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*Enzyme and Microbial Technology Research Paper*

# Utilization of hydrolysate from lignocellulosic biomass pretreatment to generate electricity by enzymatic fuel cell system

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**Highlights**

- The waste hydrolysate after the biomass pretreatment contains glucose.
- EFC system can utilize the waste hydrolysate as a fuel for power generation.
- CV, EIS, and power density did not show the inhibition of redox enzyme for hydrolysate.

**Abstract**

The waste hydrolysate after dilute acid pretreatment (DAP) of lignocellulosic biomass was utilized to generate electricity using an enzymatic fuel cell (EFC) system. During DAP, the components of biomass containing hemicellulose and other compounds are hydrolyzed, and glucose is solubilized into the dilute acid solution, called as the hydrolysate liquid. Glucose oxidase (GOD) and laccase (Lac) were assembled on the electrode of the anode and cathode, respectively. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were measured, and the maximum power density was found to be $1.254\times10^{3}\mu W/cm^{2}$. The results indicate that the hydrolysate from DAP is a reliable electrolyte containing the fuel of EFC. Moreover, the impurities in the hydrolysate such as phenols and furans slightly affected the charge transfer on the surface of the electrode, but did not affect the power generation of the EFC system in principal.

**Keywords**: dilute acid pretreatment, enzymatic fuel cell, biosensor, cyclic voltammetry, power density

## 1. Introduction

The pretreatment processes of lignocellulosic biomass are indispensable to improve the enzyme accessibility and digestibility against the economics of biomass, increasing the efficiency of sugar recovery in the saccharification process. Lignocellulosic biomass is a rigid and complex structured feedstock. Moreover, cellulose, a component of lignocellulosic biomass, consists of bunches of polymeric backbone of glucose monomers. Dilute acid pretreatment (DAP) solubilizes the hemicellulose, one of the major portions consisting of lignocellulosic biomass, which is amorphous and heteropolymeric [1]. Then, the xylose concentration after the DAP in the hydrolysate is used as the index of the pretreatment effect [2]. The glucose from glucan, a crystalline portion, can exist in the hydrolysate at some conditions of DAP. Under severe DAP conditions, the biomass and the solubilized substances in hydrolysate can be over degraded, leading to the increased degradation of the glucan portion. The increase indicates a decrease in the available portion of biomass, which could be converted into the fermentable sugar. Therefore, the aim of the DAP process optimization was to maximize the xylose concentration and minimize the glucose concentration in the hydrolysate liquid after DAP. We previously reported the statistical optimization of the DAP conditions [3]. The waste hydrolysate from DAP contains various materials such as sugars, phenolics, furans, and organic acids.

An enzymatic fuel cell (EFC) is a system, which converts chemical energy to electrical energy by electrochemical reactions, involving the redox reaction of substrate as the fuel [4]. EFCs use biosensors for various purposes, especially with micro devices due to its miniaturization ability and potentials [5]. Moreover, the advantages such as stability and simplicity are expected in EFCs utilized in various applicable fields, *e.g.* immunosensors, incapsulation devices, and self-contained implantable devices [6]. During the oxidation of the substrate on the surface of anode in EFC system, electrons are liberated; a coupling reactant accepts the electrons and undergoes reduction. The concentration of the substrate forms a gradient of electrons, which is utilized by the EFC system [7].

In this study, hydrolysate was utilized to generate electrons by using an EFC system. The redox enzymes of glucose oxidase (GOD) and laccase (Lac) were immobilized on each electrode with electron transfer mediators containing graphite, cobalt, and chitosan. The EFC performance and properties were evaluated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS); and power density was measured using the prepared electrodes from the hydrolysate. Moreover, the currents at various concentrations of glucose

were measured. Fig. 1 shows the schematics of an EFC system using the hydrolysate in the biorefinery process.

