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The extent of fermentative transformation of phenolic compounds in the bioanode controls exoelectrogenic activity in a microbial electrolysis cell

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ABSTRACT

Phenolic compounds in hydrolysate/pyrolysate and wastewater streams produced during the pretreatment of lignocellulosic biomass for biofuel production present a significant challenge in downstream processes. Bioelectrochemical systems are increasingly recognized as an alternative technology to handle biomass-derived streams and to promote water reuse in biofuel production. Thus, a thorough understanding of the fate of phenolic compounds in bioanodes is urgently needed. The present study investigated the biotransformation of three structurally similar phenolic compounds (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA), and their individual contribution to exoelectrogenesis in a microbial electrolysis cell (MEC) bioanode. Fermentation of SA resulted in the highest exoelectrogenic activity among the three compounds tested, with 50% of the electron equivalents converted to current, compared to 12 and 9% for VA and HBA, respectively. The biotransformation of SA, VA and HBA was initiated by demethylation and decarboxylation reactions common to all three compounds, resulting in their corresponding hydroxylated analogs. SA was transformed to pyrogallol (1,2,3-trihydroxybenzene), whose aromatic ring was then cleaved via a phloroglucinol pathway, resulting in acetate production, which was then used in exoelectrogenesis. In contrast, more than 80% of VA and HBA was converted to catechol (1,2-dihydroxybenzene) and phenol (hydroxybenzene) as their respective dead-end products. The persistence of catechol and phenol is explained by the fact that the phloroglucinol pathway does not apply to di- or mono-hydroxylated benzenes. Previously reported, alternative ring-cleaving pathways were either absent in the bioanode microbial community or unfavorable due to high energy-demand reactions. With the exception of acetate oxidation, all biotransformation steps in the bioanode occurred via fermentation, independently of exoelectrogenesis. Therefore, the observed exoelectrogenic activity in batch runs conducted with SA, VA and HBA was controlled by the extent of fermentative transformation of the three phenolic compounds in the bioanode, which is related to the number and position of the methoxy and hydroxyl substituents.

1. Introduction

Lignocellulosic biomass is an abundant renewable source for the production of biofuels, providing an important alternative to fossil fuels. Biomass conversion to biofuels via thermochemical processes, such as hydrolysis and pyrolysis, produces streams bearing phenolic compounds, because of lignin break-down (Monlau et al., 2014). As a result, phenolic alcohols, aldehydes, and acids are widely present in biomass-derived streams (e.g., hydrolysates, pyrolysates, and biorefinery wastewater) at concentrations ranging from milligrams per liter to several grams per liter (Ren et al., 2016; Mills et al., 2009; Borole et al., 2013). Phenolic compounds contribute to the instability and corrosiveness of pyrolysis-derived bio-oil and the observed inhibition of biomass hydrolysates to microorganisms involved in dark fermentation for H_2 and ethanol production (Jones et al., 2009; Monlau et al., 2014). Thus, phenolic compounds are regarded as a major problem in the use of biomass for biofuel production. Solvent extraction is currently practiced to

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remove phenolic compounds from hydrolysates and pyrolysates, which produces high volumes of waste streams rich in organic compounds, resulting in challenging wastewater treatment and disposal practices (Borole and Mielenz, 2011).

Microbial electrolysis cell (MEC) is a bioelectrochemical technology, which degrades organic compounds through microbial activity in the anode producing hydrogen gas (H_2) in the cathode with a small voltage input (Wang and Ren, 2013; Zhang and Angelidaki, 2014). Several studies have demonstrated significant benefits of integrating MEC technology in biofuel production. For example, MECs were studied as an alternative to or in combination with dark fermentation for enhanced H_2 production from wheat straw and corn stover hydrolysates (Lalaurette et al., 2009; Thygesen et al., 2011). A MEC was used to convert acidic and polar components of a switchgrass pyrolysate aqueous phase to renewable H_2 that can then be used in the downstream hydrogenation of bio-oil, thus reducing the need for an external H_2 supply, which is currently produced from natural gas (i.e., methane), a non-renewable fossil fuel (Lewis et al., 2015). Another potential use of MEC in biofuel production is to recycle effluent from biomass pretreatment and fermentation, thus improving water reuse in biorefineries (Borole and Mielenz, 2011).

