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Conversion of orange peel waste biomass to bioelectricity using a mediator-less microbial fuel cell

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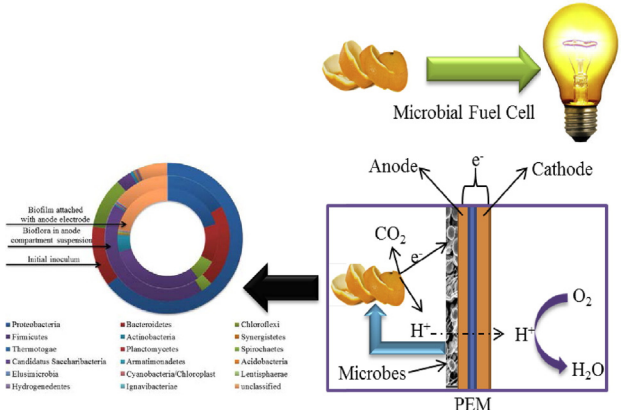
HIGHLIGHTS

- Orange peel waste (OPW) was explored for bioelectricity generation in MFCs.
- A maximum stable voltage of 0.59 ± 0.02 V was generated using OPW.
- Microbial communities were analyzed by high throughput 16S rRNA pyrosequencing.
- *Enterococcus*, *Paludibacter*, and *Pseudomonas* were dominant genera in anode biofilm.
- Exoelectrogen and fermentative bacteria played significant role in MFC performance.

GRAPHICAL ABSTRACT



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ABSTRACT

Microorganisms have the potential to become a game-changer in sustainable energy production in the coming generations. Microbial fuel cells (MFCs) as an alternative renewable technology can capture bioenergy (electricity) from carbon-based sources by utilizing microorganisms as biocatalysts. This study demonstrated that MFC technology can be explored for bioelectricity production from orange peel waste (OPW), an agricultural byproduct and an organic substrate, without any chemical pretreatment or the addition of extra mediators. A maximum voltage generation of 0.59 ± 0.02 V (at 500 Ω) was achieved in a dual chamber MFC during stable voltage generation stages. The maximum power density and current density obtained were 358.8 ± 15.6 mW/m^2 and 847 ± 18.4 mA/m^2, respectively. Key components of OPW, namely pectin and cellulose, were also tested in their pure form, with pectin giving a stable current, while no significant current generation was achieved using cellulose alone as the substrate, thus demonstrating the absence of cellulose-degrading bacteria. Maximum pectinase and polygalacturonase enzyme activities of 18.55 U/g and 9.04 U/g (per gram of substrate), respectively were achieved during orange peel degradation in MFCs. Bacterial identification using 16S rRNA analysis of the initial inoculum fed to the MFC, the biofilm attached to the anode, and the anode suspension, showed significant diversity in community composition. A well-known exoelectrogen, *Pseudomonas*, was present among the predominant genera in the anode biofilm.

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1. Introduction

The future of energy sustainability and supply is likely to rely on renewable energy sources. The production of electricity or biofuels using innovative technologies and renewable sources is a global priority in terms of energy strategies (Resch et al., 2008). A microbial fuel cell (MFC) is an emerging renewable technology, which is designed to exploit the degradation of biological substrates for the production of sustainable bioenergy in the presence of active microorganisms (Kan et al., 2011; Walter et al., 2015). Moreover, this novel electrochemical device presents a more practical alternative to existing technologies for energy recovery as it directly transforms organic waste to electricity (Kelly and He, 2014).

Electricity generation in MFCs is favored by numerous substrates, extending from easily biodegradable pure substrates such as glucose (Rabaey et al., 2003), ethanol (Kim et al., 2007), and acetate (Logan et al., 2007), to complex substances such as starch (Herrero-Hernandez et al., 2013), chitin (Rezaei et al., 2009b), and cellulose (Rismani-yazdi et al., 2007). However, utilization of these substrates in MFCs is not a cost effective option. Biomass being a carbon neutral has been attracted as one of the most promising future resources of electricity generation for addressing the rapidly growing energy needs (Mao et al., 2015). Therefore, cheap biomass sources, such as raw corn stover (Wang et al., 2009), rice (Hassan et al., 2014) and wheat (Zhang et al., 2009) straw hydrolysate, and algae powders (Velasquez-Orta et al., 2009) have also been tested as substrates in MFCs. Many other forms of waste biomass exist, each containing large amounts of energy and can be a better source of power generation in MFCs. These remain generally unexploited and yet to be tested in MFCs for microbial degradation and ultimately bioelectricity generation.

