

## Phytotechnological purification of water and bio energy utilization of plant biomass

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**Abstract.** The aim of the study was to explore the possibility of using the phytomass of aquatic plants as the substrate in the microbial fuel cells and selection of microorganisms suitable for the generation of electricity on this substrate. The conversion of chemical energy of phytomass of aquatic plants to the electrical energy was carried out in a microbial fuel cells by biochemical transformation. As biological agents in the generation of electricity in the microbial fuel cells was used commercial microbial drugs "Doctor Robic 109K" and "Vostok-EM-1". The results of evaluation of the characteristics of electrogenic (amperage, voltage) and the dynamics of the growth of microorganisms in the microbial fuel cells presents in the experimental part. As a source of electrogenic microorganisms is possible to use drugs "Dr. Robic 109K" and "Vostok-EM-1" was established. The possibility of utilization of excess phytomass of aquatic plants, formed during the implementation of phytotechnological purification of water, in microbial fuel cells, was demonstrated. The principal possibility of creating hybrid phytotechnology (plant-microbe cells), allowing to obtain electricity as a product, which can be used to ensure the operation of the pump equipment and the creation of a full cycle of resource-saving technologies for water treatment, was reviewed.

### 1. Introduction

Recently, for the protection, treatment and post-treatment of water, and the ecological reconstruction of water bodies, the phytotechnology have been used more frequently. The basis of phytotechnologies is the use aquatic vegetation and its attendant microorganisms. Today the similar research is being conducted in more than 80 countries mainly in North America, Europe, South East Asia [1, 2, 3]. The most active development in recent decades, this trend was in China. It is used for cleaning agricultural and irrigation wastewater [4]. In Russia, research on the development of phytotechnological purification of water is conducted at leading universities for more than 50 years. In particular, we studied the mechanisms and patterns of accumulation and biodegradation of wastewater components of different industries [5, 6]. With regard to the conditions of Siberia design of phytotechnological facilities, technological regulations operation of such facilities, and the options for complex purification schemes was developed [7].

Organic compounds in systems of phytotechnologies are decomposed in aerobic and anaerobic conditions. Oxygen comes to the aerobic zone from the atmosphere as a result of convective-diffusive



processes, and through the macrophyte roots. Anaerobic conditions are created in the soil pores. Typical substrates for vegetation are sand, gravel, and the production waste (sawdust, rice and buckwheat husk, ash, coal slag, dead seston, compost).

Also phytotechnology is used in detoxification and removal of the second largest group of pollutants - heavy metals. Plants which involved in the decontamination of heavy metals and radionuclides, must meet certain requirements: 1) to grow rapidly; 2) have a high tolerance to metals; 3) be resistant to diseases and pesticides; 4) have a well-developed root system and shoots; 5) have the ability to synthesize the particular substance in response to toxic external environment; 6) be unattractive to animals in order to avoid the transfer of pollutants to higher trophic levels; 7) not be specific to certain elements, so that there exists the possibility of extracting and other metal detoxification [7].

One of the problems in the operation of phytotechnologies of water purification is the removal and subsequent disposal of excess biomass of aquatic plants. Bearing in mind that plant phytomass can be used as an energy source, in this paper the possibility of its utilization in the biofuel cell was investigated. Complex biotechnological approach will not only clean the waste water, but also to convert chemical energy into electrical energy [8, 9, 10]. Currently, the most significant obstacle to the use of fuel cells is a limited number of strains of microorganisms capable of operating in conditions of microbial fuel cells (MFC). However, there are many commercial microbiological preparations for wastewater treatment, removal of various contaminants. They include a wide range of different microorganisms, utilizing many of the components of sewage [11].

In this regard, the tasks of this article included:

- Assessment of the possibility of using waste phytomass of aquatic plants as a substrate in microbial fuel cells;
- Selection of microorganisms suitable for the generation of electricity on this substrate.

