Plant Microbial Fuel Cells

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Contributor: Roman Lepikash, Daria Lavrova, Devard Stom, Valery Meshalkin, Olga Ponamoreva, Sergey Alferov

PubMed (NCBI) has pointed to an exponential growth of publications on the subject of a “biofuel cell” in the first decade of our century, and this interest persisted throughout the following years. It should be noted that biofuel elements based on microorganisms (microbial fuel cells, MFCs) are a promising technology to produce bioelectricity since they simultaneously solve the problems of contamination with anthropogenic organic waste, which can be used by microorganisms as a source of carbon and energy.

Keywords: plant microbial fuel cell ; electrogenic microorganisms ; biofuel cells

1. Introduction

The ubiquitous environmental pollution due to various anthropogenic substances, such as heavy metals [1], petroleum products [2], medicinal preparations [3], and pesticides [4], is one of the main problems of mankind nowadays. Pollutants have a negative impact not only on the environment but also on human life, accumulating in heterotrophic food chains and entering the human body, which leads to various diseases of the nervous system and respiratory organs, as well as genetic abnormalities while reducing life expectancy [1]. The above-described problems are reflected in the UN Sustainable Development Goals; according to the developed programs of the United Nations Environment Programme (UNEP) and UN-Water, the control over the global pollution of ecosystems and their restoration are of high priority [5].

Bioremediation, a complex of purification methods using the metabolic potential of biological objects, is applied to purify soil and water ecosystems from pollutants. Thus, the introduction of microorganisms into ecosystems makes it possible to dispose of various pollutants by converting them to simpler safe substances. The principle of phytoremediation is based on the binding and accumulation of pollutants in plant vacuoles [6]; it activates a complex metabolic pathway involving the antioxidant plant protection system [7]. Additionally, plants and microorganisms (fungi and bacteria) interact with each other at the root level (in the rhizosphere), showing a positive synergistic effect in the elimination of pollutants such as heavy metals and organic compounds [8][9]. The disadvantages of the bioremediation of soils include the low rate of toxicant biodegradation, as well as the need for a thorough preliminary examination of the contaminated site to clarify the modes of biotechnological work. This requires high labor and energy costs, such as the plowing and irrigation of fields and the disposal of waste plants. Therefore, this technology is not widely used in developing countries and is unattractive in poor countries [10].

A significant contribution to atmospheric pollution is made by heat and power stations (hereinafter referred to as CHP) operating on traditional fuel sources (coal, oil, and gas); their share (**Figure 1**) is about 60% of the global electricity generation [11]. The issue of using renewable energy sources (RES) for electricity generation is relevant, considering the trend programs of many developed countries towards the reduction of carbon dioxide emissions into the atmosphere and providing access to inexpensive, reliable, sustainable, and modern energy for all segments of the population [12]. These include solar panels, wind generators, and biofuels [13].

