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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 throughput 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.

Keywords

Macroalgae hydrolysis; *Laminaria digitata*; Bioelectricity; Microbial fuel cell; 16S rRNA microbial analysis

1. Introduction

The environmental threats 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 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 $54.4 \pm 0.6\%$, and volatile solid $50.4 \pm 0.2\%$.

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 1000Ω 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 (Lundtofte 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 $0.5 \pm 0.05 \text{ V}$ 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 ($300 \text{ mm} \times 7.8 \text{ mm}$), 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

Figure. 1 Enzymatic hydrolysis profile of the released sugars

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 hours, demonstrating faster hydrolysis rates compared to glucose at the hydrolysis conditions applied. After the first 2 hours, 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].

3.2 Power output and TCOD removal in MFC powered by *Laminaria digitata* hydrolysate

Figure. 2 Time course of power generation and TCOD removal in MFC with *L. digitata* hydrolysate as substrate.

As previously mentioned glucose is a suitable substrate for electricity generation in MFC [12, 21, 22]. Therefore, *L. digitata* hydrolysate with a content of glucose (44 g L^{-1}), could be favorable substrate for electricity production in MFC, which was investigated in batch mode in this section. When MFC was fed with hydrolysate at a concentration of 2 g TCOD L^{-1} , a steady state power density of $120 \pm 5 \text{ mW m}^{-2}$ ($0.5 \pm 0.02 \text{ V}$) with a period of around 9 days was rapidly achieved (Fig. 2). Accordingly, TCOD concentration gradually decreased with operation time from the initial 2.06 ± 0.04 to $0.89 \pm 0.03 \text{ g L}^{-1}$ after 11 days operation, resulting in a removal efficiency of $56.9 \pm 0.1\%$. The corresponding coulombic efficiency was $10.0 \pm 0.1\%$. Previous study [21] reported that low coulombic efficiency was generally because of substrate consumption by non-electricity-generating bacteria. The relative low coulombic efficiency in present study could be explained by the wide spectrum of fermentative bacteria that can utilize glucose and thereby compete with electroactive bacteria for glucose as substrate. Following a stable period, power generation gradually decreased from 110 to 17 mW m^{-2} in the following 11 days except sharp decrease at day 10. The decrease was most likely due to the depletion of glucose and mannitol, which was coincided with reduced

TCOD from 0.89 to 0.31 g L⁻¹. In the last 15 days, the power density kept relatively stable between 20 and 15 mW m⁻² with slight fluctuation, and the corresponding TCOD showed the similar tendency (slow decrease from 0.31 to 0.13 g L⁻¹). The subsequent coulombic efficiency increased slowly from 10.0 to 12.5%, and the overall TCOD removal efficiency reached 95% at the end of experiment.

3.3 Variations of VFA and pH during operation

Figure. 3 Variations of VFA concentrations during organic degradation in anode

During the oxidation of organics in anode, intermediate by-products are formed. In the first 5 days, the main intermediate products were acetate, butyrate, propionate, valerate and ethanol (Fig.3). The other VFAs including isobutyrate, propanol and hexanoate were lower than detection limit (10 mg L⁻¹) (data not shown here). Specifically, as shown in Fig.4, acetate and butyrate concentrations increased from 0 to 232.94 and 212.76 mg L⁻¹, respectively in the first 5 days, and decreased gradually afterwards. Likewise, the other three VFAs levels showed similar trend. The distributions of VFAs demonstrated the organic oxidation way: six-hydrocarbon sugars (glucose and mannitol) were degraded into four-hydrocarbon sugar (butyrate), followed by the butyrate oxidation to propionate or acetate, and then oxidized to CO₂. The VFA results obtained here were consistent with previous study, in which glucose as substrate was mainly degraded to acetate and then further oxidized [23]. The different distributions of intermediates will be further explained with microbial analysis.

Figure. 4 Anodic and cathodic pH changing with time

The variations of pH in anode and cathode were monitored throughout the experimental period (Fig. 4). The cathodic pH increased immediately from 7 to 8.9 while anodic pH decreased from 7 to 6.5 at day 2. The main reason could be the low efficient of proton transfer from anode to cathode which led to the consumption of proton from water for oxygen reduction and accumulation of hydroxide ions in the cathode. Similarly, the proton accumulation in anode caused a lower pH (6.5), which was also contributed by the high concentrations of VFAs. The results observed here were in agreement as previously reported [24, 25]. After 2 days, the cathodic pH exhibited a slow increase and achieved the highest value of 9.6 at day 14, where it decreased to 8.7 at the end of experiment. In the case of anodic pH, it fluctuated around 7.

3.4 Microbial community analysis

Figure. 5 Microbial community structures at phylum (A) and class (B).

