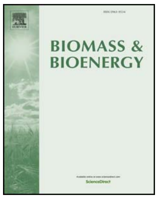
Biomass and Bioenergy 109 (2018) 1–9

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe



Research paper

Effects of salinity, growing media, and photoperiod on bioelectricity production in plant microbial fuel cells with weeping alkaligrass

J. Md Khudzari[a], J. Kurian[a], Y. Gariépy[a], B. Tartakovsky[a,b], G.S.V. Raghavan[a,*]

[a] Department of Bioresource Engineering, Macdonald Campus, McGill University, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, Québec H9X 3V9, Canada
[b] National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Québec H4P 2R2, Canada

ARTICLE INFO

Keywords:
Plant microbial fuel cells
Fults weeping alkaligrass
Bioelectricity
Salinity
Photoperiod

ABSTRACT

This study investigated the potential for bioelectricity production of a salt-tolerant plant, the weeping alkaligrass (*Puccinellia distans*), in a plant microbial fuel cell (MFC). Air-cathode MFCs with a carbon felt anode were assembled in a cylindrical vessel. The MFCs were operated using growing media of different dry organic matter (OM) mass fractions: potting mix (OM: 89%) and sandy loam (OM: 8%), and treated with different NaCl concentrations of 0, 6, and 12 kg m^{-3}. MFC performance was best at a salinity of 6 kg m^{-3}. Over 114 days, the highest power output was obtained from plant MFC (PMFC) in potting mix at 83.7 mW m^{-2} cathode area with an average power of 12.78 mW m^{-2}, followed by PMFC in sandy loam (maximum: 8.59 mW m^{-2}, average: 8.35 mW m^{-2}). The total biomass production of alkaligrass was 5–25% higher in the potting mix, when compared to the sandy loam. The presence of alkaligrass in PMFC increased the bioelectricity production by 14-fold compared to that of soil MFC (SMFC). In addition to the standard photoperiod of 16/8 h (light/dark), the MFCs were also operated under 24/0 h, 9/15 h, and 0/24 h photoperiods. Power outputs of 9/15 and 0/24 h were clearly decreased due to the effect of photoperiod, while the power outputs of 24/0 and 16/8 h were similar with some evidence of light-related inhibition. Frequent changes in the photoperiod test affected bioelectricity production and thus, a longer recovery time is recommended to reduce the adverse impact of the changes.

1. Introduction

Photosynthesis is the process by which plant uses light energy to convert carbon dioxide and water into usable chemical energy for the plant growth. The release of a wide variety of organic compounds such as root exudates, secretion, lysates and gases [1] to the soil through the plant root system, is called plant rhizodeposition. The estimates of plant rhizodeposition products or rhizodeposits differ quite markedly with portion of up to 40% of the plant's photosynthetic productivity depending on plant species, maturity, and its environmental conditions [2,3].

Generating bioelectricity from living plants represents a new and intriguing concept in microbial fuel cell (MFC) known as plant microbial fuel cell (PMFC) [2,4–6]. Fuel source in PMFC comes from rhizodeposits and organic compounds in the soil. Bacteria in the rhizosphere will break down the organic compounds and release electrons. By providing a terminal electron acceptor (anode) in the rhizosphere, it is possible to harvest these electrons and use them to generate electricity. Some of the chemical and electrochemical reactions involved in this process are described elsewhere [7].

Since 2008, different plants have been investigated to produce bioelectricity in PMFCs [8]. Since the MFC rhizosphere is submerged in water, not all plants are suitable for PMFC operation. Also, because the MFC operation requires an electrolyte medium, the suitable kind of plant species would be aquatic, semi-aquatic, or plants that can tolerate standing water and flooded areas.