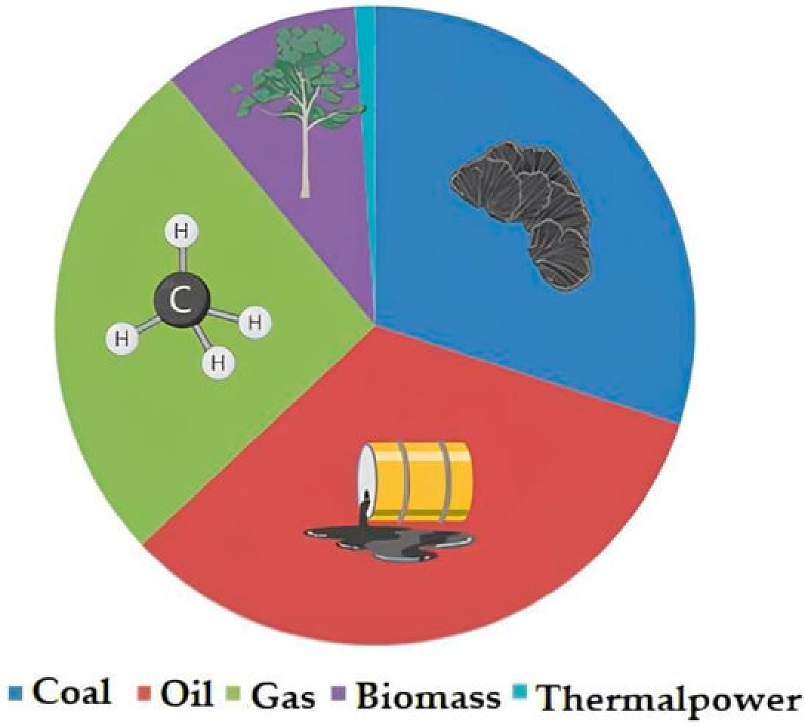

Figure 1. Global electricity generation by type of fossil fuel (according [14]).

However, renewable energy sources have a number of disadvantages. Thus, the process of their disposal is an extremely difficult task [15] since, for example, solar panels contain elements such as As, Cd, Hg, and Pb [16], which can have a negative impact on the ecosystem, and their burial is an extremely undesirable method of their disposal [17]; thermal and chemical methods of solar panel recycling have not been sufficiently mastered and are not characterized by a high degree of efficiency [18][19]. Dust is released during the mechanical processing of solar panels. It contains glass fiber, noise pollution is created, and rare earth elements are lost. However, 80 million tons of waste from used solar panels are expected worldwide by 2050, which will inevitably have a negative impact on the surrounding ecosystem.

The use of biofuel cells (BFCs) is an effective alternative in this context, as electricity generation is carried out in the process of the biocatalytic oxidation of various substrates. Despite the low power generated in the BFC system and, as a result, a long payback period, the research in the field of biofuel elements is relevant due to humanity's awareness of global environmental problems, the need to solve which reduces the role of economic levers in the development of the world community. PubMed (NCBI) has pointed to an exponential growth of publications on the subject of a "biofuel cell" in the first decade of our century, and this interest persisted throughout the following years. It should be noted that biofuel elements based on microorganisms (microbial fuel cells, MFCs) are a promising technology to produce bioelectricity since they simultaneously solve the problems of contamination with anthropogenic organic waste, which can be used by microorganisms as a source of carbon and energy. A continuous and steady supply of organic substrates is required to ensure the uninterrupted generation of electricity in an MFC, which cannot always be implemented in practice. A fairly new technology of plant microbial fuel cells (hereinafter referred to as PMFCs) eliminates this disadvantage of MFCs largely. The electricity generation is carried out via the oxidation of organic substances using microorganisms that are both synthesized in plants during photosynthesis under the action of sunlight energy and produced into the environment (root exudates, root deposits, and rhizo-deposition) and come from outside, for example, from wastewater or industrial waste. Such hybrid energy technology can be used in phytomonitoring the state of plant crops, a local power supply, charging portable devices [20], powering various low-power sensors to monitor ambient temperatures and humidity, power camera traps in remote areas [21], and serve as a biosensor for monitoring plant health in smart greenhouses [22] (**Figure 2**). It should be noted that the PMFC technology, using macrophytes, reduces the level of greenhouse gases (N_2O and CH_4) by 5.9–32.4% in terms of CO_2 [23].

