coal [28,30]	3.00 ± 0.15	4.60 ± 0.23	61.37 ± 3.07
Mountain peat [28,30]	2.80 ± 0.14	5.40 ± 0.27	55.09 ± 2.75
Black soil [29,30]	5.70 ± 0.28	5.90 ± 0.30	54.28 ± 2.71
Peat soil*	2.70 ± 0.13	8.20 ± 0.41	46.65 ± 2.33

Note. * — this study.

mountain peat and chernozem. However, the number of phenolic hydroxyl groups was maximal. This could indicate an increased ability of these HA to serve as carriers of electrons and protons.

3. Studied variants for BES with additional introduction of HA

The following variants were studied for determining the role of HA in the formation of potential differences in the root environment (Fig. 2):

- 1) BES-K — control containing only the initial substrate in the form of peat without plants;
- 2) BES-R — based on lettuce plants;
- 3) BES-HA — the total number of introduced HA was 3 g with an increased content of HA in the volume of the root environment by 2 times;
- 4) BES-HAu — with a twofold increase of the HA content in 0.5 volume area of the upper electrode;
- 5) BES-HAl — with a twofold increase of the HA content in 0.5 volume area of the lower electrode.

The HA of Roth (Germany) was introduced in the respective variants of the substrate. Vegetation experiments were carried out for 30 days with duplicate studied variants. Two lettuce plants were placed in each cell of the BES. Statistical data were processed using the Excel 2010 program, determining the average values of the studied parameters and confidence intervals. Statistical significance

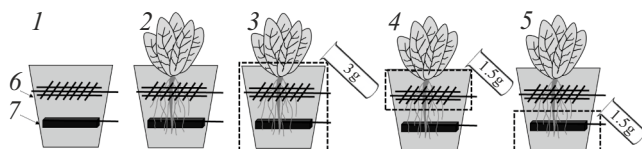


Figure 2. Studied BES variants: 1 — BES-K, a control containing only the initial peat substrate without plants, 2 — BES-R based on a peat substrate and two lettuce plants, 3 — BES-HA with a twofold increase of the HA content, 4 — BES-HAu with a twofold increase of the HA content in the 0.5-volume area of the upper electrode, 5 — BES-HAl with a twofold increase of the HA content in the 0.5-volume area of the lower electrode, 6 — top electrode, 7 — bottom electrode.

of difference between the variants was evaluated by parametric statistical methods (Student's *t*-test criterion). The differences between the variants were considered significant at $p \leq 0.05$.

4. Impact of HA on the generation of potential differences in BES

The following results were obtained during the study of the effect of HA on the formation of potential differences in the root environment (Fig. 3): the average voltage value was 360 ± 19 mV for the control variant BES-K without plant, 405 ± 2 mV for BES-R in case of growing lettuce plants, 395 ± 14 mV for BES-HA with additional HA, 418 ± 29 mV for BES-HAu with added HA in the upper electrode area, 387 ± 4 mV for BES-HAl with added HA in the lower electrode area.

The dynamics of the potential difference formation for the studied variants during the experiment was also similar: stationary generation in the first three days, smooth growth on the fourth–fifth day and stabilization on the tenth–fifteenth day. A smooth increase of voltage was observed during the first 5 days from 300 to 385 mV for the control BES-K without plants followed by slight drop to 320 mV by the thirteenth day and stabilization at ~ 380 mV. The presence of a potential difference in a BES that did not contain a plant organism was apparently associated with the occurrence of many other redox reactions in the substrate — from ion diffusion during irrigation to the electroactivity of microflora. BES-R was characterized by an increase of the voltage in the cell throughout the cultivation time from 300 to 530 mV, probably because of an increase in the intensity of processes in the root environment accompanying the development of plants. A smooth increase from 230 to 510 mV in the root environment and in BES-R, from 330 to 450 mV for BES-HAu, from 200 to 510 mV for BES-HAl was observed in case of a twofold increase of the HA concentration.

The maximum value of the potential difference of 530 mV on the twenty-eighth day of the experiment was typical for BES-R, which did not contain additionally added HA. At the same time, a fairly large variance of values of 60 mV was noted for this variant during the growing

