Processes and Electron Flow in a Microbial Electrolysis Cell Bioanode Fed with Furanic and Phenolic Compounds

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ABSTRACT
Furanic and phenolic compounds are problematic compounds resulting from the pretreatment of lignocellulosic biomass for biofuel production. Microbial electrolysis cell (MEC) is a promising technology to convert furanic and phenolic compounds to renewable H_2. The objective of the research presented here was to elucidate the processes and electron equivalents flow during the conversion of two furanic (furfural, FF; 5-hydroxymethyl furfural, HMF) and three phenolic (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA) compounds in the MEC bioanode. Cyclic voltammograms of the bioanode demonstrated that electrochemical reactions in the biofilm attached to the electrode were negligible. Instead, microbial reactions (i.e., fermentation followed by exoelectrogenesis) were the primary processes resulting in the electron equivalents flow in the MEC bioanode fed with the furanic and phenolic compounds. The distribution of electron equivalents during the fermentation and exoelectrogenesis were further quantified for each of the five compounds. More than 50% of the SA, FF, and HMF electron equivalents were converted to current. In contrast, only 12 and 9% of VA and HBA electron equivalents, respectively, resulted in current production, while 76 and 79% remained in fermentation products without being further utilized in exoelectrogenesis. For all five compounds, it was estimated that 10% of the initial electron equivalents were used for fermentative biomass synthesis, while 2 to 13% were used for exoelectrogenic biomass. The proposed mass-based framework of substrate utilization and electron flow provides an important foundation for the simulation of bioanode processes to guide the optimization of MECs converting biomass-derived waste streams to renewable H_2.

KEYWORDS
MEC; electron balance; fermentation; exoelectrogenesis

INTRODUCTION
Lignocellulosic biomass is an abundant, renewable energy source for biofuel production, providing an important alternative to fossil fuels. However, pretreatment of biomass for biofuel production produces furanic and phenolic compounds, which contribute to the corrosiveness, instability, and toxicity of various biomass-derived streams, and thus present a significant challenge in downstream processes and waste disposal [1-3]. Microbial electrolysis cell (MEC) is an emerging bioelectrochemical technology, which converts organic wastes in the bioanode and produces H_2 in the abiotic cathode with a small voltage input (0.3 – 1.0 V) [4]. Integration of MEC in biofuel production not only offers an alternative method for waste handling, but also contributes to the production of renewable H_2 for the downstream hydrogenation of biomass-derived bio-oil, thus reducing the external H_2 supply currently derived from natural gas (i.e., methane), a non-renewable fossil fuel [5,6]. In addition, furanic and phenolic compounds are highly inhibitory to H_2-producing microorganisms in dark fermentation [3]. MEC has the advantage of circumventing such microbial inhibition by producing H_2 in the abiotic cathode.

The potential of MEC technology has been demonstrated with a variety of biomass-derived waste streams, including lignocellulosic effluent, refinery wastewater and switchgrass-derived bio-oil aqueous phase [7,8,5]. The fate and effect of furanic and phenolic compounds, which are among the most problematic and challenging components of biomass-derived waste streams, have been investigated in our MEC studies with two furanic (furfural, FF; 5-hydroxymethyl furfural, HMF) and three phenolic (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA) compounds. The biotransformation of the five compounds in the MEC bioanode consisted of two sequential sub-processes: fermentation followed by exoelectrogenesis. Fermentation of the parent compounds resulted in fermentation products, among which acetate was the major substrate used in the subsequent exoelectrogenesis to produce electric current or cathodic H_2 [9]. However, these compounds were fermented to a

different extent, resulting in different levels of exoelectrogenic activity (i.e., H_2 production) [10]. The different extent of fermentation was due to the difference in fermentation pathways for these compounds, as opposed to inhibition, which affected mainly exoelectrogenesis at concentrations higher than 1.2 g/L [11,10]. Based on the findings of previous studies, electron equivalents of the parent compounds were extracted via fermentation and exoelectrogenesis, and were then transferred to the bioanode electrode. However, the distribution of different constituents in the overall electron equivalents flow has not been sufficiently quantified. In addition to the biotransformation, non-biological processes in the bioanode need to be evaluated for their potential contribution to the overall electron equivalents flow.