So far, the most popular choice of plant in PMFC studies is rice paddy (*Oryza sativa)* due to its role as a major staple food source, and the impact of its cultivation to the global methane emissions. Other plant species selected for PMFC operation include *Glyceria maxima, Spartina anglica, Arundinella anomala, Arundo donax, Pennisetum setaceum, Canna indica, Ipomoea aquatic, Lemna minuta,* and *Typha latifolia* [6,9–11]. The bacterial species such as *Desulfobulbus-like species* [12], *Natronocella acetinitrilica, Beijerinckiaceae, Rhizobiales* are examples of microbial communities that were found at the anode of rice PMFC and were considered responsible for producing the electricity [13]. In the study using *Canna indica, Geobacter* spp. (7.4% of the microbial population) was found in the PMFC, and its population was found as only 1.9% in the soil MFC (SMFC) [6]. In another study, the genus *Pseudomonas* was predominant (93.8%) among the bacteria isolated from the

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[*] Corresponding author.
E-mail address: vijaya.raghavan@mcgill.ca (G.S.V. Raghavan).

https://doi.org/10.1016/j.biombioe.2017.12.013
Received 12 May 2017; Received in revised form 15 December 2017; Accepted 18 December 2017

rhizosphere of weeping alkaligrass growing in a salt deposit area (total salt 10 kg m^{-3}) where the sodium concentration was 0.05% (~0.5 kg m^{-3} of NaCl) [14].

Another important key for electricity production in PMFC is the ionic conductivity. Strong electrolytes such as NaCl can enhance the electron mobility in the medium used. Increased salinity results in lower resistance that facilitates proton transfer to the cathode, hence improves power production [15]. The effects of the addition of salt in a PMFC was described in a study with *Spartina anglica*, where the average power density over a 13 weeks period was 10 mW m^{-2} (per membrane surface area) [16].

Nonetheless, salt tolerance varies from plant to plant. Most aquatic or semi-aquatic plants are sensitive to saline conditions. For example, rice grain yield is negatively affected when the electrical conductivity (EC) of the paddy field water exceeds 1.9 mS cm^{-1} [17]. Salt-tolerant plants have lower transportation rates of Na^{+} and Cl^{-} from roots to the leaves. They can also compartmentalize the ions in vacuoles to foil their build-up in cytoplasm or cell walls and avoid salt-related toxicity [18]. In this sense, salt-tolerant plants might have a greater potential for PMFCs than the salt-sensitive plants. Therefore, our objective was to test a plant species that grows in waterlogged yet saline condition for PMFC operation.

One such salt-tolerant plant is the weeping alkaligrass *(Puccinellia distans)*, a perennial bunchgrass species from Eurasia. Among other salt marsh grasses, alkaligrass is desirable for saline roadsides where NaCl is applied excessively for deicing in North America [19,20] and exists in the roadside where 30 kg m^{-3} of soluble salt was observed [21]. It is also able to grow in shallow water tables [22], as well as in heavy soils with poor drainage [23].

The objectives of this study were as follows: (i) to analyze the electricity production from alkaligrass PMFC in saline and non-saline conditions in growing media with different organic matter (OM) content, (ii) to examine the effect of photoperiod on the electricity production from PMFCs, and (iii) to study the changes in plant biomass production, pH, and EC in these PMFCs. The performance of the PMFCs was then compared to SMFC. This study reports on the use of weeping alkaligrass in PMFC. These results would be useful for the development of projects on the production of bioelectricity with salt-tolerant plants in saline conditions.

2. Materials and methods

2.1. Experimental design

A full factorial design with 2 types of MFCs (PMFC and SMFC), 3 salinity levels, and 2 types of growing media were investigated (Table 1). This study combined the effect of different levels of salinity and OM content on the power production from alkaligrass plants.

The salinity levels used were within the tolerance range for alkaligrass. Two growing media were used to assess the effects of organic OM content. They were a commercially available potting mix and a natural sandy loam soil. High OM contents are likely to improve plant growth, enriched microbial communities [24], and thus increase power production [25,26].

Table 1
Experimental design for plant- and soil MFCs, with 3 salinity levels and 2 soil types.