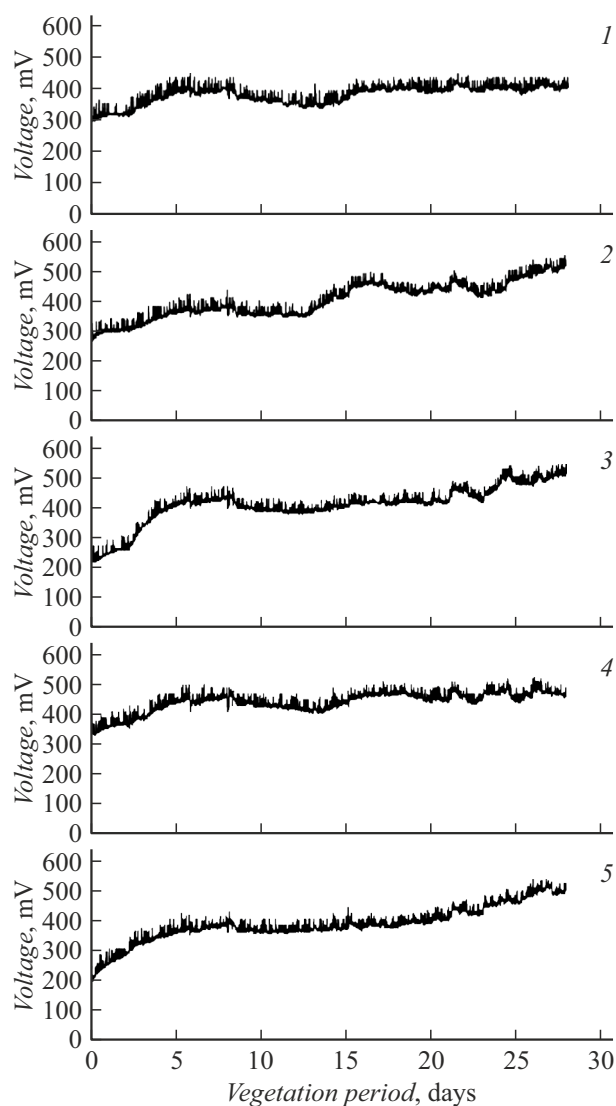


Figure 3. Dynamics of the potential difference in the root environment in BES of various compositions: 1 — BES-K control without plants, 2 — BES-R with Typhoon lettuce, 3 — BES-HA with HA introduced in the substrate, 4 — BES-HAu with added HA in the upper electrode region, 5 — BES-HAl with added HA in the lower electrode region.

season, whereas the BEP variance did not exceed 30 mV for the BES-HAu variant with the addition of HA to the upper electrode region, which was associated with a more stable generation throughout the experiment and probably with a more uniform ion diffusion.

The best result which was a more stable generation of high voltage already from the early periods of vegetation was characteristic of the BES-HAu variant with an additional introduction of HA to the upper electrode area. It could be assumed that HA transport from top to bottom in the growing container played a positive role in increasing the electrical characteristics of the BES by creating conditions for a more intensive ion distribution.

5. Physico-chemical characteristics of near-electrode regions

The electrical conductivity and HA content in the near-electrode zones were measured for determining the features of the processes occurring in the area of the upper and lower electrodes in the BES, at the end of the experiment (Table 2).

It was found upon completion of the experiment that according to the results of the estimation of the HA content in the upper and lower layers of the substrate in experimental BES the upper layer of the control version of BES-K contained approximately the same amount of HA as the original peat. At the same time, the concentration of HA in the lower layer decreased to 12 mg per 1 g of the dried substrate. A similar situation was observed for all variants of plant BES with additionally introduced HA — the HA content in the upper electrode area was greater than in the lower electrode area. It was especially interesting to note this trend for the BES-HAl variant, where HA was added only to the lower electrode area. This was probably attributable to the irrigation method by supplying water from below, since it was previously determined that this contributes to the stable generation of a potential difference due to uniform diffusion of liquid due to capillary effects [6]. Apparently, along with an increase of concentration due to the death of plant cells, HA diffused upwards the soil profile with water.

The pH values correlated with the HA content — the correlation coefficient was 0.7 for the upper electrode and 0.9 for the lower electrode. A high concentration of HA resulted in an increase of the hydrogen index due to the alkalization of the substrate.

There is also a relation with electrical conductivity in the near-electrode regions — the greater difference in the HA content between the layers, the greater difference in electrical conductivity, the correlation coefficient between these values was 0.7. At the same time, the concentration of charged ions, i.e. the intensity of metabolic processes, is higher in the area of the upper electrode. The greatest differences in HA concentrations between the upper and lower near-electrode regions were characteristic of the BES-HA and BES-HAu variants — 52 and 68 mg per gram of peat, respectively. A large difference in electrical conductivity was observed for the same cells— 127 $\mu\text{S}/\text{cm}$ for BES-HAl and 139 $\mu\text{S}/\text{cm}$ for BES-HA. It is important to note that the potential difference for these variants also had a maximum increase — the parameters increased by 166 mV for both variants since the beginning of the experiment, whereas the change was 57 mV for the BES-HAu variant, i.e. probably the voltage value in the BES was associated with the movement of HA in the substrate and the difference in their concentrations on the electrodes.