## 2. Materials and Methods

### *2.1 Chemical reagents, enzymes and electronic instruments*

Graphite, $CoCl_2 \cdot 6H_2O$, $NH_4OH$, N-(3-dimethylaminopropyl)-N′-ethylcabodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), chitosan, sulfuric acid (98.0%), 25× TAE buffer, acetic acid, hydrogen peroxide, potassium permanganate, and anhydrous dextrose (98.0%) were purchased from Sigma-Aldrich. GOD and Lac from *Aspergillus niger* and *Trametes vesicolor*, respectively, were used as the redox enzymes immobilized onto electrodes. Electrical data were gathered using a power supply by Princeton Applied Research VersaSTAT 3.

### *2.2 Dilute acid pretreatment and hydrolysate preparation*

Barley straw was pretreated with dilute sulfuric acid in a stainless tube reactor in an oil bath, comprising a preheating bath, reaction bath, and cooling bath. From the fundamental experiments, three major factors (temperature, concentration of sulfuric acid, and reaction time) were determined and optimized by a statistical method in which 20 experiments were designed [3]. The conditions included reaction temperatures, sulfuric acid concentration, and reaction time in the ranges 90–190°C, 0.02–2.3%, and 1.5–18.4 min, respectively. After the pretreatment of 20 experiments, solid/liquid separation was performed, and the hydrolysate (liquid) was neutralized using $CaCO_3$ and treated with ethyl acetate [8].

### *2.3 Fabrication of electrodes and electrochemical measurements*

Graphite was treated to obtain graphite oxide (GO) with a mixture of $H_2SO_4$, $H_3PO_4$, and $KMnO_4$ for 1 day by the Brodie method [9]. The GO particles were modified to obtain GO/Co using $CoCl_2 \cdot 6H_2O$ by the method of Wang *et al*. [10]. Chitosan was dissolved in TAE buffer with acetic acid (pH 6) at 121°C, and GO/Co particles were diffused in the chitosan solution[11].

Gold electrodes were prepared after cleaning with the solution mixture of $H_2SO_4$ and $H_2O_2$. Glucose oxidase and laccase were immobilized on the GO/Co/chitosan modified electrodes in a sodium phosphate buffer solution (0.1 M, Ph 7) for 8 h [12]. The electrochemical activities of enzymatic electrodes were measured using VersaSTAT3 (AMETEK, Princeton Applied

Research, USA). Electrochemical measurements such as CV and EIS were carried out using an Au electrode with the assembled glucose oxidase, Ag/AgCl, and a Pt wire as the working electrode, reference electrode, and the counter electrodes, respectively [13]. The following parameters were used to measure EIS using the Nyquist plot: start frequency, 20 Hz; end frequency, 0.01 Hz; and amplitude voltage, 10 mV. The resistances of electrolyte and charge transfer were derived by calculating the intercepts of the semicircle on the x-axis of real Z by the appendix software of VersaSTAT3.

## 3. Results

### *3.1 Cyclic voltammetry measurement*

CV of the redox reaction of the hydrolysate was measured at various concentrations. The initial hydrolysate concentration was 2% (w/v) and was further diluted to 1% and 0.5%. Fig. 2 (A) shows the CV curves, indicating the tendency and relation of current with varying potential (volt). As the hydrolysate dilution increased, the maximum current density decreased. CV is widely used to obtain the voltammetry data. The cyclic voltamograms provides the information of the redox reaction and adsorption/desorption effects with the applied potential. In addition, the stability, reversibility, and electron transfer kinetics can be determined using the CV peak behavior. CV can also be used to determine the electron stoichiometry of a system, the diffusion coefficient of an analyte, and the formal reduction potential of an analyte as an identification tool [13]. CV was measured at a scan rate of 100 mV/s between -0.6 V to +0.6 V. The CV results showed the irreversible curves of the reduction reaction using electrons from the glucose oxidation reaction governed by Laccase. No inverse peak was observed as shown in Fig. 2 (A), because of the absence of enzymes for the reverse reaction. Peak current decreased at the electrode potential shown, indicating a typical phenomenon of irreversible system, and Fig. 2 shows the first cycle of CV as the glucose concentrations of hydrolysate [14]. The voltamograms containing the measured current data consisted of the Faradaic current and capacitive currents, generated by the redox reactions and double-layer correction on the surface of the electrode, respectively [15]. The capacitive current depends on the charge of the electrolyte ions and the temporary storage of the generated electrons by the pseudo-capacitance on the electrode surface. When the current and potential were increased, due to the electron transfer mediators, the CV peak showed none or less pseudo-capacitance phenomenon, [11]. The peak current was found to be ~1.2,

0.5, and 0.15 mA/cm$^2$ for the hydrolysates of 2, 1, and 0.5% glucose, respectively.

