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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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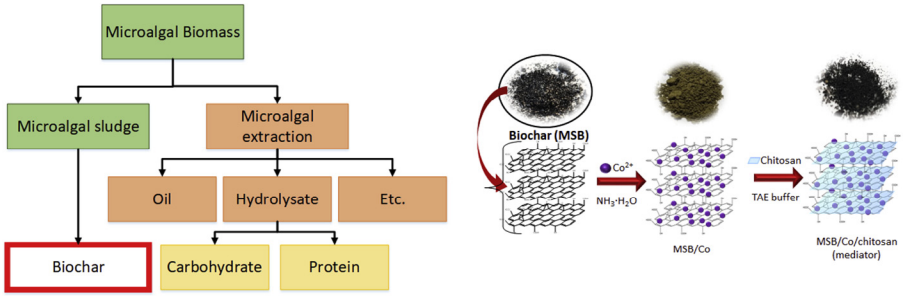
GRAPHICAL ABSTRACT



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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 ($CoCl_2{\cdot}6H_2O$), 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 $CoCl_2{\cdot}6H_2O$ 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

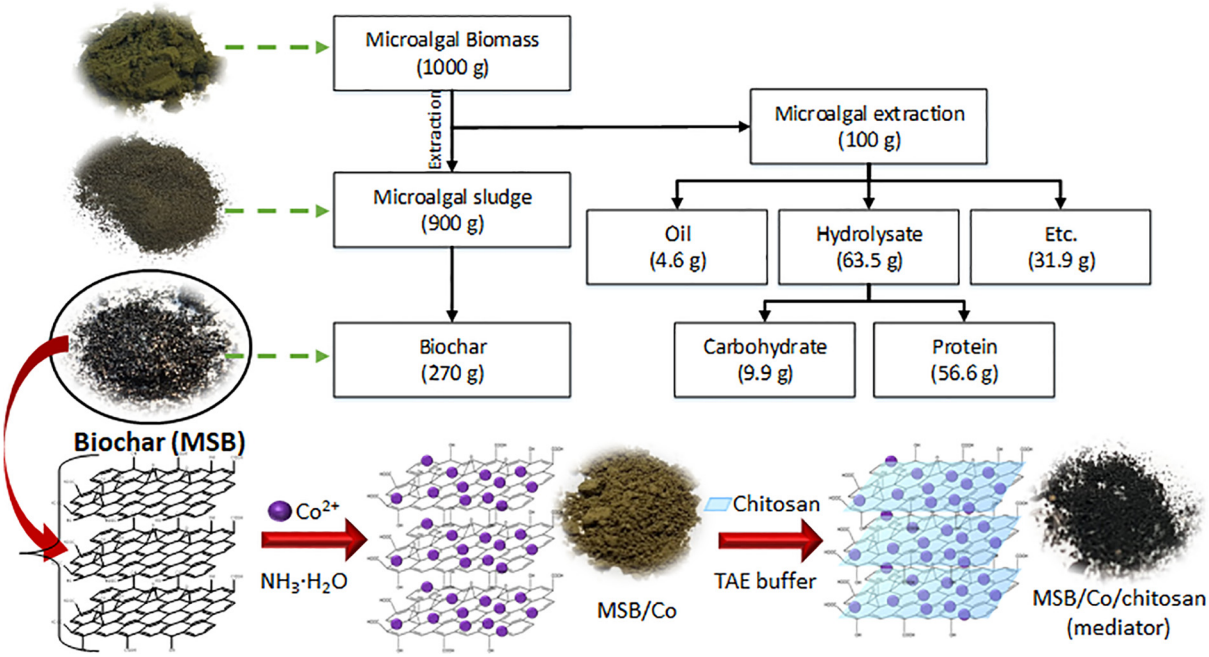

Fig. 1. Schematic diagram showing the mass balance of *C. pyrenoidosa* during biochar production and mediator synthesis process.

Table 1
Characteristics of biochar activated from various biomass under different conditions.

Types of source	Feed stock	Pyrolysis conditions (°C)	BET surface area ($m^2 g^{-1}$)	Ultimate analysis (wt%)			References
				C	O	H	
Algal biomass	*Spirulina* sp.	500	–	45.3	–	1.2	Chaiwong et al. (2013)
	Scenedesmus dimorphus	600	89	50.2	35.8	8.0	Bordoloi et al. (2016)
Lignocellulosic biomass	Corn stover	500	3.1	57.3	5.5	2.9	Mullen et al. (2010)
	Miscanthus	600	50.9	85.1	–	2.4	Kwapinski et al. (2010)
	Soft wood (pine)	500–650	4.9	71.2	11.6	2.9	Mukome et al. (2013)
	Olive husk	600	4.2	73.8	20.4	2.3	Angın (2013)
	Kenaf fiber	1000	346.6	55.5	39.3	2.0	Mahmoud et al. (2012)
	Bamboo	550	0.4	70.7	27.6	1.1	Li et al. (2014)
Waste biomass	Sewage sludge	850	35.2	33.0	25.9	3.9	Inguanzo et al. (2002)
	Conocarpus waste	800	–	85.0	4.9	0.6	Al-Wabel et al. (2013)
	MSB	800	2.9	87.8	10.2	–	This study

then occur, causing oxygen binding to break through the carbonization process, and then oxygen is released in the form of O_2, CO, and CO_2. Biochar can be produced after volatile matters are removed (Cha et al., 2016). The SEM analysis of microalgal feedstock, MS, and MS carbonization (MSB) after acid hydrolysis of microalgae. The morphology before the acid hydrolysis of microalgae showed a regular sphericity through the degradation of the cell walls by acid extraction, resulting in a modified surface structure of sphericity cells. Only biochar is left after the carbonization process, according to the HRTEM and EDX analyses of MSB. The HRTEM analysis of the structure of MSB showed that the rod particles seemed to be enveloped by a thin film. This result is consistent with that of the HRTEM analysis of graphite oxidation (GO). In addition, result of the EDX spectra index, which represents quantification of materials, shows that the components of MSB are C (87.8%) and O (10.2%). Table 1 shows the comparison between the characteristics of the biochar in this study and other biochar in the literatures. Pyrolysis conditions, BET surface area, and ultimate analysis were shown in algal, lignocellulosic, and waste biomass. The MSB demonstrated a higher carbon content than the other kinds of biochar. Therefore, the MSB could have an important role of an electron transfer precursor such as graphite or CNTs, having a carbon structure.

