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Membraneless Enzymatic Biofuel Cell Powered by Starch Biomass

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Abstract. This present work reports on an eco-friendly and membraneless enzymatic biofuel cell (EBFC) with direct utilization of starch as biofuel. This study examines the compatibility of Metroxylon Sagu (Sago) starch to be used as a substrate in the production of biofuel in EBFC via enzymatic hydrolysis, which has not yet been explored. The hydrolysis is adapted from the idea of simultaneous saccharification and fermentation (SSF), which is widely used in another biofuel production. Alpha-amylase (αAmy) and glucoamylase (Gamy) enzymes (1:1 ratio) are used simultaneously in the hydrolysis process of Sago to produce glucose. Membraneless EBFC makes the biofuel cell less bulky and reduces the cost. The presence of glucose after the hydrolysis process was identified using the DNSA method. Meanwhile, the catalytic currents have been successfully observed in the cyclic voltammetry analysis to confirm the redox reaction. Furthermore, the electrochemical performances of the membraneless EBFC were evaluated in terms of the open circuit voltage (OCV) and the maximum power density. All the measurements were carried out with a potentiostat. The best catalytic currents of an EBFC employing 1.5% (w/v) concentration of Sago substrate and 200 µl of enzymes and present a maximum power density of 39.3 µW cm^{-2} and an OCV of 0.32 V. The results proved that the direct use of Sago in EBFC successfully produces biofuel and thus generates electricity. Membraneless EBFC is a potential candidate for low-powered implantable and wearable devices.

INTRODUCTION

Hydrogen is one of the cleanest alternative energy sources and is most affectively converted into electricity using a fuel cell. Fuel cells mimic batteries as both convert the energy generated by the electrochemical reaction into usable electrical power. A fuel cell is an efficient electrochemical device that converts the supplied fuel which is hydrogen (H_2) and oxygen (O_2) directly into electricity, as long as reactants are supplied to the electrodes [1]. Interestingly, the emissions produced by a fuel cell are water and heat. Different types of fuel cells are classified according to the electrolyte, operating temperature and applications. A biofuel cell can be defined as a device capable of converting chemical energy directly into electrical energy through electrochemical reactions involving biochemical pathways. The recent manufacture of biofuel cells is considering eliminating the use of membranes in the system as it is suitable for direct chemical use in the fuel cell [2]. The absence of a membrane between the electrodes also reduces fuel crossover, which limits the available power of a fuel cell, reduces cost, simplifies manufacture and is suitable for microscale power devices [3]. Nature-assisted energy converters like enzymatic biofuel cell (EBFC) make them the best candidates for microscale energy sources for pacemakers, biosensors and wearable medical devices [4,5]. An EBFC is a fuel cell based on bio-functional anodes and cathodes in which enzymes are used as biocatalysts that convert organic substrates into electrical energy. Recently, multi-enzyme hydrolysis which is adapted from the idea of simultaneous saccharification and fermentation (SSF), is applied in EBFC to directly yield the fuel from biomass. More than one type of enzyme is used in this hydrolysis process and forms a sequence of reactions. The product of the first enzyme reaction is carried over directly to the next enzyme and immediately goes through a second reaction, and so on [6].