The kinetic parameters were derived from the CV behavior and the Butler-Volment equation, linking Faradaic current, electrode potential, and concentrations of redox materials [16]. The rate constant (*k*) is one of the important parameters in electrode kinetics, because *k*, the index of reaction rate, can be calculated using the modified Equation (1).

$$\text{Log}\, k \;=\; \alpha\text{Log}(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\left(E_p - E_0\right)}{2.3RT} \qquad (1)$$

Where F is the Faradaic constant, $E_0$ is the standard potential of glucose, α is the transfer coefficient, *v* is the scan rate, and $C_{A0}$ is the concentration of the oxidant [16]. Various scan rates (25, 50, 75, and 100 mV/s) were tested to determine the coefficient of the electron transfer rate ($\alpha$). The peak currents at the scan rates were plotted, and the regression of the scattered points was performed to derive the α value, which was found as 0.9. The kinetic parameters were derived at a scan rate of 100 mV/s, and the rate constant (*k*) of the EFC system using hydrolysis was found to be 14.83 s$^{-1}$.

Moreover, the EIS was measured at 2, 1, and 0.5% glucose concentration of hydrolysate to evaluate the charge transfer of electrode surface and electrolyte in the EFC system. Fig. 2 (B) shows the Nyquist plot of the EIS of the EFC system using the hydrolysate. The results of the EIS for the hydrolysates showed a similar tendency of impedance. The semicircle portion at high frequency and linear portion (Warburg impedance) at a low frequency were observed. The resistance of charge-transfer ($R_{ct}$) and the electrolyte resistance ($R_s$) were calculated using the diameter of the semicircle in the plot and the intercept on the real axis of the semicircle [18]. At 2, 1, and 0.5% glucose samples of the EFC system, the $R_{ct}$ values were found to be 5.4, 3.5, and 3.9kΩ, respectively. Some properties of hydrolysate affect the charge-transfer on the electrode surface. The electrodes were modified by assembling GO/Co/chitosan composites and redox enzymes on the surface for power generation. Then, $R_{ct}$ is affected by both the property of the electrode and modification of the electrode surface [19]. The enzyme loading on the electrode surface could be verified by the comparison of the $R_{ct}$ values [20]. Because the identical sets of electrodes were used for the EIS measurements, hydrolysate is the strong influential factor towards the charge transfer. The hydrolysate liquid after the DAP of lignocellulosic biomass involves various undefined materials derived from the biomass by hydrolysis and pyrolysis. These are known to be mainly sugars, phenolic compounds, furanics, and organic acids, however the hydrolysate was treated with ethyl acetate to remove the phenolics and furanics [8]. The concentration of impurities could

change the electron transfer on the surface of the electrode, expediting the formation of electrical double-layer from the ions in the hydrolysate. Moreover, the double-layer capacitance depends on the Au electrode and modification of the electrode surface as $R_{ct}$ [21]. Then, the electron transfer could be retarded, because of the modified electrode and the hydrolysate. The effect of hydrolysate on the charge transfer will change the capacitive current, which is one of the contributing current towards the total measured current and the pseudo-capacitance phenomenon [22,23]. The results of the $R_{ct}$ of EIS at 2 and 0.5% samples showed a relatively high resistance compared to 1% glucose sample, showing a pseudo-capacitance in some the hydrolysate at certain concentrations. The weak effects of pseudo-capacitance were also shown in the CV of 2 and 0.5% glucose samples as shown in Fig. 1 (A).The $R_s$ values were found to be 38.9, 31.3, and 30.6Ω for 2, 1, and 0.5% glucose concentration of hydrolysates, respectively. The measured $R_s$ values decreased, and the retardation of charge transfer in hydrolysate electrolyte diminished as the hydrolysate was diluted. The correlation between the hydrolysate concentration and $R_s$ was almost proportional, indicating a tendency of diffusion and mass transfer through the electrolyte. Moreover, the Warburg impedance indicates that the mass transfer at a low frequency disturbs the charge transfer, because the electron generation by redox reaction is responsible for the Faradaic current. The Warburg impedance and $R_s$ values are not concerned with the chemical (enzymatic) reactions directly, but focuses on the diffusion of ions between distant portion and nearby the electrode surface [18]. Then, the bulk properties of electrolyte for the overall EFC system can be evaluated, and the hydrolysate from the dilute sulfuric acid pretreatment process using lignocellulosic biomass (barley straw) is reliable as an electrolyte and a fuel for power generation.

