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Feasibility study on biostimulation of dye decolorization and bioelectricity generation by using decolorized metabolites of edible flora-extracts

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ABSTRACT

As known, naturally-present edible flora contained antioxidants (*e.g.*, polyphenolic compounds) that are significant to human health. Prior studies revealed that decolorized metabolites (DM) of textile dyes might stimulate electron transfer (ET) capabilities of reductive decolorization and bioelectricity generation (RD&BG) due to their roles as electron shuttles (ESs). Such ET stimulating phenomena were also suspected to be associated with characteristics of antioxidant. This first-attempt study selected 6 edible flora to consider whether antioxidant-containing herbs were feasible to be used for wastewater decolorization. Apparently, DM of *Gynura bicolor* showed promising electron-shuttling capabilities to increase ET efficiency of RD&BG. Moreover, according to cyclic voltammetric profiles, the dosage should be above threshold level to trigger effective ET performance. In addition, supplementation of sufficient DM of *G. bicolor* could significantly enhance the efficiency of RD of Reactive Black 5 (RBk5) and BG. This study suggested that supplementation of extracts of naturally present edible flora may act as ESs to stimulate RD and BG for environmentally-friendly bioremediation.

1. Introduction

As known, natural sources of antioxidants with health benefits are usually present in food. They can safely interact with free radicals and terminate chain reactions to resist damage of vital molecules in cells (Oroian and Escriche [1]). As a matter of fact, significant portions of such natural antioxidants are polyphenolics-rich chemical species with electrochemical characteristics. As prior studies indicated, phenolic compounds were very likely to be electron shuttles for myriads of applicability. Thus, this first-attempt study tended to disclose mysteries between antioxidants and electron shuttles (ESs) for further applications (*e.g.*, electrochemically-steered bioremediation or fermentation).

Regarding ESs and antioxidants, ESs (or redox mediators; RMs) are organic chemical(s) that can be reversibly interconverted between oxidized and reduced form(s) to mediate electron transfer processes for energy extraction/recycling. As prior studies [2–5] indicated, for effective mechanisms of electron mediating in benzene ring should at least carry hydroxyl or amino groups (*i.e.*, reduced form) *ortho* or *para* to each other to form an anion (*e.g.*, hydroquinonate anion in Fig. 2 of Chen et al. [2]). These functional groups could be oxidized to form stable active radicals, then reoxidized to form carbonyl (C=O) or iminium ions (*i.e.*, oxidized form) for electron shuttling. In addition, dimerization of 1,2-diaminobenzene (1,2db) could result in a resonant form for effective electron transfer as redox mediator [3–5]. In fact, similar iminium-associated electron transfer as redox mediators could also be stably formed for thionine-related chemicals (*e.g.*, azure A, and azure C; Chen et al. [3]) as electron shuttles. In addition, regarding hydroxyl group-containing chemicals, catechol group in many polyphenols present in edible herbs could also act as electron donors scavenging free radicals and active oxygen groups (*e.g.*, scavenging species that initiate peroxidation, quenching $\bullet O_2^-$, preventing formation of peroxides; Brewer [6]) and thus is responsible for antioxidant activity. This study explored the feasibility of such irreversible characteristics to be functioned at appropriate conditions as reversible ET capabilities (Chen and Hsueh [7]) to be used in natural bioremediation and biomass energy for cradle-to-cradle applicability.

In fact, electron shuttles play a catalytic role to decrease activation energy and accelerate oxidation rate of organic pollutants. In contrast, antioxidants as electron donors (or reductants) could scavenge free radicals and active oxygen groups as aforementioned. Due to the presence of oxygen, aerobic environments would suppress ES characteristics to be triggered and only nature of antioxidants to be effectively functioning in particular very likely for

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Table 1
Comparative analysis on decolorization performance of six edible plant extracts using dye decolorizers (number denoted % of color removal)[↑].