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The use of biomass as the substrates such as chitin and cellulose in EBFC has previously been studied [7,8]. However, starchy biomass which is found in most staple foods like rice, wheat, corn and potatoes, has received more attention as they consist of a large number of glucose units [9,10]. Abundant in nature, cheap, environmentally friendly are the additional credits of these biomasses to be employed as the substrates in EBFC. Sago is a new best candidate for substrates, the starchy staple food in Southeast Asia that is mainly found in Malaysia, Indonesia and Papua New Guinea [11]. In Sago, amylose and amylopectin are the main composition of polysaccharides that must go through the process of saccharification so that they can be converted into monosaccharides. This is because most of the oxidizing enzymes on the bioanode cannot utilize polysaccharides directly. Therefore, the process of multienzyme catalysis is required to produce glucose. Several types of amylases are most commonly used in starch hydrolysis. Amylases are found in a wide variety of organisms, including plants, animals, and microorganisms, the latter being a suitable source for the production of enzymes in industrial processes due to the ease of cultivation [12]. Alpha-amylase (αAmy), the endo-acting enzyme, catalyzes the hydrolysis of α-1, 4-glycosidic bonds of amylose and some branched α-1, 6-glycosidic bonds of amylopectin. Mainly maltose, smaller oligosaccharides and dextrin are released as products. Further, glucoamylase (GAmy), the exo-acting enzyme that mainly hydrolyzes α-1, 4-glycosidic bonds from the non-reducing ends of starch chains, resulting in the production of glucose [13]. In this study, a membraneless EBFC was fabricated in which the glucose was obtained directly from multienzyme hydrolysis of the Sago substrate. The compatibility of Sago as a substrate was determined based on the catalytic currents of the redox reaction, the open circuit voltage (OCV) and the maximum power density of the EBFC.

METHOD

Materials

Sago powder was purchased from Dhulau Ent., Sabah, Malaysia. Alpha-amylase (αAmy) (100-200 U mg^{-1}) from *Aspergillus Oryzae*, Laccase (Lac) (0.92 U mg^{-1}) from *Trametes versicolor*, Polyacrylamine (PAA), Glutaraldehyde (GA), Tetrathiafulvalene (TTF), 2,2'-azino-bis (3-ethylbenzothazoline-6-sulfonic acid) (ABTS) and phosphate buffer powder (pH 7.2) were purchased from Sigma-Aldrich, USA. Glucose Oxidase (GOx) (288 U mg^{-1}) from *Aspergillus Niger* and Glucoamylase (Gamy) (6 U mg^{-1}) from *Rhizopus* were obtained from Nacalai and TCI, Japan respectively. Meanwhile, the electrodes materials, which are graphene powder and carbon paste oil-based powder have been purchased from Sigma Aldrich, USA and BAS, Japan.

Preparation of Bioelectrodes

The bioelectrodes preparation compromised different enzymes and mediators. Bioanode used 5 mg/ml GOx, while biocathode used 5 mg/ml Lac as the biocatalyst [3,14,15]. In the meantime, 10 mM TTF and 10 mM ABTS have been used as mediators on the bioanode and biocathode, respectively. For both bioelectrodes, 80 mM PAA was used as the binding agent and 40 mM GA as a crosslinker. All solutions were prepared using a 100 mM phosphate buffer solution [16,17]. The steps are shown in Fig. 1 (a). Figure 1 (b) shows the adjustable working electrode and (c) Ag/ AgCl reference electrode. The bare working electrode is shown in Fig. 1 (d) and the coated working electrode is shown in Fig. 1 (e). The working electrode which coated with GOx was the bioanode, while the biocathode is coated with Lac.

Preparations of the Enzymatic Biofuel Cell

A complete set of an EBFC consists of the bioanode, biocathode and the sago substrate fuel. Sago substrate was prepared with 1.5 w/v % of sago powder heated in phosphate buffer solution (PBS) until completely dissolved. After the substrate solution had cooled, αAmy and Gamy enzymes were added to the solution in a 1:1 ratio of 100 µl in volume. The substrate fuel is then filled into a voltammetry cell together with the bioelectrodes and a reference electrode for the electrochemical measurement, as shown in Fig. 1 (f). The EBFC is placed in a 37 °C water bath and connected to the Gamry Interface 1000E potentiostat.