*3.2. Power output of EFC using hydrolysate*

Cell voltage and power density versus current density are shown in Fig. 3. Power density is the amount of practical power, considering the rate of electron transfer per unit surface of effective electrode of redox reaction and is also dependent on the cell voltage, as the current density changes. When the concentrated hydrolysates containing 5, 10, and 15 g/L glucose were used to generate power using the EFC system, the linear increase in the power density changed at ~750, 1,500, and 3000 mA/cm$^2$ current densities. When the results were plotted, and the calibration was performed, the slope and intercept were found to be $4.28\times10^{-3}$ and 2.5, respectively, and the coefficient of determination was found to be 0.9642. The maximum

power outputs of the hydrolysate samples were found to be 552, 828, and 1,254 μW/cm$^2$at ~1,500, 2,300, and 3,000 mA/cm$^2$ current densities, respectively. The results were similar to an electrolyte with pure glucose and higher than other studies. In our previous study, the electrode modified with DNA-wrapped single walled nanotubes generating current densities in the range 730–760 μW/cm$^2$. We have also developed a GO/Co/chitosan mediator and with a power density of ~442 μW/cm$^2$ [24]. When the electrode fabrication process was optimized, ~1,050 μW/cm$^2$ power density was obtained [11,12]. The maximum power density using the hydrolysate was similar or high compared to the literature values.

The results showed that the electron generation using the hydrolysate of the redox reaction governed by fungal redox enzymes was effective. Inhibition effects were not observed by the impurities in the hydrolysate or salts formed from the neutralization. Though the hydrolysate was treated with ethyl acetate to remove phenolic acids and furans as we previously reported [8], a small amount of the substances could be remained. The residual substances could affect the overall EFC system. However, the power densities of the samples were proportional to the concentrations of glucose. Enzymes are sensitive at various factors, and the effect of ethyl acetate treatment may minimize the inhibition of enzymes.

The results and the scheme of this research are expected to be utilized in DAP as an index of the process effect in biorefineries. As mentioned in the introduction section, the index of DAP is to maximize the xylose concentration and minimize the glucose concentration in hydrolysate liquid simultaneously after DAP. Moreover, the glucose concentration needs to be monitored and controlled in the practical operation of DAP in the biorefinery process. The EFC system is appropriate for biosensors if the stability and repeatability are demonstrated in the practical use of the process. If the EFC system is utilized to detect glucose, the monitoring and controlling could be helpful for the commercialization of biorefinery process. The EFC experiments have been performed using the fuels in pure level, and the results of this study showed the actual performance and practical use of EFC.

## 4. Conclusions

The waste hydrolysate utilization to generate electricity using an EFC system was found to be effective. The hydrolysate not only contained glucose but also various impurities and salts. The CV and EIS results showed the electrochemical properties of hydrolysate as an electrolyte, and the impurities and salts slightly affected the diffusion of ions and charge transfer on the surface of the electrode. The maximum power density was found to be

$1.254\times10^3\mu W/cm^2$ for the hydrolysate with 2% glucose concentration, and the redox enzymes were not inhibited by the remained impurities and salts. The power density values were almost proportional to the glucose concentration. Moreover, the EFC system could be applied to the glucose detection to monitor and control the DAP in the biorefinery process.

