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Exploring optimal supplement strategy of medicinal herbs and tea extracts for bioelectricity generation in microbial fuel cells

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ABSTRACT

This first-attempt study used extracts of appropriate antioxidant abundant *Camellia* and non-*Camellia* tea and medicinal herbs as model ESs to stably intensify bioelectricity generation performance in microbial fuel cells (MFCs). As electron shuttles (ESs) could stimulate electron transport phenomena by significant reduction of electron transfer resistance, the efficiency of power generation for energy extraction in microbial fuel cells (MFCs) could be appreciably augmented. Using environmentally friendly natural bioresource as green bioresource of ESs is the most promising to sustainable practicability. As comparison of power-density profiles indicated, supplement of *Camellia* tea extracts would be the most appropriate, then followed non-*Camellia Chrysanthemum* tea and medicinal herbs. Antioxidant activities, total phenolic contents and power stimulating activities were all electrochemically associated. In particular, the extract of unfermented *Camellia* tea (i.e., green tea) was the most promising ESs to augment bioenergy extraction compared to other refreshing medicinal herb extracts.

1. Introduction

According to UN report, renewable energy would become a major portion of global energy sources to worldwide use. In particular, biomass-based energy was the most appropriate renewable energy for sustainable development. In fact, microbial fuel cell (MFC) is a bioelectrochemical system that directs an electric current obtained from organics oxidation by using indigenous electroactive bacteria and/or mixed consortia. It was also revealed that redox mediator (RM)-aided MFC was the most energy-saving and economically promising as supplementation of electron shuttles (ESs) would enhance effective electron transfer (ET) efficiency and significantly reduce internal transport resistance for power generation (Chen et al., 2016a , 2010; Han et al., 2015; Xu et al., 2014). ESs or RMs (e.g., catechol, riboflavins) are organic chemical(s) that can be reversibly oxidized and reduced to mediate ET phenomena as electroactive catalysts for energy extraction. That is, with supplementation of ESs, enhancement of ET capabilities could automatically escalate the efficiency of pollutant degradation and bioelectricity generation for electrochemically-steered bioremediation. However, augmentation of arctically synthesized ESs (e.g., methylene blue, neutral red, decolorized metabolites of textile dyes) to elevate rate of pollutant degradation would be apparently not environmentally appropriate due to undesired introduction of secondary contaminant(s) for treatment. If ES supplementation to enhance operation efficiency of MFC is inevitable, augmentation of naturally present ESs would be top-priority selection to minimize environmental impact to lives. Regarding chemical structures of ESs, when electron-withdrawing groups (e.g., hydroxyl (−OH) substituent(s)) were present on benzene ring *ortho* or *para* to each other, such chemical species would strongly exhibit electrochemically stable electron-shuttling characteristics to stimulate power generation in MFCs (Chen et al., 2013a,b; Qin et al., 2016; Xu et al., 2014). That is, in the presence of hydroquinone-like chemical structures, such chemicals could own promising electron-shuttling characteristics of ESs to stimulate ET efficiency in MFCs. Thus, using polyphenolics (i.e., −OH substituents)-rich medicinal herbs and edible flora (e.g., *Camellia* and non-*Camellia* tea) as ESs seemed to be more electrochemically viable to extract bioenergy from organics oxidation in MFCs. In addition, medicinal herbs – *Lonicera japonica* (Jīnyín-huā) and *Syzygium aromaticum* (Dīng-xiāng) (Chen et al., 2017a,b) contained significant amounts of polyphenolics and flavonoids antioxidants (Przygodzka et al., 2014; Shang et al., 2011). Thus, antioxidant and ES capabilities of polyphenolics-abundant natural bioresources might be considered as inducible electrochemical characteristics that could be manipulated by exogenous conditions for bioelectricity stimulation. That is, if ES activities could be expressed properly, the presence of polyphenolics species may also synergistically interact with