Four possible processes may take place in the MEC bioanode: adsorption to the electrode, electrochemical reactions on the bioanode electrode (independent of microbial reactions), electrode-independent microbial reactions, and electrode-dependent microbial reactions (Fig. 1). As demonstrated in our previous study, adsorption of the five compounds to the carbon felt used as the anode electrode did not take place [9]. Microbial reactions were determined to be fermentation (electrode-independent) followed by exoelectrogenesis (electrode-dependent) [10]. However, the extent of electrochemical reactions (independent of microbial reactions) was not fully evaluated. A MEC with an un-inoculated anode electrode produced negligible current in the presence of the five furanic and phenolic compounds, suggesting that the presence of the bioanode microbial community was essential for the observed electroactivity [9]. However, it is noteworthy that the formation of biofilm on the electrode could have modified the electrode-anolyte interface and thus enabled electrochemical reactions. This process is different from microbial utilization of the compounds and is considered as a different path in the overall bioanode flow of electron equivalents. Therefore, whether or not and to what extent the electron equivalents of the five compounds were consumed in electrochemical reactions, as opposed to biologically-mediated reactions, needs to be further evaluated.

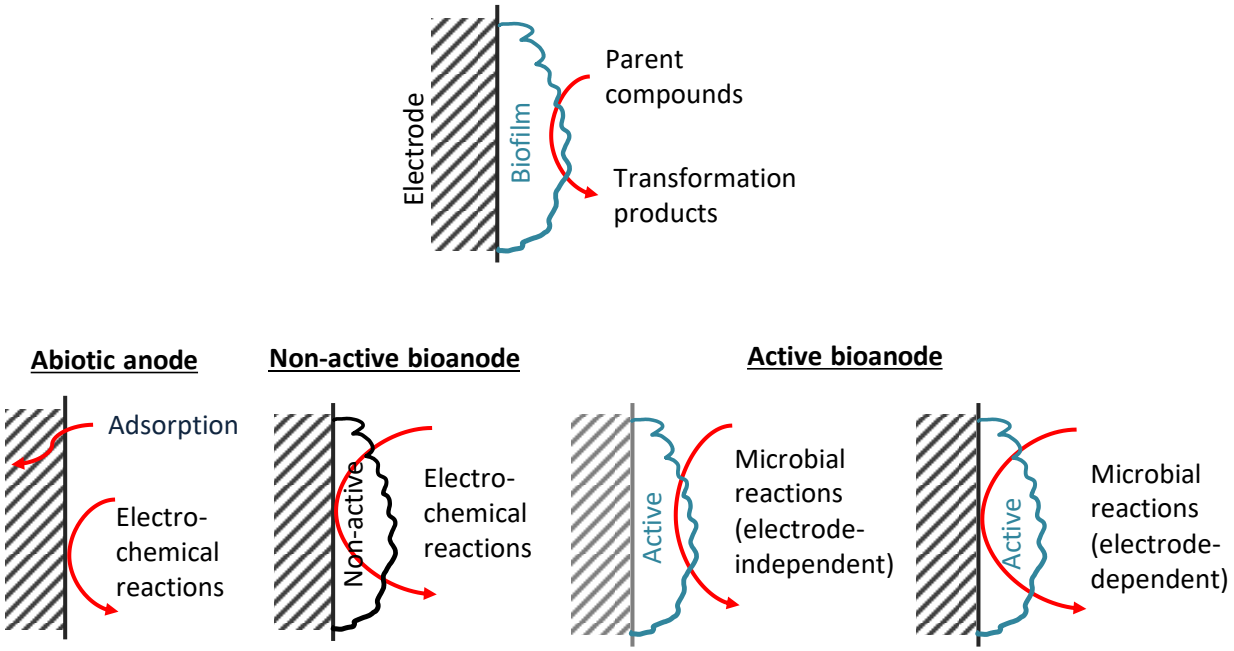

Fig. 1 Possible processes involved in the electron equivalents flow in the MEC bioanode fed with furanic and phenolic compounds