Bacterial decolorizer	P.16	P.15	MSU3	K2	KB23	NIU01	YTl1	WLP72
Gynura bicolor	30[d]	30[c],50[d]	20[c],70[d]	90[b]	60[b],90[c]	20[c],50[d]	50[b],90[c]	40[b],90[c]
Hibiscus sabdariffa	–	20[c],50[d]	10[c],30[d]	20[d]	20[d]	30[d]	10[c],50[d]	20[c],70[d]
Coptidis Rhizoma	–	–	–	20[d]	10[d]	–	10[d]	20[d]
Curcuma longa	–	–	10[d]	20[d]	–	–	–	30[d]
Beta vulgaris	20[b],80[c]	30[b],90[c]	10[c],40[d]	60[c],90[d]	20[c],70[d]	50[c],90[d]	50[b],90[c]	60[b],90[c]
Camelliaboreali -yunnanica	10[d]	30[b],90[c]	40[c],80[d]	20[c],50[d]	20[c],60[d]	50[b],90[c]	40[c],70[d]	20[b],90[c]

[a]12 h shaking culture.
[↑] Number denoted % of color removal.
[b] 12 h shaking culture, then 12 h static incubation.
[c] 12 h shaking culture, then 24 h static incubation.
[d] 12 h shaking culture, then 36 h static incubation.

polyphenol-rich edible flora. It was thus suspected that whether some chemical species was also viable to be ES could be tested via cyclic voltammetric measurement at oxygen-free conditions. For example of azo dye decolorization, if antioxidants are going to be functioned as ESs in the presence of suitable electron donor(s) and acceptor(s) at proper environments (*e.g.*, $E_{Reductant} < E_0' < E_{Oxidant}$ or $E_{Microbe} < E_{RM} < E_{Pollutant}$), appropriate oxidation–reduction potentials E_0' of the ES for azo reduction should be satisfied [8]. In fact, the E_0' of candidate mediator should not be much lower than biological reducing system (*e.g.*, −320 mV for NAD(P)H) or bulk reductant (*e.g.*, −270 mV of sulfide) (refer to Table 1 in [7]). Moreover, the mediator's E_0' should not be much higher than that of azo dye for reduction at sufficient rates. As a matter of fact, Watanabe et al. [9] also mentioned the schematics of ES-assisted microbial processes in oxidation–reduction mediation among multiple reaction species for catalysis. Apparently, this appropriate E_0 value for electron mediation may still depend upon nature of the target oxidant and/or reductant for effective ET capability to lower activation energy and increase rate of for reaction (Watanabe et al. [9]).