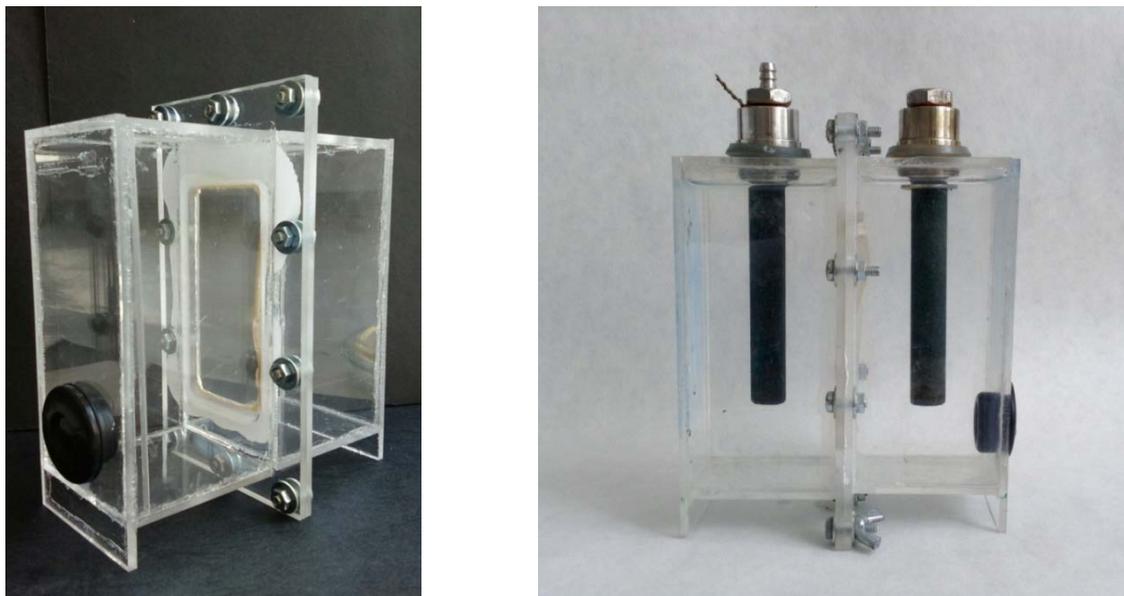
## 2. Objects and methods of research

We used phytomass higher aquatic plant *Elodea canadensis* Michx. (1803), recommended as the main component of phytocenoses for the extraction of metals from waste water and disposal of organic components of sewage in a sharply continental climate of Eastern Siberia.

We collected plants by trawl in Angara River and the Lake Baikal. Hydrophytes grown in the laboratory with moderate light and temperature 13 °C. Experiments were carried out in vessels of 5 liters in which the plants were placed at 10 g/l. Dishes filled with solutions studied metals (copper, iron) in a concentration of 1-50 mg/l. Plants exposed in thermoluminostate with illumination 2 thousand Lux and a temperature of 25 °C. After certain intervals samples were taken and analyzed the residual content of metals, the cleaning efficiency was determined.

After exposure for 10 days, the plant was used as the organic substrate for microorganisms in MFC. *Elodea* was used as a crude homogenate and dry material. Phytomass, culture media and model waste water was autoclaved at 1 atmosphere for 30 min.

We used the model sample MFC made of Plexiglas, which consists of two identical chambers of 370 ml separated by a proton exchange membrane Nafion (USA) [12, 13]. Medium filling the anode and cathode space is model waste water (GOST 50595-93). Electrodes for MFC made of electrically silicone carbide ("Podolskogneupor", Russia) (Figure 1).



**Figure 1.** The microbial fuel cell.

Tests were carried out according to the procedure described in detail in [9]. The measurements of amperage were carried out in the short-circuit mode.

In different series of experiments were added to the anode analyzed chamber substrate – an *Elodea canadensis* homogenate or in dry form. The cathode compartment was filled with a model wastewater.