Table 2. pH, electrical conductivity and HA content at the end of the cultivation time in the areas of the upper and lower electrodes in BES-K without plants, BES-R with lettuce plants, with additionally introduced HA — BES-HA in the entire cell, BES-HAu in the area of the upper electrode, BES-HAl in the area the lower electrode

Variant of BES	pH in the upper near-electrode regions	pH in the lower near-electrode regions	Electrical conductivity in the upper near-electrode region, $\mu\text{Sm/cm}$	Electrical conductivity in the lower near-electrode region, $\mu\text{Sm/cm}$	HA concentration in the upper near-electrode region, mg/g of dry substrate	HA concentration in the lower near-electrode region, mg/g of dry substrate
BES-K	6.35 ± 0.11	6.54 ± 0.21	228 ± 14	142 ± 11	53 ± 11	12 ± 4
BES-R	6.30 ± 0.11	6.61 ± 0.11	202 ± 14	109 ± 6	81 ± 9	61 ± 13
BES-HA	7.31 ± 0.10	6.87 ± 0.17	265 ± 10	126 ± 8	138 ± 21	86 ± 19
BES-HAu	6.93 ± 0.08	7.31 ± 0.10	185 ± 11	103 ± 14	195 ± 17	152 ± 18
BES-HAl	6.95 ± 0.10	7.01 ± 0.10	257 ± 21	130 ± 13	216 ± 14	148 ± 23

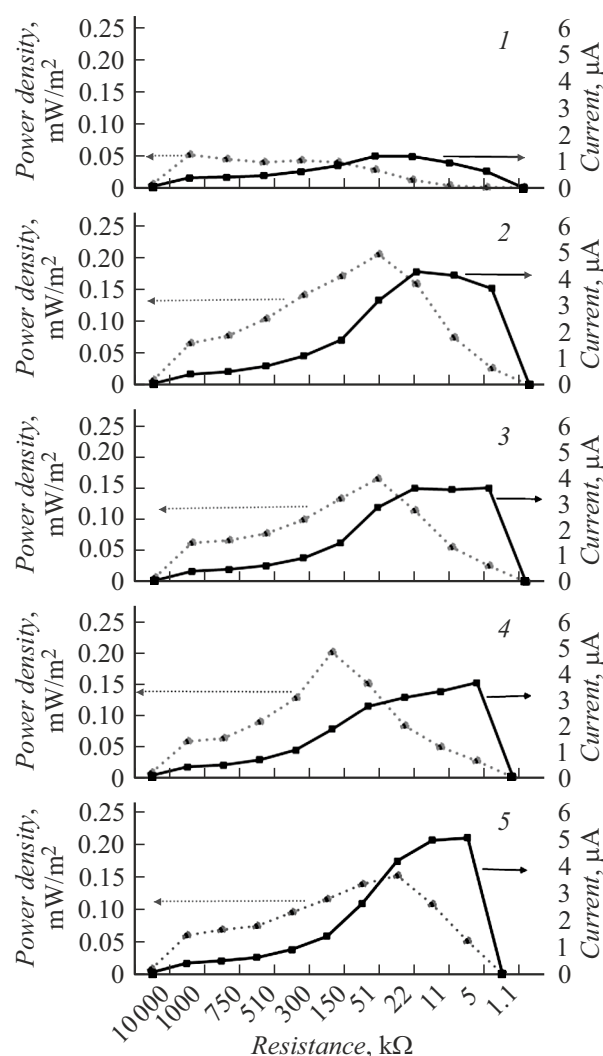


Figure 4. Dependence of BES power on the connected load: 1 — BES-K, 2 — BES-R, 3 — BES-HA, 4 — BES-HAu, 5 — BES-HAl.

6. BES load capacity

Voltage was measured with parallel connection of resistances of various nominal values from $10\text{ M}\Omega$ to $1.1\text{ k}\Omega$ for calculating the electrical capacities and load capacities of the studied BES (Fig. 4). The highest obtained specific power of 0.2 mW/m^2 was typical for the BES-R and BES-HAu variants, and the highest current value of $5\text{ }\mu\text{A}$ was obtained in the BES-HAl cell.

7. Morphophysiological characteristics of plants grown in BES

The average weight of the lettuce aerial part (2 plants per cell) in the BES was $71 \pm 9\text{ g}$ for BES-R, $53 \pm 7\text{ g}$ for BES-HA, $77 \pm 13\text{ g}$ for BES-HAu, $82 \pm 12\text{ g}$ for BES-HAl. It can be seen that the doubling of the HA amount in the entire area of the root environment resulted in some inhibition of plants, which may indicate an overabundance of them in the peat substrate and, as a result, a stressful effect.

Conclusion

As a result of the experiments conducted, it was found that HA were able to participate as possible redox mediators in electric current generation processes in PMFC — a twofold increase of the concentration of HA in the root environment allowed increasing the voltage generation by 7–16% from the control one, depending on the place of their introduction.

The highest initial potential difference was characteristic of the BES-HAu variant, which was naturally related to the fact that the largest amount of HA was concentrated in the upper layer at the time of the beginning of the experiment, accordingly, the electrical conductivity was higher there. Such a system allowed stable electricity generation, but

had a weak potential to increase the potential difference in the course of its further development. At the same time, the starting point in case of addition of HA to the bottom of the system was minimal compared to all other options. Over time, water-soluble substances, including HA, rose up together with the water flow in case of irrigation from below, and the potential difference in the system increased. The HA introduced into the lower layer of the substrate gave the maximum increase of the value of the potential difference among all the studied variants by the time of the end of vegetation.

Thus, HA could act as potential mediators of the transfer of electrons and protons from electroactive microorganisms to electrodes, and could also contribute to the concentration effects and the movement of ions in the root environment in PMFC.

Conflict of interest

The authors declare that they have no conflict of interest.

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