As MEC is increasingly used with biomass-derived streams, a thorough understanding of the fate of phenolic compounds in MEC bioanodes is needed. Removal of phenolic compounds in microbial fuel cell (MFC) bioanodes has been reported in several studies (Song et al., 2014; Friman et al., 2013; Huang et al., 2011; Borole et al., 2009). Despite the reported disappearance of the parent phenolic compounds, the biotransformation extent and pathways of individual compounds were not examined in the above-mentioned studies. It is generally accepted that when the bioanode substrate is a complex organic compound, fermentation is required to convert the complex compound to mainly acetate, which can then be used in exoelectrogenesis to produce current (Kiely et al., 2011; Ren et al., 2007). Our previous studies, using a mixture of two furanic (furfural, 5-hydroxymethyl furfural) and three phenolic (syringic, vanillic, and 4-hydroxybenzoic acids) compounds, demonstrated that the phenolic compounds were not directly utilized by bioanode exoelectrogens. Instead, fermentation in the bioanode produced acetate, which was then used as an exoelectrogenic substrate (Zeng et al., 2015, 2016a) Although several phenolic metabolites were reported, the fate of the individual phenolic compounds was not elucidated, because the phenolic compounds were used as a mixture (Zeng et al., 2015). Marone et al. (2016) reported the removal of hydroxytyrosol and tyrosol from table olive brine processing wastewater treated by a bioelectrochemical system, with the detection of 3,4-hydroxyphenyl acetic acid, 3,4-dihydroxybenzaldehyde, and *p*-hydroxyphenyl acetic acid as biotransformation products. A MFC study conducted by Hedbavna et al. (2016) showed that phenol, cresols and xylenols in groundwater were biotransformed to 4-hydroxybenzoic acid and 4-hydroxy-3-methylbenzoic acid. In this case, oxygen, nitrate, iron (III), and sulfate were present as electron acceptors in addition to the bioanode electrode.

Despite the knowledge gained from previous studies, important questions remain unanswered relative to the fate of phenolic compounds in bioanodes: (1) To what extent are these compounds transformed (e.g., aromatic rings cleaved or not)? (2) What are the biotransformation pathways? (3) Are there any limiting steps in the biotransformation pathways of these compounds in bioanodes impacting exoelectrogenic activity? To address these questions, the objective of the present study was to assess the extent of biotransformation and metabolic pathways of three phenolic compounds (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA), as well as their individual contribution to exoelectrogenesis in a MEC bioanode.

2. Material and methods

2.1. Chemicals

SA, VA and HBA were used as phenolic substrates fed in the MEC bioanode (Table 1). SA, VA and HBA have similar chemical structures, but differ in the number and position of hydroxyl (−OH) and methoxy ($-O-CH_3$) substituents, representing phenolic compounds derived from different lignin units (syringyl, guaiacyl and *p*-hydroxyphenyl, respectively) during the pretreatment of lignocellulosic biomass (Monlau et al., 2014). Analytical standards of 3,4-dihydroxy-5-methoxybenzoic acid (DHMBA), gallic acid (GA), protocatechuic acid (PA), pyrogallol, catechol, phenol, phloroglucinol, resorcinol, and benzoic acid were used as reference for the identification of SA, VA and HBA metabolites (Supplementary Material, Table S1). All chemicals were purchased from either Sigma-Aldrich (St. Louis, MO) or Alfa Aesar (Ward Hill, MA).

2.2. MEC

An H-type dual-chamber MEC was developed using two glass bottles (liquid volume of 200 mL for the anode and 250 mL for the cathode), an anode electrode made of a bundle of 5 stripes of carbon felt (1 cm × 1 cm × 5 cm, each), a cathode electrode made of platinum-coated carbon cloth (5 cm × 6 cm), and a Nafion 117 cation exchange membrane (projected surface area of 5.7 cm^2). The MEC configuration and materials were previously described in detail (Zeng et al., 2015). The anolyte was a mineral microbial growth medium consisting of the following (g/L): NH_4Cl, 0.31; KCl, 0.13; $NaH_2PO_4 \cdot H_2O$, 2.45; and Na_2HPO_4, 4.58; along with trace metals and vitamins (pH 7.0) (Zeng et al., 2015). The catholyte was a 100 mM sodium phosphate buffer, pH 7.0. The anolyte and catholyte stock solutions were both autoclaved and deoxygenated by bubbling with N_2 for 30 min before being transferred to the MEC. A glass burette, filled with acid brine (10% NaCl w/v, 2% H_2SO_4 v/v), was connected to each chamber headspace for gas collection and measurement by liquid displacement. A potentiostat (Interface 1000, Gamry Instruments, Warminster, PA) was used to set a voltage of 0.6 V at the anode against the cathode in a two-electrode setup. A reference electrode (Ag/AgCl, 0.199 V SHE) was inserted in the anode chamber for measuring anode and cathode potentials and conducting anode cyclic voltammetry.

Enrichment of the MEC bioanode microbial community was initiated in a MFC, using an inoculum from a different MFC anode, which had been fed with a switchgrass pyrolysis waste stream bearing levoglucosan, ketones, volatile fatty acids, as well as furanic and phenolic compounds (Lewis et al., 2015). The MFC used for enrichment in the present study was fed with a mixture of two furanic and three phenolic compounds (furfural, 5-hydroxymethylfurfural, SA, VA and HBA), as previously reported (Zeng et al., 2015). After 6 months of enrichment, a piece of MFC biofilm-attached carbon felt electrode (1 cm × 1 cm × 2 cm) was cut using sterile scissors and anaerobically transferred to the center of the MEC anode electrode used in the present study. The MEC bioanode was then consistently fed with the above-described mixture of furanic and phenolic compounds, maintained at room temperature (20–22 °C). During the 2-year MEC operation, the total biomass concentration in the anode chamber increased from 52.7 ± 0.9 to 352 ± 10 mg/L measured as protein, approximately 90% of which was associated with the biofilm. The MEC bioanode biofilm-associated microbial community was analyzed at 7 and 13 months of operation, following the procedure described in Supplementary Material, Text S1.