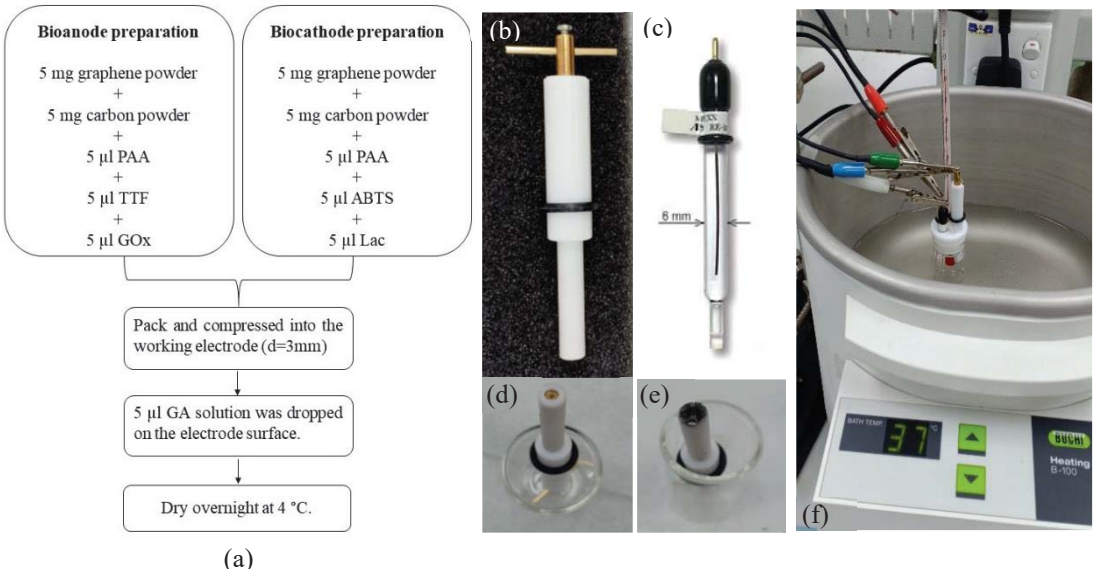

FIGURE 1. (a) Flowchart of bioelectrodes preparation (b) working electrode (c) Ag/ AgCl reference electrode (d) bare working electrode (e) coated working electrode (f) experimental setup of enzymatic biofuel cell.

Measurement of Glucose Content

The glucose content levels (mM) were measured using the dinitrosalicylic acid (DNSA) method. Every 10 minutes out of a total of 100 minutes, 1.0 ml of samples were taken from the mixture (sago substrate + αAmy + Gamy), then placed in a test tube and mixed with 3 ml DNSA reagent. The test tube was placed in boiling water for 15 minutes. Samples, blank and standard glucose solutions were heated simultaneously. After the heating process, the tube was cooled by circulating water. The absorbance of the solution was measured using a microplate plate reader (TECAN, Infinite M200 PRO) at a wavelength of 575 nm. Calibration curves were generated based on the wavelength of standard glucose solutions at concentrations of 0-100 mM.

Electrochemical Measurement

The electrochemical measurements were carried out with a Gamry Interface 1000E potentiostat. The Ag/AgCl electrode was used as the reference electrode. Cyclic voltammogram (CV) analysis was used to characterize the electrocatalytic properties of the EBFC for fuel oxidation and reduction. CV curves ranging between -0.7 V and +0.5 V were measured at different scan rates. Open circuit voltage (OCV) analysis was used to measure the electromotive force of cells composed of a bioanode and a biocathode, and the measurement time was 24 hours. A current-voltage (I-V) analysis was also performed in which currents drain in the range of 1 x 10^{-5} to 1 mA, were applied to the EBFC to measure the maximum power density of the cell. All electrochemical measurements of EBFC employing sago substrate were also compared with EBFC employing 50 mM of commercial glucose and carried out at 37 °C and physiological pH 7.

RESULT AND DISCUSSION

The analysis of the glucose content using the DNSA method is presented in Fig. 2. The glucose content from the hydrolysis process is compared to a standard curve of the commercial glucose concentration. The glucose content increases in direct proportion to the longer hydrolysis time. After a hydrolysis time of 40 min, the glucose content reached 50.87 mM and is therefore used in the EBFC. The performance of the EBFC is compared to the EBFC using 50 mM of commercial glucose as a biofuel. Table 1 shows the value of the glucose content from 0 to 100 minutes of the enzymatic hydrolysis time.

