Enzyme and Microbial Technology 85 (2016) 32–37

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

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

Sung Bong Kim [a], Dong Sup Kim [a], Ji Hyun Yang [a], Junyoung Lee [b], Seung Wook Kim [a,*]

[a] Department of Chemical and Biological Engineering, Korea University, Seongbuk-Gu, Seoul 02855, Republic of Korea
[b] Department of Plant and Food Science, Sangmyung University, Dongnam-Gu, Cheonan, Chungnam 31066, Republic of Korea

ARTICLE INFO

Article history:
Received 23 September 2015
Received in revised form 15 January 2016
Accepted 18 January 2016
Available online 21 January 2016

Keywords:
Dilute acid pretreatment
Enzymatic fuel cell
Biosensor
Cyclic voltammetry
Power density

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 1.254×10^3 μW/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.

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.

* Corresponding author. Fax: +82 2 926 6102.
E-mail addresses: seanlearns@gmail.com (S.B. Kim), wavekds@naver.com (D.S. Kim), jihyun_y@naver.com (J.H. Yang), dorgly@smu.ac.kr (J. Lee), kimsw@korea.ac.kr (S.W. Kim).

http://dx.doi.org/10.1016/j.enzmictec.2016.01.008
0141-0229/

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

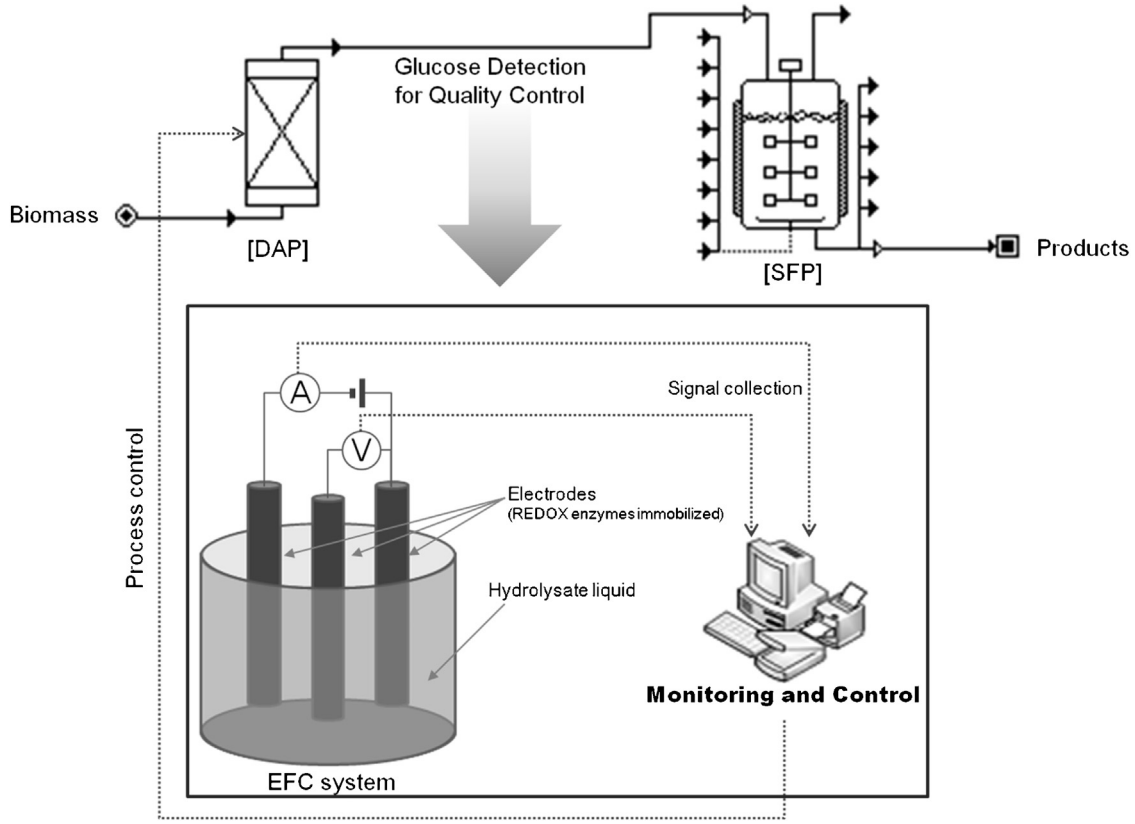

Fig. 1. The schematics of glucose detection in hydrolysate between DAP and SFP using EFC, where DAP is dilute acid pretreatment process and SFP is saccharification and fermentation process.