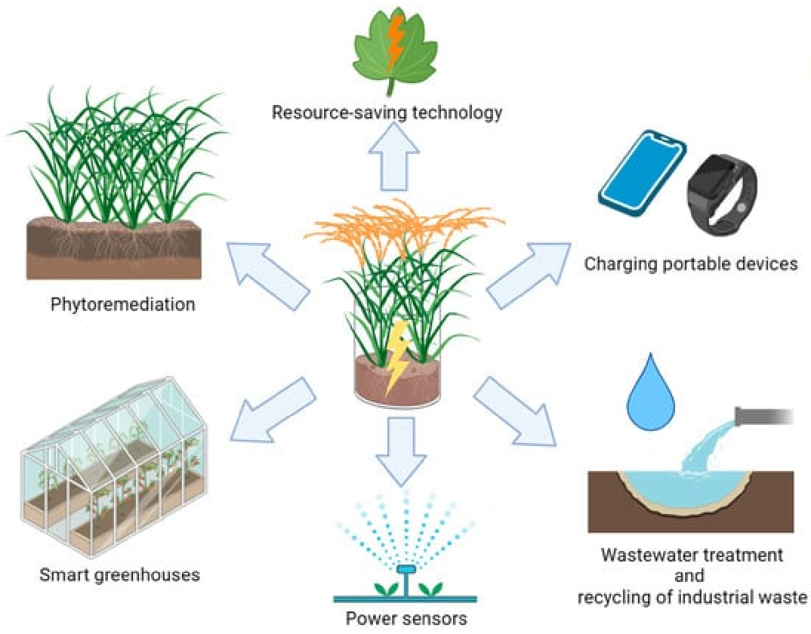

Figure 2. Possibilities of PMFC application.

2. Plant Microbial Fuel Cells: Functioning and Factors Affecting the Electrochemical Characteristics of the SYSTEM

The generation of electricity depends on many factors, such as the types of exoelectrogenic microorganisms used, the material of the electrodes and their modification, environmental factors, and the plants used. Understanding the functioning principles and the optimal choice of microorganisms and plants makes it possible to increase the efficiency of electricity generation in a PMFC.

2.1. The Principle of PMFC Operation

The principle of PMFC operation is based on two interrelated processes: the synthesis of rhizo-deposits in plants and their use as a substrate by microorganisms to generate electricity (**Figure 3**). Complex interactions in heterogeneous, polydisperse, multifactorial natural systems were previously described as a computer model of the chemical and microbiological production processes of plant biomass, soil microorganisms, and nutrients in the rhizosphere [24].

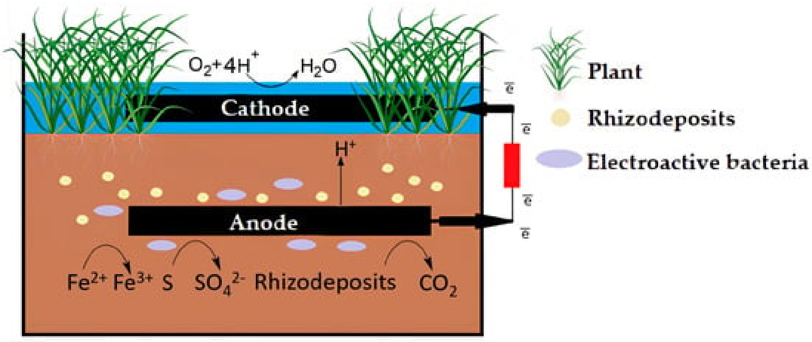

Figure 3. The scheme of PMFC functioning.

Photosynthesis regulates the vital activity of plants, during which plants fix carbon dioxide from the atmosphere and form carbohydrates, organic acids and amino acids, secrets—polysaccharide mucus (mucigel), lysates—materials of dead cells, gases—ethylene ethylene and carbon dioxide under the influence of sunlight energy [25]. Electrogenic microorganisms use deposits as substrates for growth and development, as well as electricity generation as a result of ongoing oxidative processes involving the enzymatic systems of microorganisms. As a result, carbon dioxide is synthesized, and free charge carriers (protons and electrons) are formed. Charges need to be separated to convert chemical energy into electrical energy. The process is carried out by moving the generated electrons at the anode to the cathode through an external circuit; protons migrate through a nutrient matrix or medium from the substrate to the cathode due to the presence of a potential gradient [18], where molecular oxygen or another catalyst and water molecules are formed [26]. However, it is likely that hydroperoxyl radicals (HO_2) are formed on the cathode during the reduction process as an intermediate product [27]. Microorganisms, in turn, can enter symbiosis with plant roots, forming protective biofilms and producing antibiotics to protect plants from pathogens [28].

When choosing microorganisms, it is necessary to consider their ability to transfer electrons to the anode (**Figure 4**), which can be caused by various mechanisms: direct electron transfer through cytochromes and electron-conducting molecular saws (nanowires) with the help of electroactive compounds (mediator transfer). General information about this various mechanisms is summarized in recent reviews and articles [29][30][31][32][33][34][35].