In MEC studies, chemical oxygen demand (COD) removal and Coulombic efficiency are commonly used parameters for the quantification of total electron equivalents extracted from substrates and those used in current production [12]. However, COD removal and Coulombic efficiency do not account for electron equivalents used in biomass synthesis, nor can they be used to perform electron balances for individual processes in the bioanode (e.g., fermentation and exoelectrogenesis). In a special case where glucose served as the bioanode substrate completely degraded to known, specific biotransformation intermediates, electron balances could be performed based on stoichiometry [13]. However, in the present study, furanic and phenolic compounds were partially biodegraded in bioanodes, and their biotransformation products have not been fully identified [9,10]. Therefore, a new approach is

needed to quantify the electron equivalents flow in bioanodes for organic substrates undergoing a variable extent of biotransformation.

The objective of the research presented here was to elucidate the processes and electron equivalents flow during the conversion of the above-mentioned two furanic and three phenolic compounds in a MEC bioanode. A mass-based framework of substrate utilization and electron equivalents flow in a MEC bioanode was developed, which can be applied to a wide range of complex organic compounds used as MEC bioanode substrates.

MATERIALS AND METHODS

MEC. An H-type MEC, as previously described in detail [9], was used in the present study. The MEC consisted of two glass chambers separated by a Nafion 117 cation exchange membrane (projected surface area of 5.7 cm^2). In the anode chamber, an electrode made of a bundle of 5 stripes of carbon felt (1 cm × 1 cm × 5 cm, each) was submerged in the anolyte (200 mL). In the cathode chamber, an electrode made of platinum-coated carbon cloth (5 cm × 6 cm) was placed in the catholyte (250 mL). 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). The catholyte was a 100 mM sodium phosphate buffer, pH 7.0. The anolyte and catholyte stock solutions, which were both autoclaved and deoxygenated by bubbling with N_2 for 30 min, were transferred to the MEC. A glass burette, filled with an acid brine solution (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 Ag/AgCl reference electrode (6 mm diameter; 0.199 V SHE; BASi, West Lafayette, IN) was inserted in the anode chamber for conducting anode cyclic voltammetry (CV) tests.

The MEC bioanode was inoculated with an electroactive biofilm pre-enriched with a mixture of the five furanic and phenolic compounds in the bioanode of a microbial fuel cell. After the MEC startup, the bioanode was consistently fed with the mixture of the five compounds (total concentration of 200 - 800 mg/L) at room temperature (20-22°C) for over 2 years, except for short-term experiments conducted with individual compounds. The total biomass concentration in the anode chamber, measured before the tests described in the present study, was 352 ± 10 mg/L as protein, approximately 90% of which was associated with the biofilm. The MEC bioanode biofilm microbial community consisted of fermentative and exoelectrogenic bacteria as previously described [10].

Assessment of Electrochemical Reactions Using Cyclic Voltammetry. CV was conducted with the MEC to distinguish possible electrochemical reactions due to non-active biofilm formation on the anode electrode surface from those due to microbial activities. CV tests were conducted under three sequential bioanode conditions: (1) after being starved for 1 week, i.e., non-active and unfed; (2) after being fed with substrates and mixing for 5 minutes, i.e., non-active and upon feeding; (3) after incubation for 1 day when the bioanode produced significant current, i.e., fully active. Two types of substrates were tested: a mixture of furanic and phenolic compounds (at equal electron equivalents and a total concentration of 200 mg/L); and sodium acetate (200 mg/L). The potentiostat was connected to the MEC in a three-electrode setup for conducting the CV. The working electrode lead was connected to the MEC anode, the counter electrode lead connected to the MEC cathode, and the reference electrode lead connected to the Ag/AgCl electrode. The anolyte and catholyte were not mixed during the cyclic voltammetry tests. The potential scan was changed from - 0.7 to + 0.3 V, and then to -0.7 V (vs. Ag/AgCl) at a scan rate of 5 mV/s.

