Int. J. Electrochem. Sci., 8 **(2013)** 2104 - 2111

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

Electricity Generation by Microbial Fuel Cells Fuelled with *Enteromorpha Prolifera* Hydrolysis

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Received: 5 December 2012 / *Accepted:* 27 December 2012 / *Published:* 1 February 2013

Enteromorpha prolifera bloom caused a series of environmental issues and deterioration due to its rapid growth and ability to grow in fresh or seawater. This study explores the utilization of *Enteromorpha prolifera* collected from southern portions of the Yellow Sea as a resource for electricity generation by air cathode microbial fuel cells (MFCs). The power density reached 1027 mW/m^2 with an initial hydrolysate concentration of 1,000 mg chemical oxygen demand (COD)/liter, while coulombic efficiencies and COD removal reached 69.1% and 76.1% respectively. For comparison, three monosaccharides (rhamnose, xylose and glucose) were also tested as the fuels in the anode solution, their power density, coulombic efficiencies and COD removal are almost at the same level. These results indicated that *Enteromorpha prolifera* might be suitable resources for electricity generation using the MFC technology.

Keywords: *Enteromorpha prolifera*; Hydrolysate; Microbial full cell; Electricity generation

1. INTRODUCTION

A large-scale bloom of *Enteromorpha prolifera* that led to the formation of a green tide in the middle and southern portions of the Yellow Sea occurred in May of 2008 [1]. This bloom caused a series of environmental issues and deterioration. By July 16, 2008, 1 million tons of algae had been cleared from one of the severely affected areas, Qingdao City, China [2, 3]. However, due to its rapid growth and ability to grow in fresh or seawater, *E. prolifera* is valuable as a biomass material. Indeed, it is used in the food industry, animal feed industry, chemical fertilizer industry and biosorption of heavy metals [4]. Furthermore, *Enteromorpha prolifera* has been considered as a feedstock for the

production of biofuels and industrial chemicals [5]. Electricity generation from renewable resources and byproducts can contribute to the establishment of a sustainable society. The hydrolysate resulting after the prehydrolysis and the hydrolysis of biomass, contain varying amounts of monosaccharides, both pentoses and hexoses, and a broad range of substances either derived from the raw material or resulting as reaction products from sugar and lignin degradation [6].

Enteromorpha prolifera is composed of ~50% carbohydrate with a relatively high hemicellulose content (>20%) [5]. The hemicellulose fraction of the *Enteromorpha prolifera* is easily hydrolyzed to its constituent sugars by a dilute acid pretreatment process, forming a carbohydrate-enriched liquid hydrolysate. The hydrolysate of *Enteromorpha prolifera* has been shown a possible carbon source for lactic acid productions. In the last few decades, Chemical and biological approaches for sustainable energy production from biomass hydrolysates to energy carriers, such as methane, ethanol, and H_2, have been developed. However, many of these approaches encounter technical and economical hurdles. An alternative strategy is direct conversion of biomass hydrolysate to electrical energy in microbial fuel cells (MFCs) [7-9].

Microbial fuel cells (MFCs) are bioelectrochemical systems (BES) that employ microorganism as catalysts to oxidize organic or inorganic matters for electricity generation. The electrons released by bacteria are transferred to the anode and then transferred to the cathode where they are used to reduce electron acceptors, commonly oxygen. The system has been proven to be feasible in the fields of renewable energy production, biosensor and wastes treatment [10-13]. Electricity production has been achieved using various organic compounds including monomeric carbohydrates such as glucose and xylose and carboxylic acids such as acetic acid. The use of straw hydrolysates in an MFC has been tested with corn stover [14] and wheat straw [15] as raw materials. Promising electricity generation was obtained in both cases with current densities of (63 and 14) A/m^3 reactor volume, respectively.

Here we used *Enteromorpha prolifera* hydrolysate as fuel in air cathode MFCs equipped with carbon cloth anodes for electricity generation with simultaneous COD removal. This is the first report of exploiting microbial communities for direct conversion of *Enteromorpha prolifera* hydrolysate to electrical energy in an MFC.

2. MATERIALS AND METHODS

2.1 Acid hydrolysis

E. prolifera was collected from the Yellow Sea of China. Acid hydrolysis of *E. prolifera* was carried out using sulfuric acid as catalyst. *E. prolifera* hydrolysate was prepared by refluxing the dried *E. prolifera* with 2% (w/w, based on the water content) H_2SO_4 for a period of 4 h, in a 500 mL flask, at 10% solid. The samples subjected to acid hydrolysis contained 100 g of dry *E. prolifera* mixed with 900 mLof 2% H_2SO_4. The hydrolysate was neutralized with $CaCO_3$, centrifuged, and filtered through a 0.22 μm syringe filter. Products in the hydrolyzed sample were then identified using high-performance liquid chromatography (HPLC).