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electrochemical activities for ET stimulation in MFCs. As a matter of fact, major constituents of *Camellia* tea- catechins could play dual roles of either antioxidants or ESs at appropriate conditions (Chen et al., 2017b). Although Chen and Hsueh (2016) suggested plausible reasons to elucidate polyphenols-rich edible flora as ESs (e.g., flavonoids antioxidants as possible ESs) for bioenergy applications (e.g., MFCs, electrofermentation), comparison upon diverse natural bioresources to suggest promising candidates had still remained virtually open for discussion. Of course, this would lead to unpredictable practicability of using appropriate ES sources to maximize bioenergy extraction. Here, comparative assessment upon test samples exhibited that extracts of *Camellia* tea (e.g., green tea, oolong tea, Pu'er tea and black tea) would be the most appropriate to stimulate electron transport phenomena in MFCs. Follow-up studies would decipher whether only some crucial chemical species as main-effectors or synergistic interactions of several electrochemically active species directly augment power generation in MFCs.

2. Materials and methods

2.1. Preparation of edible plant extracts

To obtain extract samples, Camellia tea- Camellia sinensis (L.) Kuntze (Green tea), Oolong tea, Camelliaboreali yunnanica, Camellia assamica (Mast.) Chang and non-Camellia tea- Coreopsis tinctoria Nutt. (snow chrysanthemum; 'Kunlun Xunju'), Chrysanthemum (Chrysanthemum morifolium Ramat, "Chuju") and Bellis perennis and medicinal herbs-Lonicera japonica (Jīnyín huā or Rěndōng), Syzygium aromaticum (Dīng xiāng), Citrus reticulate (Chénpí) at 2.5 g were ground to be powdered and then dissolved in 50 mL distilled water and 50% ethanol solution for 65 °C total reflux about 2 h. Then, such mixtures of herb or tea extract were concentrated via reduced pressure of rotary evaporator. After cooling to ambient temperature, such extracts were centrifuged at 13,000 rpm, 25 °C 10 min to obtain supernatant. Such supernatants were then filtered via 0.2 μm filters (Nylon Acrodisk 13 MiniSpike, 13 mm Gelman Sci.) to remove residual particles. Deionized and distilled water was then added to have resultant solutions in 50 mL for study afterwards.

2.2. Cyclic voltammetric (CV) measurement

Medicinal herbs or tea extract-bearing solutions were first purged with nitrogen for 15 min to remove residual oxygen. CV electrochemical inspection were then implemented immediately for comparative inspection on electrochemical characteristics of different plant extracts. As medicinal herbs and tea extracts contained mixtures of diverse chemicals, ca. 6 CV scan cycles to oxidize or reduce unstable chemical species and to ensure reductive and oxidative potential peaks with stable electrochemical properties were conducted prior practical testing to be carried out. To exhibit electrochemical stability of test sample(s), cyclic voltammetry of samples for 100 cycles of scan was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 10 mV s^{-1} scan rate. The working, counter, and reference electrodes were a glassy carbon electrode (0.07 cm^2), platinum electrode (6.08 cm^2), and a Hg/Hg_2Cl_2 electrode filled with saturated $KCl_{(aq)}$, respectively. The glassy carbon electrode (GCE, ID = 3 mm; model CHI104, CH Instruments Inc., USA) was successively polished with 0.05 μm alumina polish and then rinsed with 0.5 M H_2SO_4 and deionized water prior to study. Experiments were performed in phosphate buffer solutions (PBS; pH 7.0) at 0.1 M and the solutions were purged with nitrogen for 15 min prior to analysis. The scanning rate was 10 mV s^{-1} over the designated range (e.g., +1.5 to −1.5 V). The redox potentials recorded as Hg/Hg_2Cl_2 reference electrode were corrected by 0.241 V (i.e., E_0 of Hg/Hg_2Cl_2) to the standard hydrogen electrode (SHE). To quantitatively evaluate electrochemical capabilities of candidate biomaterial(s) for comparative assessment, areas of redox potential curves in closed CV loops Area = $\int_{V_L}^{V_H}(i_H - i_L)dV$ were determined via SigmaPlot 10.0.