Moreover, prior studies [5,10,11] indicated that decolorized metabolites (DM) of azo dyes (*e.g.*, aromatic amines, 1-amino-2-naphthol (1A2N) and 4-amino-1-naphthol (4A1N) as DM of azo dye—orange I and II, respectively) could act as electron shuttles (ESs) to stimulate reductive decolorization and bioelectricity generation (RD&BG) in microbial fuel cells (MFCs)-aided bioremediation. However, these artificially synthesized dyes and derived intermediates might be still mutagenic or biotoxic to lives worldwide. Therefore, extracts of naturally present plants/herbs as possible sources of ESs would be more environmentally friendly and ecologically acceptable for practicability. As a matter of fact, when hydroxyl groups were present on the benzene ring of the compounds (*e.g.*, 2AP, 4AP, hydroquinone), the molecules can express significant electron-shuttling capabilities as ESs to augment BG and RD in MFCs [2]. Moreover, some natural edible flora contained pigments with chromophore (s) (*e.g.*, electron transfer (ET)-oriented functional substituent(s)). Thus, they are very likely to own ET-capabilities to reversibly mediate electrons between electron donor and acceptor as ESs [9]. For example, betalain-containing red beetroot (*Beta vulgaris* L.; Tián-Cài-Hóng) and catechin-bearing Yunnan black tea (red-flowered wild *Camellia boreali-yunnanica*; Diān-Hong or Diān-Shān-Chá) should be feasible as ESs or antioxidants as proper condition (*e.g.*, major compositions of tea extract—catechin as ES at pH ∼ 5–8 and antioxidant at pH ∼ 10–13 as indicated in [12,13]). As known, plants are usually abundant in polyphenols and of course have significant characteristics of antioxidants scavenging free radicals and active oxygen groups. Moreover, such properties were also possibly associated to ET capabilities to stimulate performance of reductive degradation and bioelectricity generation in MFC-assisted bioremediation [9]. Thus, this first-attempt study tended to explore such edible plant extracts and derived DM as possible environmentally friendly ESs to enhance MFC-assisted wastewater decolorization and bioelectricity generation. In particular, this study chose some pigments-bearing edible plants as sources of natural dyes for feasibility assessment (*e.g.*, Gynura's Deux Couleurs (*G. bicolor*; Hóng Fèng Cài), roselle (*Hibisus sabdriffa* L.). In fact, leaves of *G. bicolor* and *H. sabdriffa* L. (rosella or rosella fruit in Mandarin Chinese *Méi-Guī-Qié*) contained abundant amounts of pigment-antioxidants—anthocyanidins [14,15]. In addition, many Chinese medicinal herbs were also rich in components of antioxidants (*e.g.*, polyphenols, antioxidant vitamins, β-carotene, flavonoids, anthocyanins); therefore, this study also chose turmeric (*Curcuma longa*; Jiānghuáng) and coptis root (*Coptidis rhizoma*; Huánglián) as natural plants to evaluate whether intact extracts or DM could act as ESs for applications to MFC-aided pollutant degradation for sustainable wastewater treatment.

2. Materials and methods

2.1. Preparation of edible plant extracts and juices

G. bicolor (Hóng Fèng Cài; purchased from Yuanshan Village, I-Lan, Taiwan) 20 g, *B. vulgaris* L. (Tián-Cài-Hóng) 20 g, *C. longa* (Jiāng Huáng) 1 g, *C. boreali-yunnanica* (Diān- Hóng) 1 g, *Coptidis rhizome* (Huánglián) 1 g, *H. sabdriffa* L. (Méi Guī Qié) 1 g were ground to be powder and then boiled in distilled water at 100 °C for 10 min to obtain model extracts. After cooling to ambient temperature, such mixtures of plant extracts were centrifuged at 13,000 rpm, 25 °C 10 min to obtain supernatant. Such supernatants were first filtered through Whatman No. 1 paper and then filtered via 0.24 μm sterile filter to remove residual particles and microbes for study afterward.

2.2. Microbial cultures and screening upon decolorizing capabilities

To obtain stable synchrony of cultures for feasibility study, a loopful of *Aeromonas hydrophila* YTl1, *Exiguobacterium acetylicum* K2, *Aeromonas hydrophila* KB23, *Shewanella haliotis* WLP72, *Planococcus rifietoensis* P.15, *Aeromonas* sp. NIU01, *Psychrobacter* sp. P.16, *Aeromonas salmonicida* MSU3 seed taken from an isolated colony on a LB-streak plate was precultured in 50 mL Bacto LB broth, Miller (Luria-Bertani) (per liter; 10 g Bacto tryptone, 5 g Bacto yeast extract, 10 g sodium chloride) for 24 h at 30 °C, pH 7.0, 125 rpm using a water bath shaker (SHINKWANG, SKW-12). To obtain the most decolorizable plant extracts and the most promising bacteria for color removal, well-precultured cell broth in 0.05 mL of 8 bacterial strains YTl1, K2, NIU01, KB23, WLP72, P.15, P.16 and MSU3 were used to decolorize 6 plant extracts (*ca.* 0.5 mL) in LB broth medium (5 mL) as aforementioned.

2.3. Shake-flask decolorization

To explore whether decolorized intermediates of plant extracts could stimulate dye decolorization of Reactive Black 5 (RBk5),

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