**Author's contributions**

SBK: Overall experiments and manuscript draft

DSK: Electrode fabrication

JL: Results and discussion and manuscript check

JHL: Manuscript check

SWK: Results and discussion and manuscript finalization

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**Figure legend**

Fig. 1 The schematics of glucose detection in hydrolysate between DAP and SFP using EFC, where DAP is dilute acid pretreatment process and SFP is saccharification and fermentation process.

Fig. 2 The measurement of cyclic voltammetry (A) and impedance (B). The CV was measured with 0.1 M $K_3Fe(CN)_6$ in potassium nitrate buffer.

Fig. 3 Effect of various glucose concentrations (5, 10, and 20 g/L) on the power density of EFC.

Fig. 1

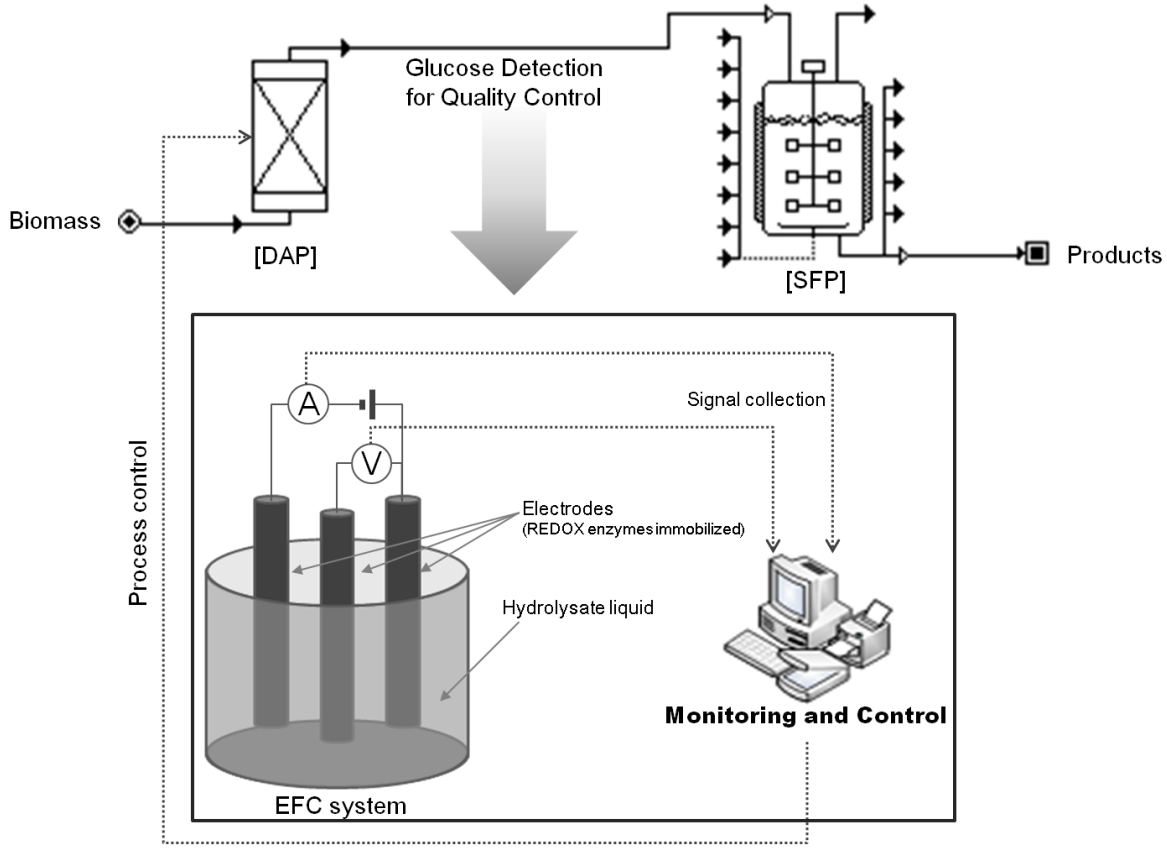
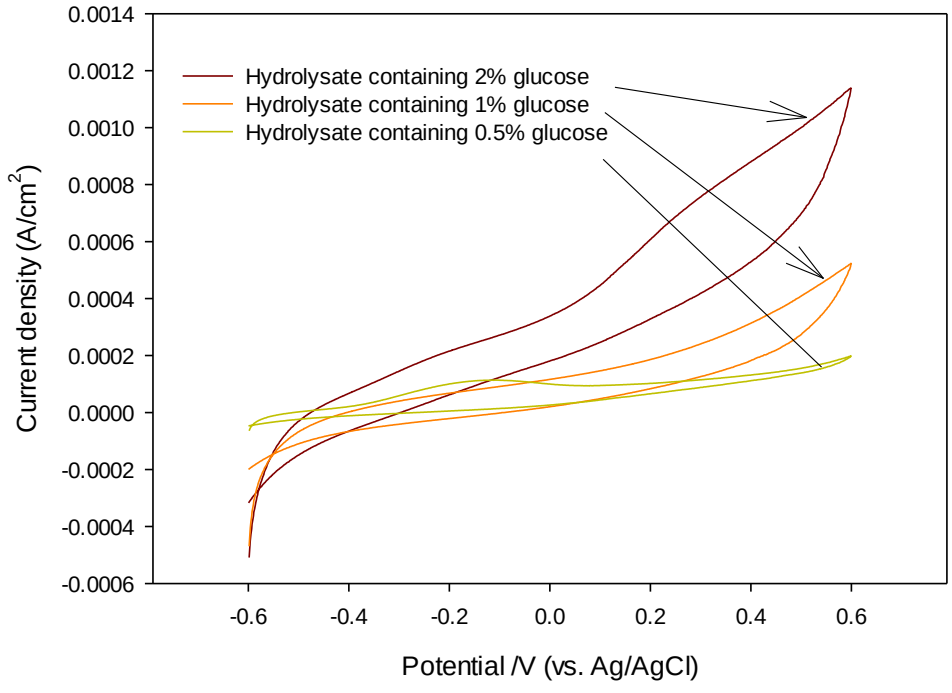
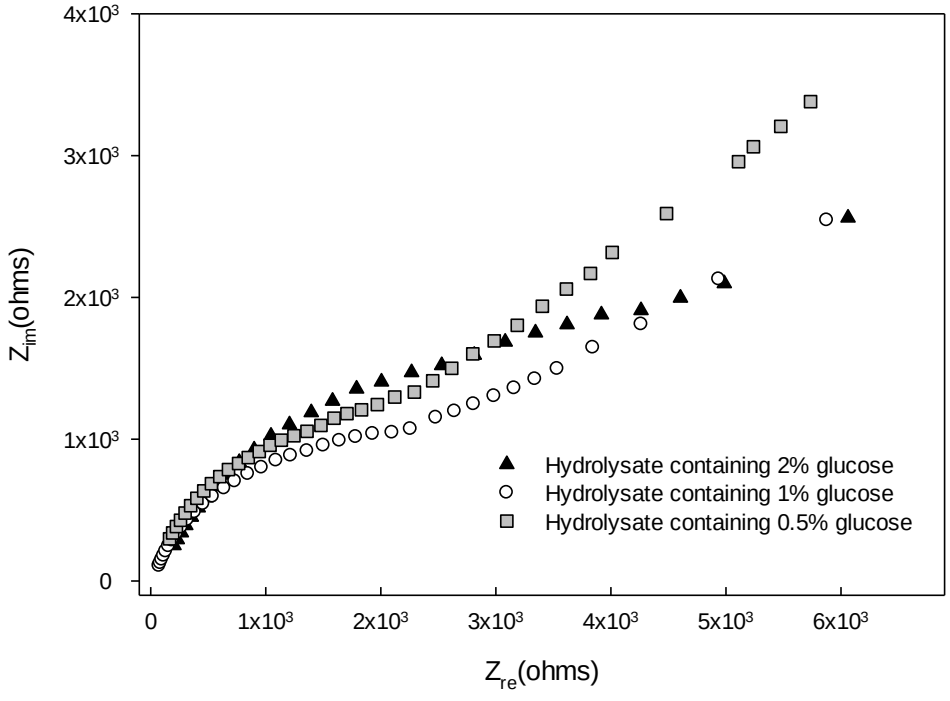


Fig. 2




(A)




(B)

Fig. 3