As biological agents at generating electricity in the MFC was used the commercial microbial preparations "Doctor Robic 109K" and "Vostok-EM-1". Doctor Robic "109K" produced for cleaning of septic tanks and cesspools. It consists of: wheat bran 60%, baking soda 37-38%, 2-3% of non-pathogenic microorganisms containing  $3 \times 10^{10}$  CFU/g: *Bacillus amyloliquefaciens*, *B. pumilis*, *B. licheniformis*, *B. subtilus*. Microbiological preparation "Vostok-EM-1» is used for seed treatment and improve soil quality. It contains lactic acid, photosynthetic bacteria, yeast, fungi (*Lactobacillus plantarum*, *L. casei*, *Rhodopseudomonas palustris*, *Saccharomyces cerevisiae*), and the waste products of microorganisms. Preparations previously centrifuged to separate the microbial biomass, which is introduced into the anode chamber in an amount of MFC  $\sim 10^6$  CFU/ml.

It is known that a number of strains of *Clostridium* effectively utilize cellulose. In this regard, we evaluated the possibility of intensifying the transformation of Elodea in addition clostridia to biological agents in MFC. We used *Clostridium acetobutylicum* strain VKPM-B-1787. The microorganism is introduced into the anode chamber MFC with Elodea and microbial preparations in an amount  $\sim 10^5$  CFU/m.

Incubation of microorganisms in the anode chamber of MFC was carried under anaerobic conditions. Cathode chamber was aerated. We used Microsoft Excel software package for statistical analysis of the data. The reliability of the results of the differences was determined by Student's t test. Conclusions are made with probability of faultless prognosis  $R \geq 0.95$

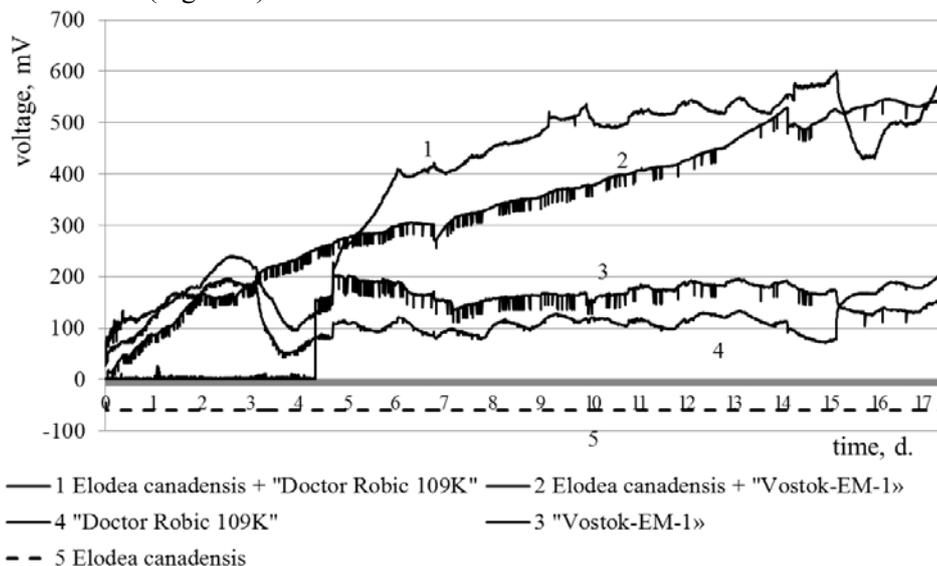
### 3. Results and discussion

Table 1 shows the absorption coefficients of biological metals in different groups of plants. Studies have shown that plants belonging to various ecological groups have different storage capacity in relation to heavy metals. Thus, the maximum amount of metal was accumulated in the tissues of the immersed plants. In this connection, preference should be given to the plants submerged primarily in *Elodea canadensis* (Table 1).

