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Electricity generation and microbial communities in microbial fuel cell powered by macroalgal biomass

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ABSTRACT

The potential of macroalgae *Laminaria digitata* as substrate for bioelectricity production was examined in a microbial fuel cell (MFC). A maximum voltage of 0.5 V was achieved without any lag time due to the high concentration of glucose and mannitol in the hydrolysate. Total chemical oxygen demand removal efficiency reached over 95% at the end of batch run. Glucose and mannitol were degraded through isobutyrate as intermediate. The 16S rRNA gene high throughout sequencing analysis of anodic biofilm revealed complex microbial composition dominated by *Bacteroidetes* (39.4%), *Firmicutes* (20.1%), *Proteobacteria* (11.5%), *Euryarchaeota* (3.1%), *Deferribacteres* (1.3%), *Spirochaetes* (1.0%), *Chloroflexi* (0.7%), *Actinobacteria* (0.5%), and others (22.4%). The predominance of *Bacteroidetes*, *Firmicutes* and *Proteobacteria* demonstrated their importance for substrate degradation and simultaneous power generation. These results demonstrate that macroalgae hydrolysate can be used as a renewable carbon source of microbial electrochemical systems for various environmental applications.

1. Introduction

The environmental threads due to extensive use of fossil fuels have motivated the search for alternative sources of energy [1–3]. Bioenergy, which refers to the energy generated by biomass has great potential as renewable resource [4,5]. Among the various types of biomass, macroalgae is an attractive candidate due to its high content of carbohydrates, high biodegradability, and high growth rates [6,7]. Many studies have focused on the utilization of macroalgae for bioenergy purposes including mainly biogas production via anaerobic digestion and bioethanol and biochemicals production by fermentation [8–10]. Though macroalgae can be used for biogas or bioethanol production, economic constraints point to alternative products and technologies for feasible utilization of this biomass.

Microbial fuel cells (MFCs) offer a possibility to directly generate electrical power from various kinds of organic-rich wastes and biomasses while treating them [6,7,11]. Generally, an MFC is composed of an anode and a cathode separated by a proton exchange membrane. In the anode, the exoelectrogenic microorganisms, as biocatalysts, oxidize organic matter into electrons, protons and carbon dioxide. The electrons are transferred to the cathode via external circuit, and protons are diffused into cathode through membrane. Oxygen or another oxidant combines with electrons and protons in cathode. By this process, the energy stored in organics can be converted to electricity, which could be used directly.

Compared to the conventional biogas or bioethanol production from macroalgae, producing bioelectricity from macroalgal biomass has the advantage of avoiding energy conservation and transportation cost. The concept of using macroalgae (*Ulva lactuca*) as substrate in MFC for electricity production has been previously reported [6,7]. However, in Velasquez-Orta's study, the biomass utilization efficiency in terms of TCOD removal in MFC powered by *Ulva lactuca* was relatively low (maximum 73% removal of TCOD), probably because the macroalgae in the form of powder were not pretreated and thereby not fully biodegradable. It has been reported [8] that after hydrolysis of macroalgae (*Laminaria digitata*), the complex sugars could be decomposed into simple monosaccharides (such as glucose). Thus, pretreatment of macroalgal biomass such as by enzymatic hydrolysis might improve the substrate (biomass) utilization in MFC. The feasibility of such process has never been reported. Furthermore, it is important to explore the microbial communities on the anode capable of electricity production and macroalgal biomass utilization. Elucidation of the microbial composition would assist future construction of effective microbial consortia in order to enhance the output of the process.

Based on previous biogas study [8], it was shown that the hydrolysate of *Laminaria digitata* was rich in glucose. It has been demonstrated that glucose, is a suitable fuel for MFCs [12–14]. In this context, *L. digitata* hydrolysate could be a potential substrate in MFC for electricity production, which has never been investigated. Accordingly, the aim of this study was to generate directly electrical power from *L. digitata* in an MFC. Moreover, the microbial community was analyzed to explore the function of microorganisms that were responsible for the degradation of macroalgae hydrolysate in MFC. This study is the first attempt

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to broaden the utilization of the macroalgae species *L. digitata* into bioelectricity production. It may offer another sustainable and effective way to produce power from macroalgal biomass.

2. Material and methods

2.1. Algae characteristics and hydrolysis

L. digitata was collected at Hamborg Strand (north of Hanstholm at the Danish North Sea coast) and was stored at −20 °C before use. As described before [8], *L. digitata* was dried at 50 °C for four days until the moisture content was <10%. Then it was mechanically cut into pieces (<2 mm) and grounded into powder for the following hydrolysis. The hydrolysis was performed with the help of relevant enzymes as described before [8]. *L. digitata* hydrolysate was used for starting-up the MFC. The characteristics of hydrolysate were tested: pH 5.0 ± 0.1, total solid 54.4 ± 0.6%, and volatile solid 50.4 ± 0.2%.

2.2. MFC construction and operation

An MFC made of nonconductive polycarbonate plates was set up as previously described [15]. The anode chamber (5 × 5 cm) and cathode chamber (5 × 5 cm) was separated by a cation exchange membrane (CEM, CMI 7000, Membrane international, NJ). To avoid leakage, rubber gaskets and screws were used to tighten the reactors. The anode and cathode materials were carbon brush (5.0 cm in diameter, 5.0 cm in length, Mill-Rose, USA) and a titanium woven wire mesh (4 × 5 cm, 0.15 mm aperture, William Gregor Limited, London) coated with 0.5 mg/cm^2 Pt, respectively. The anode and cathode were connected by a 1000 Ω resistance externally. An external recirculation bottle (500 ml) was connected to the anode chamber to increase the volume of anodic compartment. The external bottle was agitated at 250 rpm to ensure anodic medium fully mixed. The recirculation flow rate was 40 ml/min.