Oranges are citrus fruits that are consumed worldwide in large magnitudes in their natural, peeled, and juiced forms (Rezzadori et al., 2012). The statistical database of the Food and Agriculture Organization of the United Nations (FAOSTAT) shows that worldwide orange production was approximately 68.2 million tons in 2012, representing nearly 52% of the total citrus fruit production (http://faostat3.fao.org/home/E). During orange juice production, approximately 50–60% of the processed fruit weight is converted to peel waste, comprised mainly of peel, seeds, and membrane residues (Garcia-Castello et al., 2011; Wilkins et al., 2007). This large volume of waste is either deposited on soil near the production site for use as an animal feed raw material after drying, or is incinerated. Such a method of waste handling results in wastewater pollution in terms of chemical and biological oxygen values, which can negatively affect the soil, ground, and superficial waters (Martín et al., 2010). A number of promising proposals for use of this waste have been described, including the production of fertilizers, essential oils, pectin, industrial enzymes, single cell proteins, pollutant absorbents, and paper pulp supplements. In addition, in terms of useful bioenergy recovery, ethanol production (Choi et al., 2013; Oberoi et al., 2010) and anaerobic digestion to produce methane gas (Koppar and Pullammanappallil, 2013) are believed to be viable treatment methods, and benefit from abundant orange peel waste (OPW). An excellent alternative approach to ethanol and methane gas production from OPW is bioelectricity production using an MFC, thus avoiding environmental pollution, and reducing operating costs.

This study explores OPW as an economical and feasible substrate for bioelectricity generation, in parallel with a reduction in chemical oxygen demand, thus resulting in environmentally friendly degradation of the OPW. To the best of our knowledge, electricity production in MFCs using OPW has not yet been reported. Voltage generation, power density, polarization curves, coulombic efficiencies, and organic matter removal were monitored to investigate the performance of this system. In addition, microbial community structure on the anode, in suspension, and in the initial inoculum of the MFC is analyzed using high throughput pyrosequencing.

2. Materials and methods

2.1. Orange peel waste and anaerobic sludge

Fresh oranges (*Citrus sinensis*) were purchased from a local market in Daegu, South Korea. Oranges were manually peeled and processed for use in the MFC. The peeled waste was divided into two parts: 1) One part was converted into juice by blending and adding deionized water before use in the MFC; and 2) One part was dried at 50 °C in an oven for 24 h, ground into a powder, and homogenized. The physicochemical composition of the powder is given in Table 1. Both samples were stored at 4 °C until further use. Anaerobic sludge for the MFC anode was collected from the Sincheon wastewater treatment plant in Daegu, South Korea. The sludge characteristics were as follows: chemical oxygen demand (COD) = 692.7 ± 7.5 mg/L; total organic compound (TOC) = 242.8 ± 12.2 mg/L; pH = 6.45 ± 0.05; total suspended solids = 16.4 ± 0.5 g/L; and volatile suspended solids = 9.5 ± 0.2 mg/L.

2.2. Assembly and operation of the MFC

A dual chamber laboratory scale MFC was used for this study, with an anaerobic wastewater anode (OPW), and an aerobic air cathode (oxygen) (Supplementary Fig. S1). A proton exchange membrane (PEM) was used to separate the two chambers, thus avoiding intermixing of the two solutions, and aiding transport of protons from the anode to the cathode. Each electrode chamber measured 5 cm × 5 cm × 8 cm (l × w × h), which equates to a volume of 200 mL. Platinum-coated graphite cloth (20 wt.% Pt., 5 cm × 5 cm) was used as the cathode, and graphite felt (5 cm × 5 cm, 3.18 mm thickness) as the anode. Nafion 117 (DuPont Co. USA) was used as a proton exchange membrane after pre-treatment with boiling deionized water, aqueous H_2O_2 solution (3% *v*/*v*), and dilute H_2SO_4 (0.5 M). This gave the highest power density and coulombic efficiency (CE) of a dual chamber MFC reported to date (Ghasemi et al., 2013). The anode and cathode were connected by insulated copper wires with a fixed resistance of 500 Ω, unless stated otherwise.

The MFC anode was inoculated initially with 20% *v*/*v* anaerobic consortia and defined medium of the following composition (all measurements are in mg/L): $NaHCO_3$ = 420, $MgSO_4 \cdot 7H_2O$ = 200, $(NH_4)_2SO_4$ = 560, $MnSO_4 \cdot H_2O$ = 20, and $CaCl_2$ = 15, along with other trace minerals and a buffer solution. Glucose was initially used as the carbon source, and was later replaced by OPW. The medium in the anode chamber was stirred continuously (magnetic stirrer) to maintain a homogenous mixture, and the cathode chamber was aerated using an air regulator. Stringent anaerobic conditions were maintained in the anodic chamber by introducing N_2 (in anolyte) to every batch for 10 min, and filling the anode chamber headspace with N_2 gas using a pure nitrogen gas bag. Provisions were made in the MFC for sampling and for inlet/outlet ports. The MFC was operated in batch mode in a temperature-controlled compartment at 30 ± 2 °C. Samples were

Table 1
Characteristics of OPW powder.

Parameter	Value (%)
Moisture	7.7 ± 0.20
Ash	4.4 ± 0.50
C	40.3 ± 0.02
H	5.8 ± 0.08
N	1.1 ± 0.20
S	0.1 ± 0.03
Protein	6.73 ± 1.26
Pectin	25.4
Cellulose	17.5
Hemicellulose	8.6