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Short Communication

Enhanced electron transfer mediator based on biochar from microalgal sludge for application to bioelectrochemical systems

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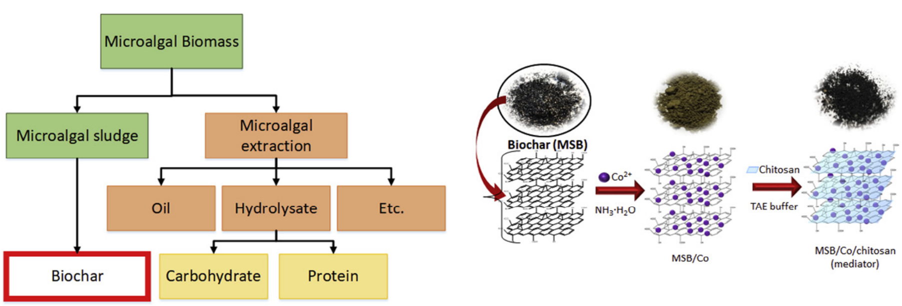
GRAPHICAL ABSTRACT



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ABSTRACT

This study is focused on the utilization of waste microalgal sludge (MS) from microalgal extraction and its potential as an electrode material. The MS was activated under N_2 at high temperature for conversion to biochar (MSB). In addition, cobalt (Co; metal hydroxide) and chitosan were used as a mediator for electron transfer by immobilization on MSB (MSB/Co/chitosan). Through analysis of the surface and components of the MSB/Co/chitosan, it was shown that Co and chitosan were properly synthesized with MSB. The enzymatic fuel cell (EFC) system successfully obtained a power density of 3.1 mW cm^{-2} and a current density of 9.7 mA cm^{-2}. In addition, the glucose biosensors applied with the developed electron transfer mediator showed a sensitivity of 0.488 mA mM^{-1} cm^{-2}.

1. Introduction

Microalgae are autotrophic and unicellular organisms that are small enough to freely move in water and produce nutrients through photosynthesis. They were considered to be unnecessary organisms influencing the generation of red tide and green tide. However, microalgae is used in various fields such as the production of bioenergy (due to their characteristic of containing diverse materials and nutrients), future food, raw materials of cosmetic products, and in the reduction of greenhouse gases. Research on microalgae has therefore been reactivated worldwide, and their status as a biological resource has increased (Chew et al., 2017; Choi et al., 2016). In order to obtain active

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components that can be applied to various fields, microalgae are processed physically and chemically. The physical and chemical process of microalgae leads to the generation of relatively large amounts of waste algal sludge compared to the useful components. The waste microalgal sludge can be carbonized and contributes to the areas of energy and environment such as portable lab-on-a chip, biofuel cell, power source device, heavy metal and CO_2 adsorption.

Recently, pyrolysis technology used for the utilization of biomass with direct combustion has attracted considerable attention. Biomass pyrolysis is a thermal decomposition process of polymeric compounds by heating the biomass at over 350 °C in an anaerobic condition. Some of the biomass is then converted to pyrolysis steam, and the remains are emitted as solid by-products, the main component of which is carbon. As these solid by-products have high carbon contents (over 80%) and large micro-surface areas (200–400 $m^2 g^{-1}$), they are used for electrode materials for batteries, catalyst materials for various processes, and high-quality fuels for steel and power generation industries, reducing greenhouse gas emissions (Tan et al., 2017; Lee et al., 2016). Several studies have reported the use of algae as the precursor of activated carbon. Aravindhan et al. (2009) researched the adsorption characteristics of activated carbon within algae to remove phenol. Ferrera-Lorenzo et al. (2014) produced activated carbon for adsorption by using potassium hydroxide (KOH) from *Gelidium Sesquipedale*, a macroalgal waste. Balahmar et al. (2017) produced activated carbon for CO_2 adsorption by using *Paeonia lactiflora* and seaweed. However, although biomass has been used as an energy source, building materials, and fertilizer for years, few studies have been carried out on the use of microalgae for the production of electrochemical energy.

Many researchers have proposed various methods to associate direct electron transfer (DET) and mediated electron transfer (MET) fuel cells or biosensors. The MET EFC system relies on the mediator that stands between enzymes as it transfers electrons from the oxidation reactions to the surface of the electrode (Zhao et al., 2018). Especially, graphite oxide (GO) is specified by special surface characteristics and layer structures. This GO sheet has a large surface area and thus can become a potential supportive material that can contain nanocrystals. As GO has oxygen containing functional groups, it can work as a centric material to immobilize active materials such as metal hydroxide on the surface. As GO is composed of carbon, it has an excellent ability for electron transfer, and thus it has been used in many studies. Most commercial carbon materials for electron transfer, such as carbon induced from phenolic resin at 2000 °C, are obtained from petroleum products (Zheng et al., 2012). However, a simple method for the synthesis of carbon within renewable resources is to carbonize biomass. Microalgae having a rapid growth cycle, can be harvested without using valuable land, and can reuse carbon dioxide as biomass.