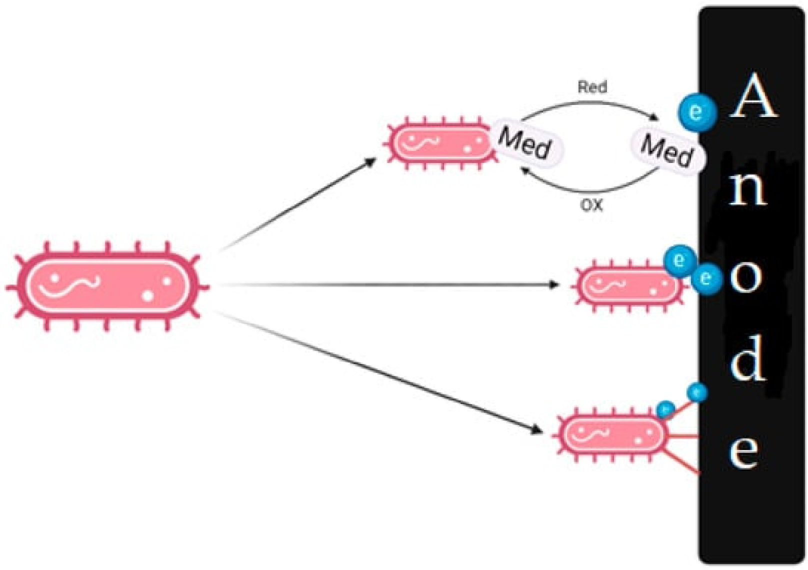

Figure 4. Various ways in which electron transfer to the anode can occur.

The focus is on natural ecosystems when choosing microorganisms for a PMFC system. Relatedly, bacteria inhabit the environment in the rhizosphere; they are anaerobes that produce protons and carbon dioxide and can transfer electrons to the anode during the oxidation of organic compounds. **Table 1** presents a description of some rhizospheric bacteria.

Table 1. Rhizospheric microorganisms capable of direct extracellular electron transfer.

Microorganism	Description	Consumable Substrates	References
***Desulfobulbus* sp.**	**Obligate anaerobes capable of oxidizing sulfur to sulfate using an anode as an electron acceptor.**	**Acetate, propionate, butyrate, lactate, and pyruvate**	[36][37][38]
***Geobacter* sp.**	**Anaerobic metal-reducing bacteria. Fe (III) and Mn (IV) are used as electron acceptors. They can transmit electrons using pili—filamentous protein formations.**	**Benzoate, p-cresol, trichloroethane, benzene, lactate, acetate, and starch**	[39]
Geothrix fermentans	**Anaerobic metal reducers. Fe (III) is used as an electron acceptor. They are capable of forming extracellular mediators of the quinone series and riboflavin, which makes it possible to transfer electrons to the electrode more efficiently.**	**Acetate, propionate, lactate, and fumarate**	[40][41]
Rhodoferax ferrireducens	**Facultative metal-reducing anaerobe with a wide temperature range of growth. Fe (III), Mn (IV), nitrate, fumarate, and oxygen can be used as electron acceptors.**	**Acetate, lactate, propionate, pyruvate, malate, succinate, and benzoate**	[42]
***Shewanella* sp.**	**Facultative anaerobic bacteria using Fe (III) and Mp (IV) as electron acceptors are capable of producing flavins that act as electronic transfer mediators.**	**Lactate and formate**	[43][44]
Clostridium butyricum C. beijerinckii	**Obligate anaerobes can use an anode as an electron acceptor. Hydrogen, which is able to oxidize at the anode, is produced during the enzymatic fermentation of substrates.**	**Glucose, starch, sucrose, and lactate**	[45]

The basic property of microorganisms that allows their use in bioelectric systems [46][47][48] is their ability to produce electroactive compounds, as well as to use an anode as an electron acceptor. Moreover, the use of inorganic anions as an electron acceptor makes it possible to reduce the salinity of treated wastewater [49][50], for example, when using sulfate-reducing bacteria that are capable of the assimilatory reduction of sulfates to sulfides [51].