**Table 1.** Coefficients of biological accumulation in plants at pH = 7.2.

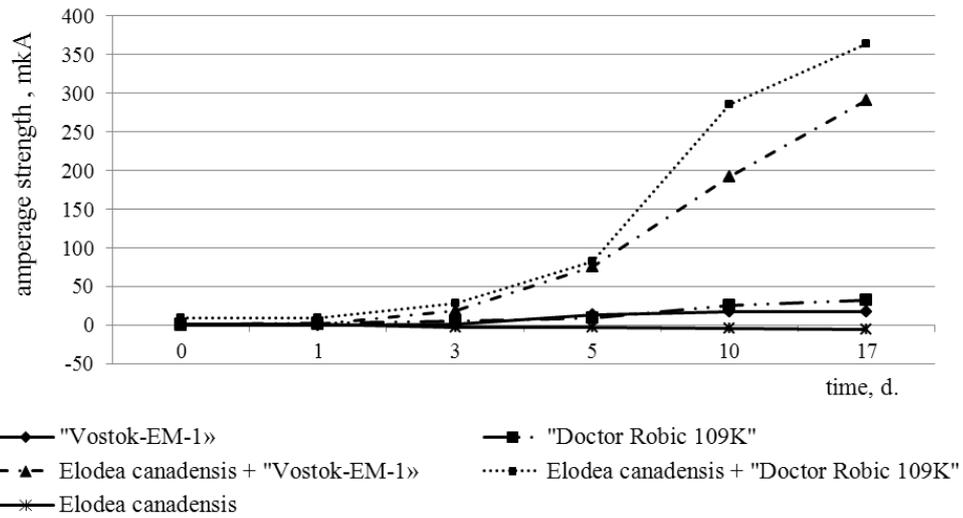
Plant	Bioaccumulation factor						
	Fe	Al	Ni	Cu	Cd	Zn	Pb
<i>Acorus calamus</i>	1.1	8.5	2849.0	1.9	9.0	38.0	10.2
<i>Typha angustifolia</i>	1.3	8.5	22.7	9.5	949.0	649.0	409.0
<i>Comarum palustre</i>	0.9	28.8	35.5	4479.0	46.5	9.2	3.5
<i>Potamogeton natans L.</i>	0.6	8.7	5.8	2239.0	18.0	3.1	40.0
<i>Nuphar luteum (L.)</i>	1.1	7.8	34.6	3732.0	236.0	1.0	135.6
<i>Potamogeton lucens L.</i>	3.3	27.8	22.7	21.4	18.0	2.4	40.0
<i>Potamogeton perfoliatus L.</i>	1.1	24.8	311.5	447.0	94.0	974.0	101.5
<i>Potamogeton htctinatus L.</i>	1.2	775.0	39.7	2132.0	13.6	194.0	19.5
<i>Myriophyllum spicatum L.</i>	1.0	484.0	14.0	1947.0	46.5	974.0	18.5
<i>Ceratophyllum demersum L.</i>	1.7	4.5	406.1	173.1	474.0	71.2	44.5
<i>Elodea canadensis L.</i>	2.0	8.0	474.0	51.7	157.0	47.7	24.6
<i>Chara vulgaris</i>	1.7	27.9	63.7	741.0	89.0	56.2	10.9
<i>Chara hispida</i>	1.8	78.2	71.3	235.0	134.0	91.1	130.0

Experiments have shown that in MFC generated EMF increases with time when adding a phytomass of Elodea to microbiological preparations (Figure 2). Both drugs had electrogenic activity for disposal Elodea biomass. Thus, after 6 days of exposure in the case of drug Doctor Robic voltage was 407 mV, and an EM-preparation – 304 mV. Prolonged incubation (for 17 days) increased the voltage to 550 - 600 mV (Figure 2).



**Figure 2.** The dynamics voltage in MFC with Elodea when used "Dr. Robic 109K" and "Vostok-EM-1" as electrogenic drugs.