In order to get further insight to the role of microorganisms in electricity generation, the microbial community established on the anodic biofilm was analyzed using 16S rRNA gene sequences approach (Fig. 5 and supplementary info). As reported previously [12, 26, 27], it showed diverse bacterial community in MFC anode mainly because glucose supports growth of many different fermentative bacteria. At phylum level, bacterial species in anode biofilm was dominated by *Bacteroidetes* (39.4%), *Firmicutes* (20.1%), and *Proteobacteria* (11.5%), followed by *Euryarchaeota* (3.1%), *Deferribacteres* (1.3%), *Spirochaetes* (1.0%), *Chloroflexi* (0.7%), *Actinobacteria* (0.5%), others (22.4%). In the community, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were the three most abundant phyla (Fig. 5A). Similarly, it was previously reported the enrichment of *Bacteroidetes* in the biofilm of glucose-enriched MFC [12]. Additionally, the presence of *Actinobacteria* also reflected its assistance in organic degradation since they are known to be members among versatile hydrocarbon degraders [28, 29].

Fig. 5B presented the community distribution at class level. Correspondingly, *Bacteroidia* (39.4%) which belonged to the phylum *Bacteroidetes* was dominant, followed by *Clostridia* (14.3%) and *Erysipelotrichi* (5.9%) which belonged to *Firmicutes*. Three classes within *Proteobacteria* were *Betaproteobacteria* (10.0%), *Gammaproteobacteria* (0.7%) and *Epsilonproteobacteria* (0.7%), respectively. The predominance of

Firmicutes and *Proteobacteria* class in the bacterial community in MFCs was also reported by others. Chae et al. [21] found the highest dominance of *Proteobacteria* (62%) in the glucose-fed MFC, whereas the proliferation of *Firmicutes* (59.3%) in propionate-enriched MFC. Jung et al. reported that *Firmicutes* were found only in glucose-fed MFCs regardless of other substrates like acetate and lactate [30]. *Firmicutes* have been reported to be exoelectrogens capable of exocellular electron transfer [31, 32] and able to convert glucose into simple organics [30]. Herein, due to the high concentration of glucose in hydrolysate, *Firmicutes* were enriched on the biofilm. *Proteobacteria* were also proposed as possible exoelectrogens [28], particularly the classes of *Gamma*- and *Betaproteobacteria*.

Figure. 6 Heat map analysis of bacterial community structures at genus level.

The genus level characterization further illustrates the functional roles of microbes in the MFC community. As shown in Fig. 6, *Prevotellaceae* sp. (23.0%) were the most dominant taxon of the community, followed by *Rhodocyclaceae* sp. (6.6%), *Prevotella paludivivens* (4.6%), *Clostridium chromiireducens* (4.1%) and others (lower than 4%). *Prevotellaceae* are known as fermentative microorganisms using glucose producing hydrogen besides volatile fatty acids. Considering their dominance in the anode, once the electrons were released from organics, *Prevotellaceae* consumed the electrons at the anode, leaving fewer electrons to flow to cathode, and thereby resulting in low coulombic efficiency. The predominance of *Prevotellaceae* was consistent with the lower coulombic efficiency observed before (Section 3.2). *Rhodocyclaceae* sp., described as short-chain fatty acid utilizers [33], were found in the sample, which was consistent with VFA results. *Rhodocyclaceae*, known to reduce iron [34], were previously observed to dominate anodic biofilm in MFCs [35, 36]. The *Clostridium chromiireducens*, which was isolated from Cr (VI)-contaminated soil and has the ability to reduce Cr (VI), found in biofilm was associated with glucose fermentation [37]. *Geobacter* species [38-40] and *Shewanella* species [41-43], known as transferring electrons directly to anode surface, were not found in our study. It's presumably due to that their presence was below the detection limit or they grow in suspension.

3.5 Perspective

Our results successfully demonstrate electricity production is possible from lab-scale MFC fed with macroalgae hydrolysate. Though promising, more efforts are still required. For example, substantial optimization will be required for further commercialization. Some parameters such as external resistance, anode material, initial TCOD loading need to be further investigated for providing a deep insight of the practical application.

Generating other products other than electricity probably is feasible. For example, hydrogen production in microbial electrolysis cell powered by the same macroalgae hydrolysate could be an alternative approach for using macroalgal biomass.

4. Conclusions

This study demonstrated effective electricity generation and utilization of *L. digitata* biomass in MFC. After hydrolysis, high concentrations of glucose (42.67 g L^{-1}) and mannitol (15.70 g L^{-1}) were formed. TCOD removal efficiency achieved was over 95% with coulombic efficiency of approx. 11%. The main degradation pathway was from six to four hydrocarbon compound (mainly isobutyrate) and then to CO_2 . Anodic pH tended to become acidic, while cathodic pH increased was over 8. The 16S rRNA gene analysis showed a diverse microbial community which were consistent with VFA results. The results provide an insight of macroalgae utilization for sustainable energy production.

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Graphical abstract

Highlights

- The utilization of macroalgae *Laminaria digitata* in microbial fuel cell.
- High contents of glucose and mannitol enriched in hydrolysate of *L. digitata*.
- Good performance of MFC in terms of COD removal and power density.
- Glucose and mannitol degraded through isobutyrate as intermediate.
- Microbial communities consistent with volatile fatty acid results.