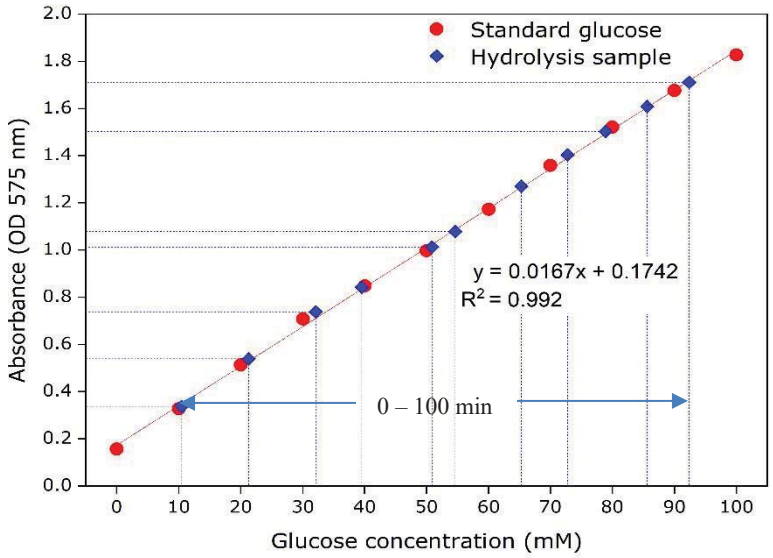

FIGURE 2. Glucose concentration of sago substrate samples after 100 minutes of enzymatic hydrolysis.

TABLE 1. Glucose concentration (mM) from enzymatic hydrolysis at a different time (min).

Enzymatic hydrolysis time (min)	Glucose concentration (mM)
0	10.48
10	21.29
20	32.12
30	39.54
40	50.87
50	54.61
60	65.27
70	72.77
80	78.87
90	85.59
100	92.35

The CV curves of EBFCs employing 1.5 w/v % sago substrate and 50 mM commercial glucose are shown in Fig. 3 (a) and (b), respectively. In the CV curve of EBFC using sago substrate, the upper peaks represent the cathodic peaks while the lower peaks are the anodic peaks. The appearance of the peaks proved the presence of glucose via the enzymatic hydrolysis and the redox reaction took place in the EBFC. For each EBFC, both the anodic and cathodic current peaks increase with an increasing scan rate. The scan rate of the CV controls how fast the applied potential is scanned per second. Faster scan rates lead to a decrease in the size of the diffusion layer, and higher currents are observed as a result [18]. The anodic current peaks tend towards a more positive voltage potential, while the cathodic current peaks tend towards a less positive voltage potential. The anodic, cathodic and overall reactions of the EBFCs are shown in Equation (1), (2) and (3) and are explained schematically in Figure 4. Free αAmy and Gamy in the Sago substrate solution work to hydrolyze the starch into oligosaccharide and glucose, respectively. The immobilized GOx on the bioanode oxidizes the glucose. The electrons released during the oxidation will flow through the external circuit of the EBFC and generate electricity. Further, the oxygen from the ambient at the biocathode is reduced by the immobilized Lac enzyme and water is produced as waste.

$$Anodic\ reaction: \quad 2C_6H_{12}O_6 + 2H_2O \xrightarrow{GOx} 2C_6H_{12}O_7 + 4\bar{e} + 4H^+ \tag{1}$$

$$Cathodic\ reaction: \quad O_2 + 4\bar{e} + 4H^+ \xrightarrow{Lac} 2H_2O \tag{2}$$

$$Overall\ reaction: \quad 2C_6H_{12}O_6 + O_2 \rightarrow 2C_6H_{12}O_7 + 2H_2O \tag{3}$$