2.3. Total phenolic concentration (TPC)

Total phenolic concentration (TPC) of medicinal herbs and tea extracts was determined according to Naczk and Shahidi (2004). First, 0.25 mL of sample extracts was mixed with 0.25 mL Folin-Ciocalteu reagent which was previously diluted with distilled deionized water (1:1, v/v), 0.5 mL saturated sodium carbonate (Na_2CO_3) and 4 mL of water. The mixture was incubated at room temperature for 25 min and centrifuged at 2000 X*g* for 10 min. Supernatant absorbance was measured at λ = 725 nm using a spectrophotometer (GENESYS 10S UV-Vis). TPC was standardized against gallic acid (GA) and expressed in terms of GA equivalents (GAE).

2.4. MFC construction and microbial cultures

Membrane-free air cathode single-chamber MFCs were constructed in cylindrical tubes made by polymethyl methacrylate (PMMA) (cell sizing ID = 54 mm, L = 95 mm) with the operating volume of ca. 230 mL (i.e.,. $\frac{(5.4cm)^2\pi}{4} \times (9.5 + 2 \times 0.3)cm \approx 231.3mL$) Porous carbon cloth (CeTech) (without waterproofing or catalyst) with a projected area of ca. 22.9 cm^2 (i.e., $\pi \times 2.7^2$) on one side was used as anode electrodes. The air cathode was almost identical to the anode in size and consisted of a polytetrafluoroethylene (PTFE) diffusion layer (CeTech) on the air-facing side.

Detailed procedures of bacterial cultures for MFCs (e.g., bacterial acclimation, cell immobilization, bioelectricity stimulation) were described elsewhere (Chen et al., 2010). Culture medium in MFCs used in the study (unit: g L^{-1}) is LB (tryptone 10, yeast extract 5, sodium chloride 10). LB medium was used for cell growth and organic matter provided for energy sources. MFC was seeded with *Shewanella haliotis* WLP72 as nanowire-generating bacterium for bioelectricity generation. A loopful of bacterial seed taken from an isolated colony on a LB-streak plate (i.e., LB medium supplemented with Bacto agar 20 g L^{-1}) was precultured in 50 mL LB broth (pH 7.0 ± 0.2) using 250 mL Erlenmeyer flask for 12 h overnight at 30 °C, 125 rpm. After 12 h preculture, 2.2 mL cell broth was taken for inoculating 220 mL LB medium in MFC. MFC at 25 °C was operated with batch-fed mode of impulse addition of 5 mL 8.8 × LB medium every two days to maintain 0.2 × LB medium. MFC reactors were operated in ambient temperature and stably acclimatized for more than 2.5 years. That is, electrochemically active cultures in MFCs for stable power generation were achieved in conditions of "ecologically stable equilibrium".

2.5. Electrochemical measurements

Power generation measurement: Cell voltage was automatically measured (set at one data point per minute) using a data acquisition system (DAS 5020; Jiehan Tech. Corp.) through external resistance R_{out} = 1KΩ. Note that a relatively high resistance (1000 ohms) was intentionally used in order to compare with prior results. The power densities (P) and current densities (I) of MFCs were determined by P = (V × C)/A and I = C/A using linear sweep voltammetry (LSV) measurement and the corresponding voltages were recorded using a multimeter (V and C denoted electric voltage and current, respectively). Note that all MFCs were operated in model of membrane-less single chamber at 25 °C. To implement feasibility study, 5 mL 8.8 × LB medium with aforementioned extract in appropriate concentrations was supplemented to MFC (all of extract concentrations set at 5% (v/v)). Then, after 2 h response delay for supplement to achieve maximal voltage, comparative MFC study was then carried out.