The purpose of this study is to produce biochar through the utilization of carbonized waste microalgal sludge and to evaluate its potential as an electrode material. Because microalgae grow constantly and rapidly, it has potential as a raw material for the production of biochar. Metal and chitosan were synthesized in order to produce a high-potential electron transfer mediator. The developed mediator was successfully proved by the applied EFC system and biosensor with sufficient results showing high power generation and sensor sensitivity, respectively.

2. Experimental

2.1. Microalgal biomass and reagents

Microalgal biomass (*Chlorella pyrenoidosa*)-dried powder was obtained from WUDI LV QI BIOENGINEERING Co., Ltd. (China). Cobalt (II) chloride hexahydrate ($CoCl_2·6H_2O$), ammonium hydroxide (NH_4OH), chitosan, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcabodiimide hydrochloride (EDC), 25 × Tris-Acetate-EDTA (TAE) buffer, acetic acid, hydrogen peroxide, and anhydrous dextrose (98.0%) were all purchased from Sigma–Aldrich. Glucose oxidase (GOD) from *Aspergillus niger* and laccase (Lac) from *Trametes vesicolor* were used for immobilized redox enzymes. All the chemicals were utilized with reagent grades.

2.2. Analysis of microalgal biomass composition

Biomass analysis of the solid algae was performed to determine its absolute composition (carbohydrate, protein and lipid), based on the standard procedures of the National Renewable Energy Laboratory (NREL, USA) and the Official Methods of Analysis of AOAC INTERNATIONAL (OMA)

2.3. Preparation of biochar

To investigate the conditions of the acid extraction of *C. pyrenoidosa*, the solid-liquid ratio and hydrochloric acid concentration were evaluated in the range of 100 g/L and 2% (w/w), respectively. An alumina boat was inserted into a horizontal quartz tube furnace and heated at 5 °C/min with an activation temperature of 800 °C under N_2 (300 ml/min). The furnace was then maintained at the activation temperature for 3 h, followed by cooling to room temperature.

2.4. Modification of MSB

MSB was modified to obtain MSB/Co using $CoCl_2·6H_2O$ using the method of Kim et al. (2015). Chitosan solution was dissolved in TAE buffer with acetic acid (pH 6.0) at 121 °C, and the MSB/Co particles were diffused in the chitosan solution. GOD and Lac were immobilized on the MSB/Co/chitosan-modified electrodes in 0.1 M sodium phosphate buffer (pH7.0) for 8 h.

2.5. Analytical methods

The synthesized structures of the mediator were investigated by scanning electron microscopy (SEM, Hitachi S-4300) and high-resolution transmission electron microscopy (HRTEM, FEI Instruments, USA). Surface analysis of the elements was performed with energy dispersive X-ray spectroscopy (EDX) and X-ray photoelectron spectroscopy (XPS). The composition of the mediator was analyzed using Fourier transform infrared spectroscopy (FTIR). The electrochemical activities of the enzymatic electrodes were measured using potentiostat/galvanostat (VersaSTAT3; AMETEK, Princeton Applied Research, USA). Electrochemical measurements such as cyclic voltammetry (CV) and power density were carried out using an Au electrode with the assembled GOD, Lac, Ag/AgCl, and a Pt wire as the working, reference and counter electrodes, respectively.

3. Results and discussion

3.1. Compositional analysis of microalgal feedstock

The mass balance shown in Fig. 1 was used in the analysis of energy resources produced from microalgae. Analysis showed that 1 kg of microalgal biomass was comprised of 4.6 g oil, 63.5 g hydrolysate (9.9 g carbohydrate and 56.6 g protein), and 900 g microalgal sludge (MS). It is well-known that oil and hydrolysate are used in the conversion to biofuel and biochemical, respectively. However, 900 g MS is made into pellets or trashed. If a large amount of MS that is currently not used can be utilized after carbonization, it can become a promising raw material in the future.

3.2. Characteristics of carbonized MSB

For carbonization, MS is heated under N_2 at high temperature to remove hydrocarbon and volatile matters. Dehydration and deoxidation