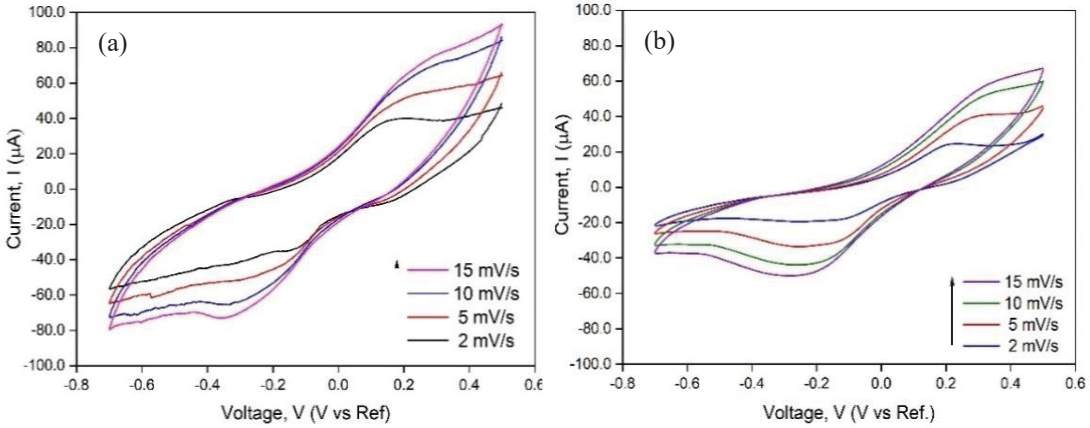

FIGURE 3. Cyclic voltammogram curves of EBFCs employing different fuel (a) 1.5 w/v % sago substrate and (b) 50 mM glucose.

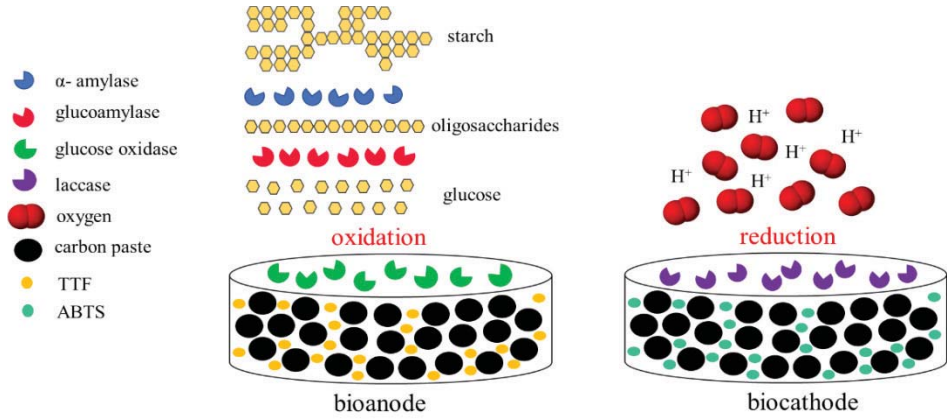

FIGURE 4. Schematic of multienzyme hydrolysis at the bioanode and biocathode in the EBFC.

Figures 5 (a) and (b) show the curves of the maximum power density curves of EBFC employing 1.5 w/v % sago substrate and 50 mM glucose, respectively. The maximum power density of EBFC using sago substrate was 39.3 μW cm^{-2}, while the maximum power density of EBFC using glucose was only slightly lower at 37.6 μW cm^{-2}. The active areas of both electrodes were 0.28 cm^2. Furthermore, Fig.5 (c) shows the OCV profile of both EBFCs employing sago substrate and glucose. Throughout the 24 h storage without any connection to an external load, both EBFCs exhibit good durability with OCV of 0.32 and 0.28 V. The stable voltage of the EBFCs can be explained by the good tolerance of the reactants within the system, with the EBFCs showed no voltage loss or self-discharge properties and can withstand long storage. Although our designed bioanode saccharized and oxidized starch biomass simultaneously on the electrode surface, the value of the maximum power density and the OCV were almost of the same order of magnitude as the EBFCs described in the literature that uses commercial glucose as fuel [19,20].