2.2 MFC configuration and running

2.2.1 Electrode preparation

Carbon cloths (3×5 cm) (non-wet proofed, type A, E-TEK) were used as anodes. All the carbon cloths were first cleaned by soaking them in pure acetone (Aladdin) overnight, then acid treated by soaking the cloths in a solution of ammonium peroxydisulfate (200 g/L) and concentrated sulfuric acid (100 mL/L) for 15 min. After that, carbon cloths were heat-treated in a muffle furnace at 450 ºC for 30 min. Following treatments, all cloths were washed three times with distilled water before being used in MFCs. Cathodes were also made of carbon cloth with a projected surface area of 4.5 cm^2; the water proof layer was made as described by previous report [16,17]: Coated with one layer of a mixture of Vulcan XC-72 (2.5 mg/cm^2) and 40% by Weight poly tetrafluoroethylene Solution (Aldrich PTFE dispersion in water) onto one side of the carbon cloth, air-drying at room temperature for 2 h, followed by heating at 370 ºC for 0.5 h, this is diffusion layers (DLs). Additional DLs were made by brushing a PTFE solution (60 wt%) onto the coating side, followed again by drying at room temperature and heating at 370 ºC for 10 min, and four layers of PTFE (60%) applied on the air-facing side. The catalytic layer was then prepared as follows: 30 mg of commercial Pt/C (20 wt%, with a Pt loading of 0.5 mg/cm^2) , 200 μL of 5 wt% Nafion solution and 100 μL pure iso-propanol were blended in a plastic sample vial, the suspension was coated onto the surface of a carbon cloth. All electrodes were dried at room temperature for at least 24 h before use.

2.2.2 MFC construction and operation

Air-cathode single chamber cylindrical MFCs (length 6 cm, diameter 2.4 cm, volume 27 mL) were constructed, and wired to an external resistor (1000 Ω). The cathode was placed on one side of MFC with the oxygen catalyst coating layer facing to the anode, with the PTFE layer exposed directly to air. The anode was positioned (perpendicularly to the cathode) in the other side of chamber, with a distance of 1.0 cm from the cathode and no membrane between the two electrodes. MFC reactors were inoculated using pre-domesticated bacteria from another double chamber MFC. MFCs were inoculated using solution containing COD (1 g/L) and a phosphate buffered nutrient medium (PBM) containing NH_4Cl (0.31 g/L), $NaH_2PO_4 \cdot H_2O$ (4.97 g/L), $Na_2HPO_4 \cdot H_2O$ (2.75 g/L), KCl (0.13 g/L), and a metal (12.5 mL) and vitamin (12.5 mL) solution [18, 19]. The solution was replaced at the end of each fed-batch cycle, defined as a voltage less than 50 mV. All tests were conducted in a 30 °C temperature, and were carried out in two parallel samples.

2.3 Analyses

Chemical oxygen demand (COD) was measured according to standard methods [20]. The concentrations of all sugars were measured by HPLC, using a chromatograph (Type Waters ALC 201) with a RI detector. A Bio-Rad HPX-42 column was used with 70:30 acetonitrile/water as the mobile phase at a flow rate of 0.8 mL/min. The polarization curves and the power density curves were

obtained by varying the external resistance (R) from 20000 to 20 Ω, when the voltage output was in steady-state after keep 20000 Ω for two hours, and the voltage (V) changing with the external resistance in the MFC was recorded by a data acquisition system connected to a computer every 10 min. At each resistance (R), MFCs were operated for two batches to ensure repeatable voltage output. The power density (P, mW/m^2) was calculated by the equation 1.

$$P = \frac{V_{\text{cell}}^2}{R_{\text{ex}} A} \quad (1)$$

and the current density was calculated by equation 2.

$$P = \frac{V_{\text{cell}}}{R_{\text{ex}} A} \quad (2)$$

in which A is the project area of the cathode. Internal resistance (R_i) was calculated by linear regression of voltage *vs.* current and open circuit voltage (OCV) was the voltage obtained at zero current. The Coulombic efficiency (CE) was calculated from the total current production (Q_{ex}) and the total initial added COD of glucose (equation 3). The chemistry oxygen demand of 1 g/L glucose is 1.063 g/L.

$$CE = \frac{Q_{ex}}{COD_{glucose} \times V \times F \times b \div M} \quad (3)$$

in which V is liquid volume (0.02 L), F is Faraday's constant (96485 C/mol of e^-) and b is mol of e^- produced per mol of O_2 (4 mol/mol), M is the mole mass of O_2 (32 g/mol).