3.3. Production of electron transfer mediator

In previous research, the composite of GO, metal, and chitosan has been used as a mediator of the EFC system. The mediator was shown to perform the important function of electron transfer between the redox enzyme and electrode (Kim et al., 2015). In addition, the mediator was bound on the redox enzyme molecule self-assembled on the electrode. It is known that GO, as a mediator in the EFC system, has a good electron transfer ability between redoxase and electrode, while MSB (as a mediator replacing GO) enables the improvement of electron transfer. Thus, in this study, Co was modified on the surface of MSB in order to investigate the potential of MSB as a mediator. SEM and HRTEM analyses were performed to investigate the shape of the modified Co on the surface of MSB (MSB/Co) and the crystal phase. SEM analysis shows the condensation of particles, which is the mechanical characteristic of Co-modified composite materials. In addition, HRTEM analysis showed that the surroundings of the MSB/Co composite are comprised of particles in a rod shaped thin film with amorphous regions. The rod shape of the cohesive thin film indicated the crystal structure of the composite by metallization of Co (Mabayoje et al., 2012). The Co of the MSB/Co composite not only provides excellent coherence but can also be an important element of structural capability for the miscibility of materials. EDX spectra index showed quantification of Co (68.7%), C (3.4%),

O (14.6%), and Cl (5.6%) in the structure of MSB/Co. The large amount of oxygen represents the presence of a hydroxyl functional group on the structure of the mediator, taking charge of the electron transfer with Co. MSB/Co/chitosan composite was produced from the covalent bond between the MSB/Co composite and chitosan. The chitosan was a linear hydrophilic polysaccharide induced by the deacetylation of natural biopolymer chitin. Especially, chitosan has a large amount of reactive amine groups playing an important role in ion sorption and dissolubility. The MSB/Co/chitosan composite, had chitosan coated on the surface of the MSB/Co composite. The EDX analysis showed 5.4% N, indicating the formation of amine groups. This result implies that MSB was successfully developed as a mediator.

3.4. Application of developed mediator

In order to evaluate the electron transfer of the developed mediator, it was applied to the electrodes of the EFC system. GOD and Lac were immobilized on the anode and cathode, respectively. The CV and power density were measured using 0.1 M glucose as a substrate in order to evaluate the voltage-current relationship and power production. CV was used to specify the voltage-current relationship and the mechanism of oxidation-reduction reaction. The CV at a voltage range of -0.6–0.6 V and a scan rate of 100 mV s^{-1}, indicates that the peak of the oxidation reaction drastically increased. The current and power density of the EFC system were 9.7 mA cm^{-2} and 3.1 mW cm^{-2}, respectively. Kim et al. (2015) used GO/Co/chitosan as mediators on electrodes, and as a result, showed a power density of 1.1 mW cm^{-2} in the EFC system. This value increased by 2.8-folds compared to when the GO was used as a mediator, indicating that MSB improves electron transfer.

In addition, the developed mediator was applied to a glucose biosensor for chronoamperometric measurement. Every successive addition of 4 mM glucose was performed at an actuation potential of 0.9 V. The solution was then stirred and the electrochemical reaction was recorded. The current density for the electrochemical oxidation of glucose increased depending on the glucose concentration, while the sensor sensitivity was measured to be 0.488 mA mM^{-1} cm^{-2}. Al-Sagur et al. (2018) and Lu et al. (2007) reported SiO_2(LuPc$_2$)PANI(PVIA)-CNB/GOx and exfoliated graphite nanoplatelets (xGnP) biosensor with sensitivity of 0.038 and 0.014 mA mM^{-1} cm^{-2}, respectively.

MSB can be used as a renewable and cheap precursor for the electron transfer mediator. The average price of biochar is \$2.65 kg^{-1} worldwide, ranging from \$0.09 (Philippines) to \$8.85 kg^{-1} (UK) depending on the place of origin (Ahmed, 2016). Activated biochar is cost-effective and could potentially be applied to various areas as a new environmentally-friendly carbon-based material. Compared to traditional activated carbon, the feedstock for biochar production is rich and can be obtained from cheap biomass and solid wastes. The performance of biochar applied to various fields has been reported to be equal to or higher than that of expensive commercial activated carbon, CNTs, and graphene. Thus, biochar is expected to play an important role based on its industrial and economic aspects.

4. Conclusions

This study shows that it is possible to carbonize sludge without the active components of microalgae and use it as the mediator composite of electrodes. The characteristics of the mediator synthesis of MSB/Co/chitosan were systematically investigated and analyzed. When the developed mediator was applied to the EFC system and glucose biosensor, the maximum power density was 3.1 mW cm^{-2} and sensitivity was 0.488 mA mM^{-1} cm^{-2}. The concept discussed in this study could use MSB in the development of future electrode materials. In addition, it is expected to solve environmental problems by utilizing recycled materials.

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