Development of Electron Equivalents Flow and Mass Balance. Based on the observation that electrochemical reactions were negligible (discussed in Section 3.1, below), a framework of substrate utilization and distribution into biomass and other products in the MEC bioanode was developed as presented in Fig. 2. The parent compound (S_p) was first fermented to exoelectrogenic substrates (S_e; e.g., acetate) and non-exoelectrogenic end products (S_{ne}; e.g., aromatic fermentation products). Fermentative biomass (X_f) was produced with an observed biomass yield coefficient of $Y_{obs,1}$. In the second step, only exoelectrogenic substrates were utilized to produce electric current (I), which in turn resulted in cathodic H_2 production. Exoelectrogenic biomass (X_e) was produced with an observed biomass yield coefficient of $Y_{obs,2}$. All coefficients and variables are expressed in COD units to illustrate the distribution of electron equivalents. S_p in COD units was calculated as the product of the amount of the parent compound utilized and the electron equivalence of the compound. I in COD units was calculated based on the total electrons recovered as current over the incubation period.

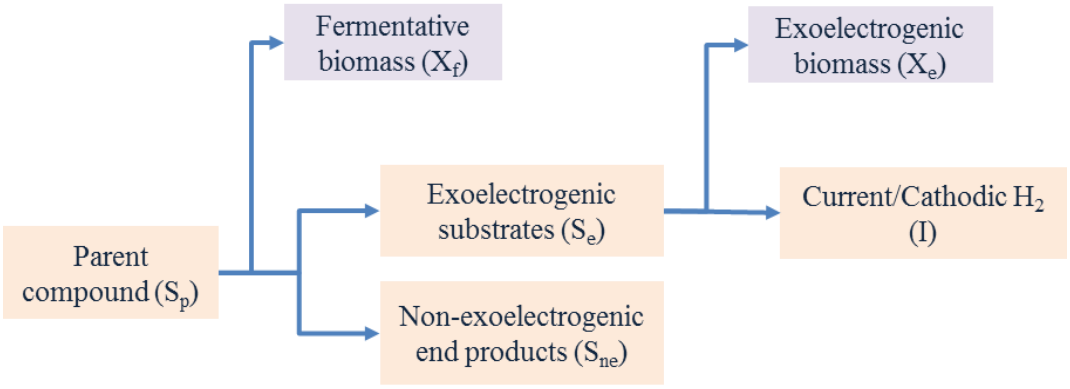

Fig. 2 Substrate utilization and electron equivalents flow in a MEC bioanode in two sequential sub-processes: fermentation, followed by exoelectrogenesis

To quantify each component in the electron equivalents flow, a mass balance was performed for the two sub-processes (i.e., fermentation and exoelectrogenesis). Biomass production was expressed using Y_{obs} and the respective substrate utilized, as follows:

$$X_f = Y_{obs,1} S_p \quad (1)$$

$$X_e = Y_{obs,2} S_e \quad (2)$$

The mass balance equation during the exoelectrogenic step was as follows:

$$S_e = X_e + I = Y_{obs,2} S_e + I \quad (3)$$

With the definition of anode efficiency, $\alpha = I/S_p$ [10], I was expressed as

$$I = \alpha S_p \quad (4)$$

By substituting Equation (4) to Equation (3), S_e was expressed in terms of S_p, $Y_{obs,2}$, and α, as follows:

$$S_e = \frac{\alpha S_p}{1 - Y_{obs,2}} \quad (5)$$

Then, a mass balance was developed for the fermentative step, as follows:

$$S_p = X_f + S_e + S_{ne} \quad (6)$$

Substituting the expression of X_f in Equation (1) and S_e in Equation (5) to Equation (6), S_{ne} was expressed as follows:

$$S_{ne} = S_p \left(1 - Y_{obs,1} - \frac{\alpha}{1 - Y_{obs,2}}\right) \quad (7)$$