PGPR (plant-growth-promoting rhizobacteria), which promote plant growth, play an important role in maintaining the vital activity of plants and are used for the development of PMFC. Such microorganisms include, for example, bacteria of the species *Bacillus thuringiensis*, which are involved in nitrogen fixation processes, sulfur and phosphorus exchanges, and the synthesis of plant growth stimulants [52]. Bacteria of the genus *Pseudomonas* sp. can be also considered as a PGPR-group bacteria [53]. Some species of *Pseudomonas* sp. are capable of surfactant destruction [54][55]; they can form biofilms on the surface of an anode and secrete compounds of the phenase-new series [56]. These compounds play an important role both in protecting plants from pathogen infection [57] and stimulating the growth of shoots [58]. Moreover, phenazines act as mediators of the electronic transport between bacteria and an electrode [59]. Bacteria of the family *Ruminococcaceae* spp. are not electroactive but are capable of utilizing cellulose (35–50% of the dry plant weight) while producing organic substrates, which are additionally used by electroactive microorganisms as electron donors [60]. Therefore, the use of PGPR-group bacteria can be used in PMFC systems to stimulate plant growth and protection, which theoretically can have a beneficial effect on electricity generation.

2.2. Electrodes in PMFC

It is important to choose the right electrode material for the efficient generation of electrical energy when creating PMFCs along with biological components [61]. Generally, the electrode material should have high electrical conductivity, electrochemical stability, porosity, and biocompatibility [62]. Metals (zinc [63], stainless steel [64], and platinum [65]) and carbon materials [66] are usually used as electrodes in bioelectrochemical systems. Despite the high electrical conductivity of metals in comparison with carbon materials, the use of stainless steel, for example, increases the period of microorganism adaptation on the metal anode surface [66]. It causes a decrease in current generation at the initial stage of the PMFC operation, which is explained by the lower biocompatibility of stainless steel to microorganisms. Moreover, metals are subject to corrosion processes [64] and have a high cost, thus limiting their use in PMFC development.

The geometric area of the electrodes affects the output of electricity—the larger the area, the more contact there is for electroactive microorganisms, which leads to an increase in current density [67]. In turn, graphite electrodes (felt/fiber) have a developed surface that promotes the adhesion of microorganisms and the sorption of organic compounds. This material is not subject to corrosion; therefore, it is promising for the creation of PMFCs [68]. The addition of granular graphite or activated carbon to the surface of the anode improves the adsorption of organic compounds and increases the specific surface area for colonization via bacteria. Electrode modification is used to improve the producible power of bioelectrochemical systems, which is described in detail in recent articles [69][70][71][72][73]. The use of carbon materials produced from crop waste is also promising in this field [74].

Thus, the choice of electrode material is the key element determining the efficiency of the entire PMFC system. Existing materials can be modified to reduce their internal resistance in order to increase the current output and power.

2.3. Application of Proton Exchange Membranes in PMFC System

Various PMFC configurations have been developed so far: sediment PMFCs, constructed-wetland MFCs, tubular PMFCs, floating-treatment wetland MFCs, flat plate PMFCs, and power-generating trees. The advantages and disadvantages of each model are detailed in the review [75]. One of the components of bioelectrochemical systems for power generation is a proton exchange membrane, which allows the improvement of charge segregation and power performance [76]. The most preferred proton exchange membrane is Nafion, but its use in BES significantly (by 40%) increases the cost of the device [77]. Thus, the search for new membranes that will have a lower cost and provide high stability and efficiency in BES is currently underway.

In [78], modified Nafion 117 proton exchange membranes were tested. The modification included the treatment of the membrane with solutions of polyvinylidene difluoride (PVDF) and sulfonated PVDF with the addition of silicon oxide (SiO_2). The third modification involved the polymerization of a Nafion membrane in a methyl methacrylate (MMA) solution with the addition of sodium sulfite as an initiator. According to the results obtained, all three methods increase the power generation parameters of MFC systems. The highest increase in current density, from 0.81 mA/m^2 to 18.82 mA/m^2, was demonstrated using the modification of Nafion with MMA.