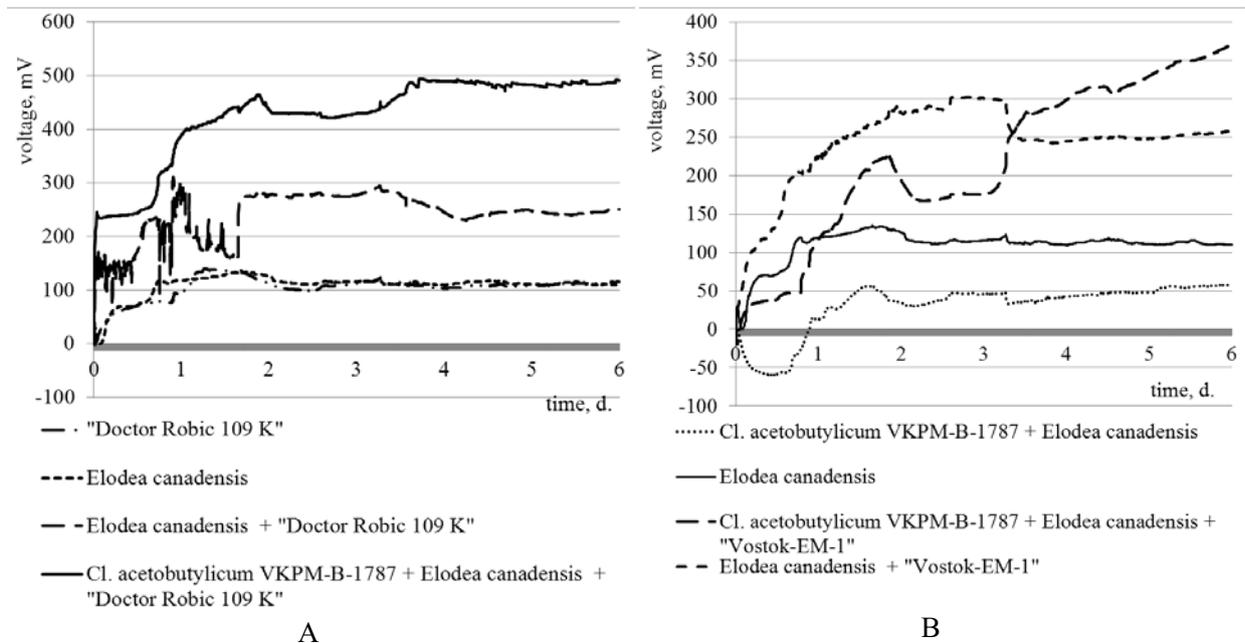
A similar trend was observed in the measurement of the amperage. A gradual increase of this index occurred in the MFC, which containing both microbial drugs and Elodea. On day 17 of the experiment amperage, generated by the drug "Doctor Robic 109K" was 364 mcA, drug "Vostok-EM-1" - 291 mcA. In control cells containing microbial preparations without elodea, amperage does not rise higher than 32 microamps. In the version of the MFC with Elodea, but without the addition of drugs, amperage is not fixed throughout the experiment (Figure 3).



**Figure 3.** The dynamics of the amperage strength in the MFC with *Elodea* when used as electrogenic drugs "Doctor Robic 109K" and "Vostok-EM-1».

To intensify the process of disposal of plant mass by preparations in MFC additionally introduced the culture of *Cl. acetobutylicum* strain VKPM-B-1787.