3. RESULTS AND DISCUSSIONS

3.1 Hydrolysate composition

Acid hydrolysis of *E. prolifera* (10% solid content) was carried out using 2% (w/w, based on the water content) sulfuric acid as a catalyst at 100 ºC for 4 h. After the analysis, the acid hydrolysate of *E. prolifera* contained a total of 8.5 g/L monosaccharide and had the highest content of L-rhamnose (3.74 g/L), followed by D-xylose (2.11 g/L), D-glucose (1.78 g/L) , D-glucuronic acid (0.581 g/L), and D-glucuronic acid lactone (0.29 g/L). Trace amounts of furans (furfural and hydroxymethylfurfural) were formed during the acid hydrolysis of the seaweed. The D-glucuronic acid lactone in the hydrolysate was found to be formed from D-glucuronic acid during the acid hydrolysis process.

3.2 Power generation

Initially the MFC was operated with glucose as substrate, in order to form biofilm on the anode electrode. Biofilm was successfully formed after approx. 7 days of the MFC operation. Thereafter the MFC was operated with 20 mL hydrolysate (COD 1g/L) as substrate. For comparison, three monosaccharides were also tested for their power generation efficiency, all the MFCs were on the same conditions except the fuel species. Electricity generation in MFCs with different substrates were

Table 1. Comparison the parameters of the four MFCs

	Enteromorpha	Rhamnose	Xylose	Glucose
CE (the second circle)	69.1%	46.1%	40.6%	44.0%
COD removal	76.1%	80.0%	79.9%	78.4%
The highest power density (mW/m^2)	1027	1400	1406	1488
Internal resistance (Ω)	244	198	193	194
Open circle voltage (V)	0.72	0.75	0.76	0.74

shown in Fig 1., the MFCs fuelled with hydrolysate, xylose and glucose can reach the maximal voltage within about five hours in every circle, but the MFC fuelled with rhamnose need more time, which is about ten hours. The stable period for power generation with hydrolysate was normally longer than that with other single substrate at the same COD concentration [21]. In this study, the MFC fuelled with hydrolysate's electricity generation circle (ca. 250 hours) is much longer than the others (ca. 200 hours), which might have been due to the humic acid content of the hydrolysate. The Coulombic efficiency of these MFCs were measured to be 69.1%, 46.1%, 40.6% and 44.0% for hydrolysate, rhamnose, xylose and glucose, and the COD removal is 76.1%, 80.0%, 79.9% and 78.4%, respectively. The MFC fuelled with the hydrolysate's CE could reach 69.1% (the highest in these four MFCs) but its COD removal was only 76.1% (the lowest in these four MFCs), this may be due to a change in the dominant carbon source for the power production from easily degradable organics to persistent organics, as well as a shift of microbial consortia from bacteria utilizing easily degradable organics to bacteria degrading persistent organics, as illustrated in the literature [22]. The maximal output voltage of MFC with the *E. prolifera* hydrolysates is only 10-25 mV lower than the MFC with monosaccharides, and the maximum voltage of every cycle were maintain at about 0.55 V. Thereby we could conclude that the *E. prolifera* can direct conversion of biomass hydrolysate to electrical energy in microbial fuel cells.