Therefore, all parameters in Fig. 2 were expressed in terms of S_p, α, $Y_{obs,1}$ and $Y_{obs,2}$. To calculate the fraction of electron equivalents of each component per unit of parent compound utilized, X_f, X_e, S_e, and S_{ne} were divided by S_p. Thus, α, $Y_{obs,1}$ and $Y_{obs,2}$ were the input parameters to calculate all components of the electron equivalents flow (i.e., X_f, X_e, S_e, and S_{ne}).

The values of α for individual furanic and phenolic compounds were obtained from our previous experimental measurements, i.e., 50, 12 and 9 % for SA, VA and HBA, respectively [10]; 85 and 66% for FF and HMF, respectively (unpublished work). The values of the biomass yield coefficients for the fermentative and exoelectrogenic bacteria were obtained based on the following reasoning. An overall Y_{obs}, accounting for both X_f and X_e, was experimentally estimated as 0.23 g biomass-COD/g substrate-COD, using a mixture of the five furanic and phenolic compounds [14]. Typical values of true yield coefficient (Y, not accounting for decay; g biomass-COD/g degradable-COD) are as follows: fermentative bacteria, 0.18; sulfate reducing bacteria, 0.11; acetoclastic methanogens, 0.07; and hydrogenotrophic methanogens, 0.11 [15]. Considering the measured overall Y_{obs} and literature reported Y values for fermentative and anaerobic respiring bacteria, values of $Y_{obs,1}$ and $Y_{obs,2}$ were assumed to be 0.1 and 0.15 g biomass-COD/g substrate-COD, respectively.

RESULTS AND DISCUSSION

Extent of Electrochemical Reactions. To evaluate the extent of electrochemical reactions on the biofilm electrode, independent from microbial reactions (e.g., exoelectrogenesis), a CV test was conducted with active (i.e., fed) and

non-active (i.e., starved and unfed) bioanodes. As shown in Fig. 3A, except for one small peak observed around – 0.35 V, the voltammogram of the non-active bioanode upon the addition of the compounds mixture was almost identical to that of the non-active bioanode in the absence of the compounds. Thus, the formation of biofilm (without bioactivity) did not have a significant contribution to the current production. In contrast, the CV test conducted with the bioanode after 1 day of incubation with the five compounds, when the bioanode produced significant current, resulted in a significant oxidation peak (Fig. 3A). In comparison, the observed current in the non-active bioanode CV was negligible. Therefore, the bioactivity of the biofilm (i.e., exoelectrogenesis) contributed much more significantly to the observed current production than the modification of the electrode surface by biofilm attachment.