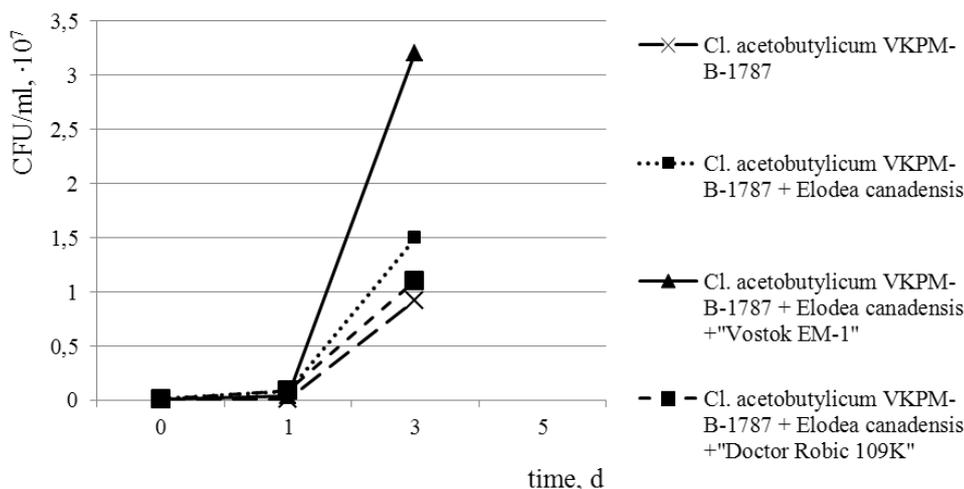
Within 7 days of incubation the greatest indicators of the voltage (~500 mV) were shown in the cells containing the drug "Doctor Robic 109 K", *Elodea* and culture of *Cl. acetobutylicum*. During the same time, the drug "Doctor Robic 109 K" in MFC with no added *Clostridium* was generated only 300 mV (Figure 4A.). When used as a electrogenic instead of the drug "Doctor Robic 109 K" microbial associations "Vostok-EM-1" watched voltage increase to 300 – 370 mV (Figure 4B).



**Figure 4.** Dynamics of stress in the MFC with *Elodea* and *Clostridium acetobutylicum* strain VKPM-B-1787 for use as electrogenic drug "Doctor Robic 109K" (A), "Vostok-EM-1" (B).

Parallel analysis of electrogenic characteristics was evaluated the dynamics of the growth of the *Cl. acetobutylicum* strain VKPM-B-1787 and microorganisms of preparations "Doctor Robic 109K" and "Vostok-EM-1".

The most active growth of *Clostridium* cells was observed in cells with Elodea and preparation "Vostok-EM-1". In this case, at 3 days of incubation the number of cells increased from  $7.9 \cdot 10^5$  to  $3.2 \cdot 10^8$  CFU/ml. The MFC containing Elodea and *Cl. acetobutylicum* without the addition of microbial agents, the number of viable *Clostridium* cells after 3 days of cultivation was slightly below the  $1.5 \cdot 10^8$  CFU/ml. In a medium containing *Clostridium* without Elodea culture titer in the specified time interval was  $9.2 \cdot 10^7$  CFU/ml. That is, it is actually no different from the same period in the cell with the drug "Doctor Robic 109K", Elodea and *Clostridium* ( $1,1 \cdot 10^8$  CFU/ml) (Figure 5).



**Figure 5.** Dynamics of cell culture growth of *Clostridium acetobutylicum* strain VKPM-B-1787 in MFC.

Similar relationships were observed in experiments with the drug "Vostok-EM-1". Over the 5 days of incubation a number of microorganisms was increased from  $2.1 \cdot 10^6$  to  $8,5 \cdot 10^9$  CFU/ml.

#### 4. Conclusion

Thus, the possibility of disposal of excess biomass of *Elodea* in MFC which generated during the implementation of phytotechnology of water purification was experimentally demonstrated. It was established that as a source of electrogenic microorganisms can be used drugs "Doctor Robic 109K" and "Vostok-EM-1". At the same time the drug "Dr. Robic 109K" has generated somewhat higher emf values than the "Vostok-EM-1".

It should be mentioned that electric indicators of MFC in which biological agents were microbiological drugs "Doctor Robic 109K" and "Vostok-EM-1", were comparable to data given by other specialists were used as biocatalysts other microorganisms [14, 15].

Consequently, there is in principal possibility of creating hybrid phytotechnology (plant-microbe cells), allowing to obtain electricity as a product, which can be used to ensure the operation of the pump equipment and the creation of a full cycle of resource-saving technologies for water treatment.

The authors are grateful to V Ya Severina for providing the drug "Vostok-EM-1", V A Bybin and N S Stepanova for participation in research. The work was supported by the RF Ministry of Education as part of the design of the public tasks in the field of scientific activity (Task № 13.1263.2014/K from 07.11.2014), RFBR and Government of Republic of Buryatia according to the research project № 16-48-030887.

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