To enrich exoelectrogenic biofilm on the anode, 300 ml domestic wastewater collected from a primary clarifier (Lundtofe Wastewater Treatment Plant, Lyngby, Denmark) was used as the medium and 1 g L^{-1} glucose was added as substrate to acclimate the electroactive bacterial consortia. In the cathode, 45 ml ferricyanide solution (50 mM) was used as the catholyte. It should be noted here, the ferricyanide was only used during the enrichment period. Every week when the voltage decreased below 0.1 V, the anodic and cathodic solutions were replaced with fresh ones. After around one month, when the voltage of MFC could achieve maximum 0.5 ± 0.05 V every time after changing the fresh solutions, indicating that electrochemically active microorganisms had colonized the surface of anode for the successful starting-up of MFC. After the successful enrichment, 9 ml *L. digitata* hydrolysate and 291 ml domestic wastewater were mixed as anodic electrolyte for the experiment. Every two or three days, the anodic solution was sampled and stored in the freezer at −20 °C for later analysis. Once the experiments were completed, the samples for the microbial analysis were obtained as described below. All the experiments were conducted in duplicate at room temperature.

2.3. Electrochemical analysis

The glucose and mannitol concentrations in hydrolysate were measured using high performance liquid chromatography (HPLC, Agilent) as previously described [12]. H_2SO_4 of 0.04 M was used as eluent and flow rate was set to 0.6 ml/min with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm), and temperature was set to 63.5 °C. The volatile fatty acids (VFA) were measured by a GC with FID detection (Agilent 6890). The pH was tested using a PHM 210 pH meter (Radiometer). Total chemical oxygen demand (TCOD) was determined as previous study [16]. Voltage across the resistor was recorded by a digital multimeter (model 2700, Keithley Instruments, Inc; Cleveland, OH) every 30 min. Power density (P_d = IV/A), and Coulombic efficiency were calculated as previously described [17] with the power density normalized by the projected surface area of the anode.

2.4. Microbial analysis

Biofilm samples were cut off from carbon brush as previously described [18] by using sterilized scalpel at the end of experiment. Total DNA extraction was performed using the Powersoil DNA isolation kit (MoBio PowerSoil, Carlsbad, CA USA) and stored at −20 °C before doing the freeze dry. Total genomic DNA was further amplified using 16S rRNA gene universal primers 515F/806R (V4 hypervariable region) and amplicons were sequenced by Illumina MiSeq desktop sequencer (Ramaciotti Centre for Genomics, Kensington, Australia). Raw data can be obtained from the Sequence Read Archive [6] database (http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA412459. The raw 16S rRNA gene sequences were analyzed using microbial genomics module plug of CLC Workbench software (V.8.0.2, QIAGEN). Reads trimming and operational taxonomic units (OTUs) clustering were performed as previously described [19]. The final taxonomical assignment of the most interesting OTUs was performed including a manual comparison of CLC results with 16S ribosomal RNA sequences (Bacteria and Archaea) database at the National Center for Biotechnology Information (NCBI) by using BLAST. In order to facilitate data interpretation, the microbial relative abundance was depicted as a heat map using Multi experiment viewer software (MeV 4.9.0) [20].

3. Results and discussion

3.1. Hydrolysis of the macroalgae

During the enzymatic hydrolysis of the raw *L. digitata*, glucose and mannitol were released as shown in Fig. 1. The glucose concentration showed a gradual increase up to 43.65 g L^{-1} after 24 h and a slight decrease between 24 and 48 h. Mannitol was released quickly in the first 2 h, demonstrating faster hydrolysis rates compared to glucose at the hydrolysis conditions applied. After the first 2 h, the mannitol concentration was stable around 14 g L^{-1}. Overall, the final concentrations of glucose and mannitol in the hydrolysate achieved 42.67 and 15.70 g L^{-1}, respectively. In terms of the levels of released sugars, the hydrolysis of *L. digitata* kept the same level with that of previous studies [8]. Similarly, the combined sugar yield was achieved at 77% w/w of dried macroalgae, with regard to 69% accounting for glucose and 8% for mannitol, respectively [8].

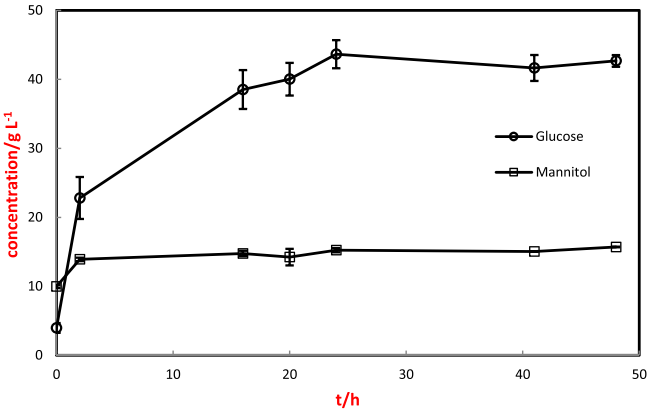

Fig. 1. Enzymatic hydrolysis profile of the released sugars.