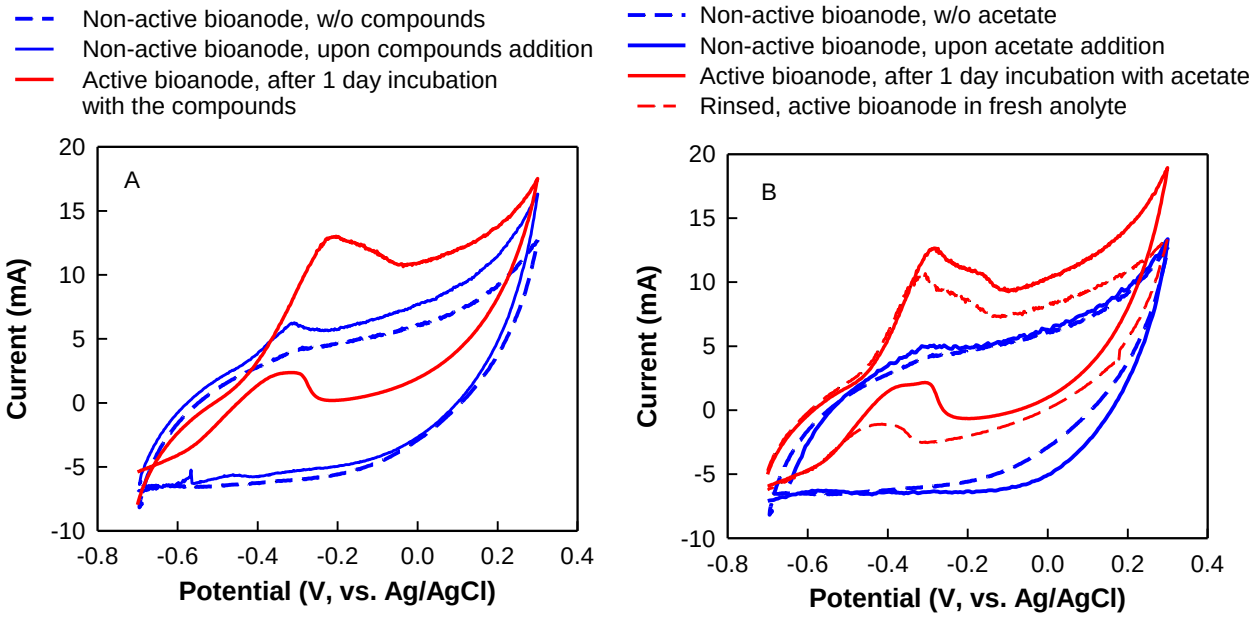

Fig. 3 Cyclic voltammograms of non-active and active bioanodes tested with the mixture of furanic and phenolic compounds (A) and sodium acetate (B)

Because acetate was a direct exoelectrogenic substrate, another CV test was conducted using sodium acetate as the substrate to examine the exoelectrogenic activity of the bioanode. As shown in Fig. 3B, the non-active bioanode produced only capacitance current, either in the presence or absence of acetate. In contrast, after 1 day of incubation with acetate, the voltammogram of the bioanode showed a significant oxidation peak at around – 0.3 V (Fig. 3B). Thus, the oxidation peak was not associated with electrochemical acetate oxidation, but rather resulted from the exoelectrogenic activity. To further confirm that electrochemical acetate oxidation was negligible, the active bioanode was anaerobically rinsed with anolyte to remove acetate, and the CV test was conducted again with the bioanode in fresh anolyte. It is noteworthy that the same oxidation peak was observed in the voltammogram of the rinsed bioanode at a comparable height to that in the active bioanode, despite the slight shift of the baseline capacitance current (Fig. 3B). Therefore, the extent of electrochemical reactions was negligible relative to the extent of microbial reactions (i.e., exoelectrogenesis).

A plausible explanation for the oxidation peak in the voltammogram of the rinsed bioanode was that a direct electron transfer mechanism was used during exoelectrogenesis. In direct electron transfer, exoelectrogens use outer-membrane bound c-type cytochromes or nanowires to transport electrons to the electrode. The other electron transfer mechanism, indirect transfer, relies upon soluble electron transfer mediators [16,17]. The fact that active exoelectrogenesis was observed in a rinsed bioanode in fresh anolyte (i.e., free of soluble electron transfer mediators) suggests that the direct electron transfer mechanism was used in the bioanode in the present study.

The difference in voltammograms between the active and non-active bioanodes was consistently observed for acetate and the furanic and phenolic compounds. Thus, it was concluded that microbial activity was the primary process contributing to the electron equivalents flow in the bioanode, whereas the extent of electrochemical reactions was negligible. In addition, our previous study evaluated and excluded possible contribution of adsorption

and abiotic electrode reactions [9]. Therefore, the quantification of bioanode electron equivalents flow was based on fermentation and exoelectrogenesis, as presented in Fig. 2.