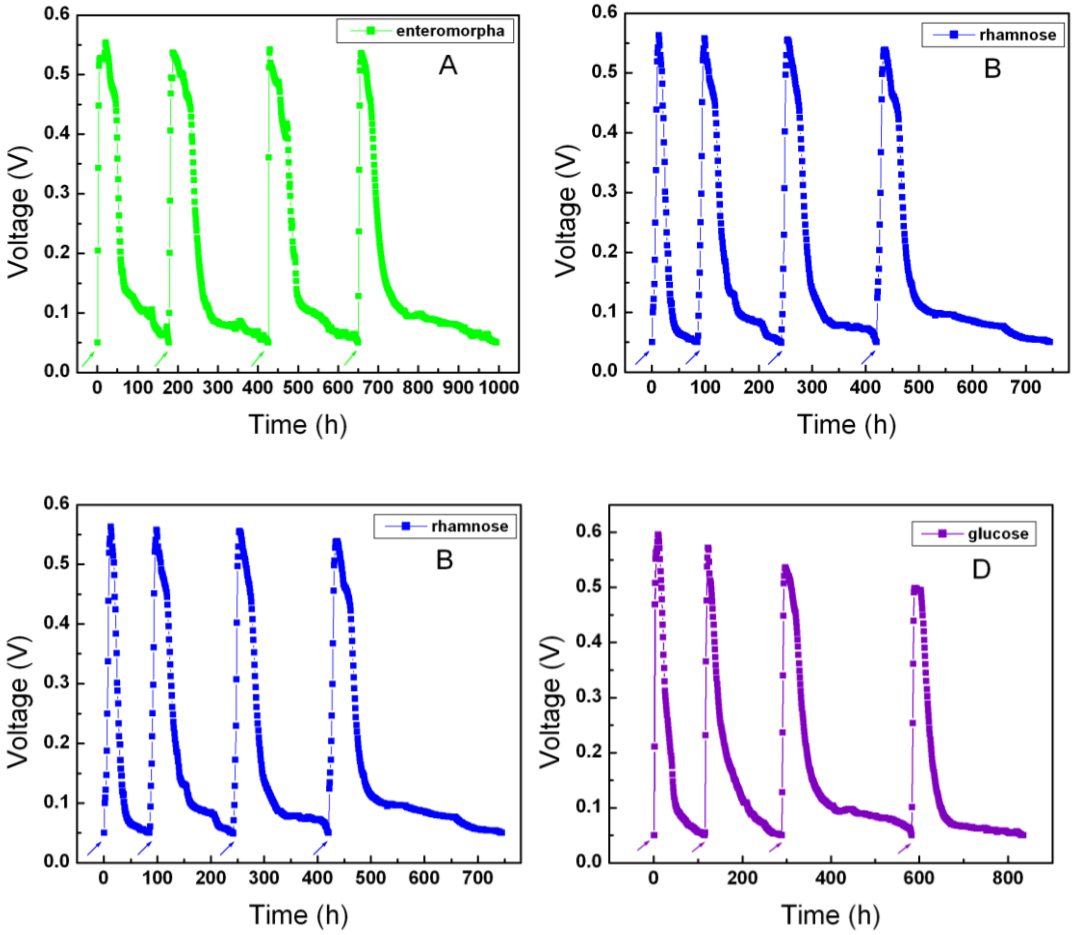

Figure 1. Voltage output over time of the MFC with external resistance of 1000 Ω. (A) the substrate was *E. prolifera* hydrolysate, (B) rhamnose, (C) xylose, (D) glucose. The concentration for each substrate was 1g-COD/L. The arrows indicate when MFC were fed with fresh medium.

3.3 Polarization curve and power density

Polarization curve and power density can direct reflect the performance of the MFC. Polarization data were obtained by varying the circuit external resistance, and the power densities curve was calculated from the polarization data. The obtained power densities and polarization curves are shown in Fig. 2. The maximum power density achieved from 1g/L COD of the *E. prolifera* hydrolysate as the sole fuel in the single chamber air-cathode MFC was 1027 mW/m^2 at a current density of 3.8 A/m^2. And the other three monosaccharides were 1400 mW/m^2 at 4.0 A/m^2 (rhamnose), 1406 mW/m^2 at 4.1 A/m^2 (xylose) and 1488 mW/m^2 at 2.8 A/m^2 (glucose), respectively. There was a sudden drop of cell voltage at relatively higher current and lower external resistance (20–100 Ω) in all polarization tests. Internal resistance was estimated from the slope of the plot of voltage versus current, and it was observed to be 244 Ω, 198 Ω, 193 Ω and 194 Ω for the four MFCs fuelled the hydrolysate, rhamnose, xylose and glucose, respectively. The internal resistance of the hydrolysate MFC was about 50 Ω bigger than the three monosaccharides' MFCs. These results reflect the conductivity of hydrolysate in the MFC is not good as the monosaccharides. The electrochemical reaction rates could

be evaluated by the open circuit potential (OCP). A higher OCP value was related to a higher reaction rate. The OCP of the MFC with the *E. prolifera* hydrolysate was 0.72 ±0.02 V, and the MFC with rhamnose, xylose, and glucose were 0.75 ±0.02 V, 0.76 ±0.02 V, 0.74 ±0.02 V, respectively.