In [79], a proton exchange membrane based on agar and polyvinyl alcohol (PVA) with the addition of vermiculite nanoparticles was tested. According to the results obtained, the proton exchange properties of the tested membranes were 216% higher than those of the commercial Nafion membrane. In addition, the MFC current density increased (from 605 mA/m^2 to 1515 mA/m^2) when agar and PVA-based membranes were used. A low cost and environmental safety, in combination with the increased efficiency of MFC energy generation, allow the use of agar and PVA-based membranes as an alternative to expensive Nafion membranes.

Ceramic membranes based on clay, bentonite, coal ash, Na_2CO_3, Na_2SiO_3, and H_3BO_3 were considered in [80]. The use of hybrid ceramic membranes with the addition of different compounds contributed to the increase in PMFC power density by 78% (up to 22.38 mW/m^2) compared to the control (100% clay membrane). There was a decrease in internal resistance from 346 Ω (control) to 234 Ω. The addition of bentonite, coal ash, Na_2CO_3, Na_2SiO_3, and H_3BO_3 improved the membrane's cation transport, reducing oxygen diffusion to the anode chamber. The membrane demonstrated high stability during 6 months of PMFC operation. In addition, the ceramic membrane is significantly cheaper than the Nafion membrane.

Thus, one of the important aspects of PMFC operation, power increase, and internal resistance reduction is the use of proton exchange membranes. At the same time, for the commercialization of PMFC systems, it is necessary to take into account the cost of the production of such membranes and the expenses associated with the complication of the design when using membranes.

2.4. The Influence of Environmental Factors on the Electricity Generation in a PMFC

The metabolic activity of exoelectrogenic microorganisms, which play an important role in BES functioning and electricity generation, depends on the temperature, the pH, and the rate of organic substrates' receipt. Thus, the work [81] showed that, when the air temperature rises to 30 °C, the voltage of the bioelectrochemical system increases from 100 to 150 mV, which may be due to an increase in the metabolic rate of exoelectrogenic microorganisms. The pH value affects the development of microorganisms. pH of 6–9 is mostly suitable for the functioning of BES [82]. The power decreases to 158 mW/m^2 at a pH value of 6.0 for the MFC system [83], while the power value is 600 mW/m^2 at a pH of 8.0. The inhibition of the metabolic activity of exoelectrogenic microorganisms is observed with a decrease in pH, which contributes to a decrease in the BES power [84].

Periodic watering is necessary for the normal functioning of plants since soil moisture affects the generated potential in a PMFC system. The article [85] states that, in the absence of irrigation, the soil dries up, which leads to a two-fold decrease in the PMFC potential, but after watering (60–70% of the soil moisture capacity), the potential is restored. Thus, energy generation changes depending on the time of day [86]. An increase in electrogenic activity is observed after sunrise due to the launch of photosynthesis processes, the peak of which is observed from 14 to 15 h. Depending on the system under study, the open circuit potential is 600–700 mV at the specified time. Then, the photosynthetic activity of plants decreases at nightfall, which leads to a decrease in electricity generation to 300–400 mV.

The rate of photosynthesis is affected by the concentration of carbon dioxide in the atmosphere [87]. The trend towards carbon dioxide emissions increases every year and is 390 ppmv, according to the latest data (mass fractions of a percent per volume), which is 30% more than the CO_2 concentration in the early twentieth century [88]. The increasing CO_2 concentration and climate warming significantly affect plant growth [89]. The work [90], using agricultural plants (*Saccharum officinarum* and *Sorghum bicolor*), showed that the rate of photosynthesis grows significantly with an increase in the CO_2 concentration, which in theory can have a positive effect on the power produced via a PMFC. It should be noted that plants with the C_3 and C_4 types of photosynthesis react differently to an increase in the carbon–acid gas concentration. C_4 plants attach CO_2 to phosphoenolpyruvate [85], resulting in the formation of oxalic acid containing four carbon atoms. The photosynthesis efficiency of C_4 plants is significantly higher since the C_4 pathway is an extra pump that supplies additional portions of CO_2, increasing its concentration in the plant since the CO_2 concentration in the assimilation chamber is lower than in the air, which is a limiting factor of photosynthesis.

It should be noted that the countries with warm climates and high solar insolation, as well as "green roofs" cities, have the greatest potential for the PMFC technology's implementation to reduce the concentration of carbon dioxide in the air [91].

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