MFC	NaCl concentration (kg m^{-3})	Potting mix (PM)	Sandy loam (SL)
Plant MFC	0	PM0-PMFC	SL0-PMFC
	6	PM6-PMFC	SL6-PMFC
	12	PM12-PMFC	SL12-PMFC
Soil MFC	0	PM0-SMFC	SL0-SMFC
	6	PM6-SMFC	SL6-SMFC
	12	PM12-SMFC	SL12-SMFC

Notations: PMFC = plant microbial fuel cells, SMFC = soil microbial fuel cells, PM = potting mix, SL = sandy loam, numbers 0, 6, 12 = NaCl concentration prepared in a modified Hoagland solution. All the treatments were done in duplicate.

2.2. Cultivation of alkaligrass

The Fults weeping alkaligrass seeds were obtained from Basin City, located in the lower Columbia Basin of Washington, USA. The seeds were purchased from the Evergreen Seed Supply company (Deshler, Ohio, USA). Germination was done in trays filled with potting mix and regular tap water was used for watering. Each PMFC received 20 alkaligrass plants.

2.3. MFC growing media

Two different types of growing media were used to fill the MFCs. The first type was the potting mix (G10 AGRO MIX®, Fafard et Frères Ltd., Québec, Canada) contained a mass fraction of 89% dry OM by mass fraction. According to the manufacturer, the potting mix included limestone, balanced nutrients, micronutrients, gypsum, and a wetting agent. The second type was a natural sandy loam soil, contained about 5% dry OM by mass fraction. The sandy loam came from the Macdonald Research Farm, McGill University (45°28′ N, 73°45′ W), Sainte-Anne-de-Bellevue, Québec, Canada. This soil was a mixed and frigid Typic Endoquent, classified as a Chicot series with 67% sand, 25.5% silt, and 7.5% clay, respectively.

MFC with potting mix medium was filled with 310 g of the dry potting mix, whereas the MFC with sandy loam soil was filled with a mixed volumetric ratio of 7 parts of sandy loam (1360 g) and 3 parts of potting mix (103 g). Adding the potting mix to the sandy loam soil produced a medium with a total dry OM of 8% by mass fraction.

2.4. Design and construction of MFC

Sections of PVC pipe were used to construct single-chamber MFCs (Fig. 1a). A rectangular opening i.e. electrode window (17 cm × 3 cm) was made on the wall of the cylinder to receive an air-cathode (Type E4B, Electric Fuel Ltd., Bet Shemesh, Israel). The oversize air-cathode (21 cm × 5 cm) was glued to the outside perimeter of the opening. The air-cathode had an active layer of manganese-based catalyzed carbon bonded to a current collecting screen made of nickel mesh. This air-cathode was also had a blocking layer and a separator layer. The carbon felt anode (SGL Canada, Kitchener, Ontario, Canada) of same dimensions was fixed to the inside of the electrode window. Titanium wires (0.5 mm thick, $< 5\,\Omega\,m^{-1}$) (Grade 1, Solution Materials, Santa Clara, California, USA) were used as a current collector to connect electrodes to an external resistor.

It should also be noted that these electrodes were installed at 7 cm from the bottom of the MFCs to assure that they were operated under anaerobic conditions (Fig. 1b). The placement of electrodes is important because an operation under aerobic conditions can result in the occurrences of voltage reversal and low electricity production. Additional details on MFC design and voltage reversal are available in the Supplementary information.

2.5. MFC operation

All experiments were conducted in the Research Greenhouse at Macdonald Campus, McGill University. The first set of experiments was carried out for a period of 114 days (August to December) under the standard photoperiod of 16-h-light/8-h-dark. The minimum temperature was 21 ± 2 °C during the day and 14 ± 2 °C at night, controlled by Priva Maximizer (Priva North America, Ontario, Canada).

To ensure waterlogged conditions, MFCs were irrigated with tap water three times per day. The MFCs were treated twice a week with