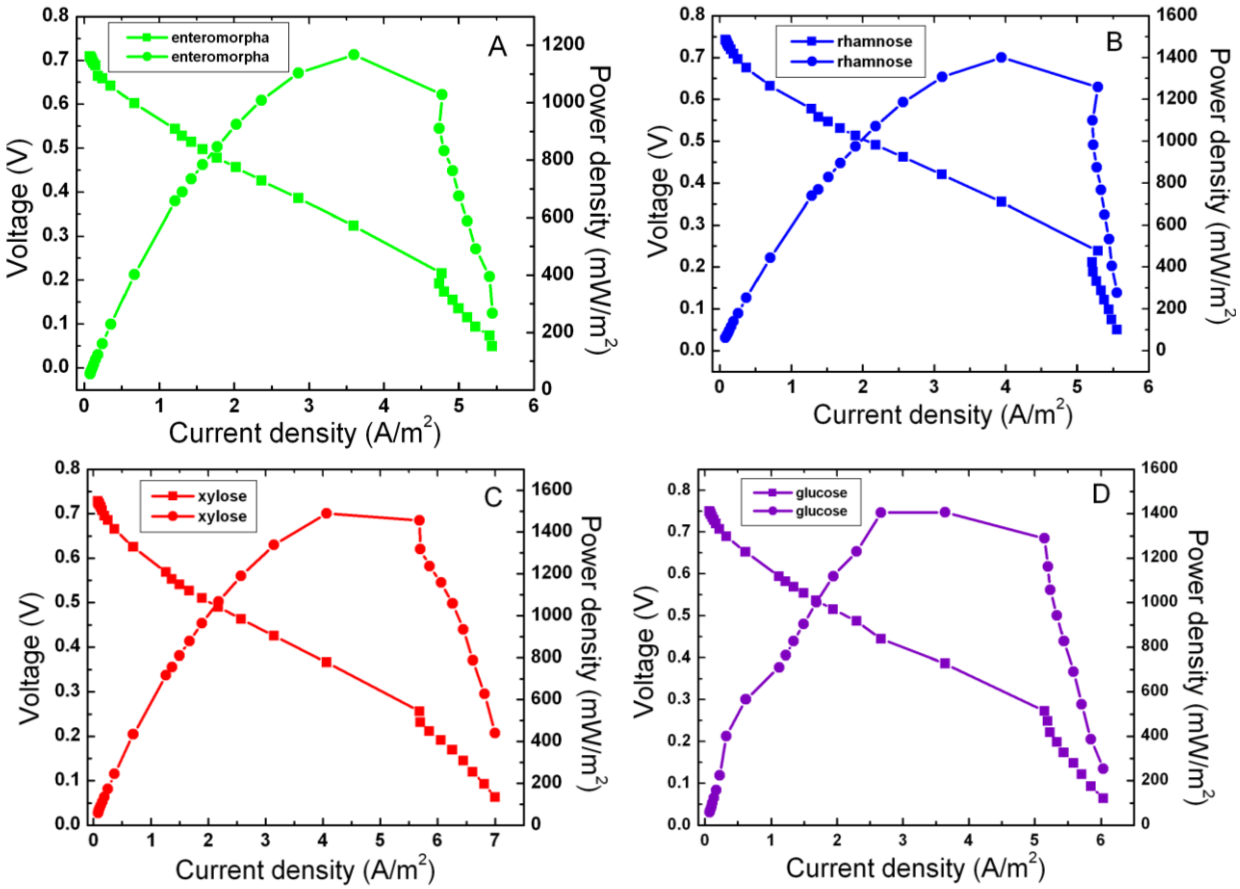

Figure 2. Power density curves and polarization curves of the MFC. (A) the substrate was *E. prolifera* hydrolysate. (B) the substrate was rhamnose. (C) the substrate was xylose. (D) the substrate was glucose. The concentration for each substrate was 1 g-COD/L.

4. CONCLUSIONS

The composition of acid hydrolysis of *E. prolifera* were analysed by HPLC. And electricity was successfully generated using *E. prolifera* hydrolysate as the sole fuel in air cathode single chamber microbe fuel cells. This study demonstrated that *E. prolifera* hydrolysate can generate more electricity than the monosaccharides with the same COD. And the highest power density, COD removal, internal resistance and open circle voltage of the MFCs were determined, all the results showed that *E. prolifera* hydrolysate is a favorable fuel for MFC. So that the MFC technology should be a potential method for the biological treatment of *E. prolifera* bloom for energy output.

ACKNOWLEDGEMENTS
The work was financially support by the National Natural Science Foundation of China (20906043, 31170110), the promotive research fund for young and middle-aged scientists of Shandong Province (2009BSB01453), the Natural Science Foundation of Shandong province (ZR2010BQ009, ZR2011EL002) and the Taishan Scholarship of Shandong Province.

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