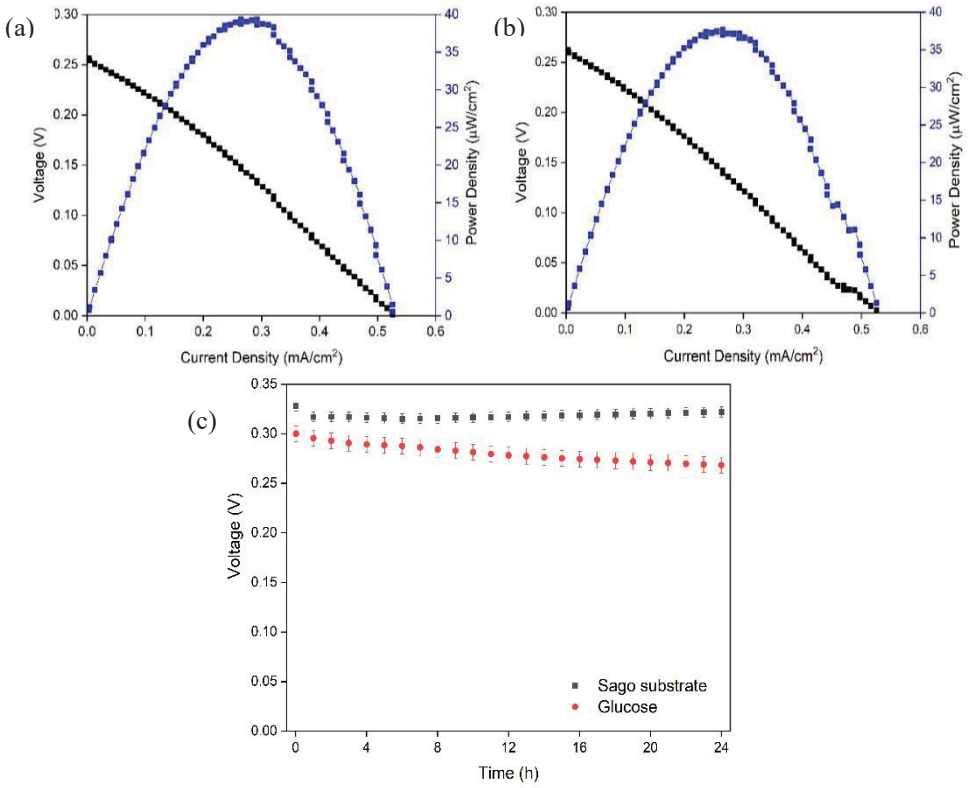

FIGURE 5. (a) and (b) Maximum power density curves of EBFC employing different fuel 1.5 w/v % sago substrate and 50 mM glucose, respectively. (c) The OCV plot of EBFC employing 1.5 w/v % sago substrate and 50 mM glucose in 24h.

CONCLUSION

In summary, we have developed a membraneless EBFC that operates while directly utilizing fuel produced from Sago for the first time. The multienzyme hydrolysis of Sago using αAmy and Gamy effectively produced glucose in a very simple step. The EBFC successfully generated electricity where the redox reactions took place at the bioelectrodes, as proved from the cyclic voltammogram. The EBFC employed 1.5 w/v % sago substrate delivered an OCV of 0.32 V and a maximum power density of 39.3 μW cm^{-2}. The results are comparable to the EBFC using commercial glucose (50 mM), which had an OCV of 0.28 V and a maximum power density of 37.6 μW cm^{-2}. These results indicate that a readily available fuel that can be used for EBFCs will promote new studies and designs for low-powered implantable and wearable devices.

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