Quantification of Electron Equivalents Flow. Each component in the electron equivalents flow presented in Fig. 2 was calculated for the microbial utilization of FF, HMF, SA, VA and HBA. The fractions of electron equivalents in biomass (X_f and X_e), current (I), and non-exoelectrogenic end products (S_{ne}) are presented in Fig. 4. More than 50% of the electron equivalents of SA, FF, and HMF were converted to current, whereas only 12 and 9% of VA and HBA electron equivalents, respectively, were used for current production. The fractions of electron equivalents used for current production were equal to the anode efficiency (α). Based on these calculations, during the biotransformation of VA and HBA, 76 and 79% of electron equivalents remained as persistent products (S_{ne}) without being further utilized for current production (Fig. 4). Consistent with this estimation, our pervious experimental work showed that the fermentative biotransformation of VA and HBA in the MEC bioanode resulted in catechol and phenol, respectively, as the major dead-end products, which accounted for 80% of the VA and HBA based on carbon balance [10]. Compared to VA and HBA, the biotransformation of SA was estimated to result in a smaller quantity of persistent products (S_{ne}), accounting for 31% of the SA electron equivalents (Fig. 4). Our previous study reported a major pathway for complete SA fermentation to acetate in a MEC bioanode [10]. The estimation that 31% of the SA electron equivalents remained in persistent products indicated that other minor pathways for SA fermentation might exist and warrant further investigation. The estimated fraction of persistent products (S_{ne}) during the biotransformation of FF and HMF was as low as 5 and 24%, respectively (Fig. 4). Fermentative pathways of FF and HMF in bioanodes have not been elucidated. However, the low fraction of S_{ne} suggested that the majority of electron equivalents of FF and HMF were effectively utilized for current production. For all five compounds, 10% of the initial electron equivalents were used for fermentative biomass synthesis (X_f ; Fig. 4), because the same value of $Y_{obs,1}$was used for these compounds. Future improvement can be made to estimate specific $Y_{obs,1}$ for each of the compounds based on bioenergetic principles [15], if specific stoichiometric reactions become well-understood. Exoelectrogenic biomass synthesis (X_e) accounted for 2 - 13% of the initial electron equivalents (Fig. 4), related to the amount of exoelectrogenic substrate produced from fermentation (S_e, 11 – 85%). It is noteworthy that the above-discussed results were based on α, $Y_{obs,1}$ and $Y_{obs,2}$ values measured under un-inhibited conditions. To account for exoelectrogenesis inhibition by high concentrations of furanic and phenolic compounds [11], values of α and $Y_{obs,2}$ need to be modified based on the extent of inhibition.

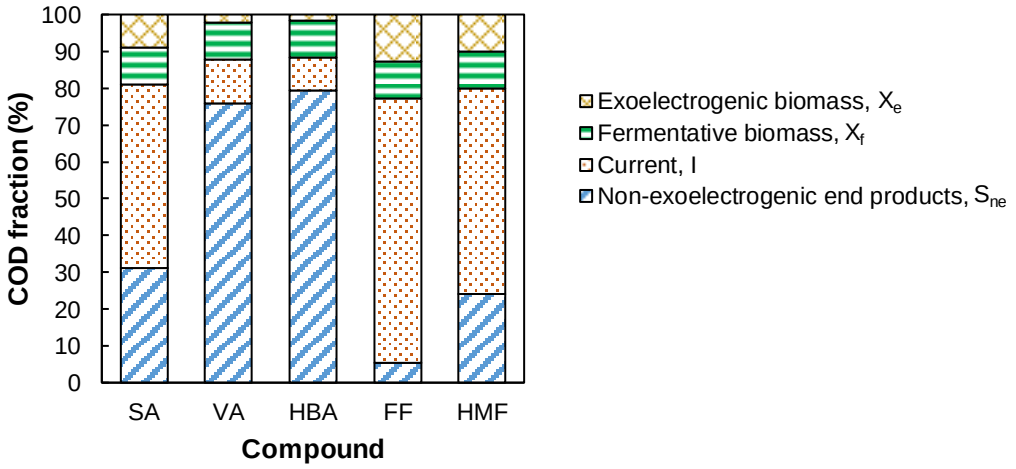

Fig. 4 Electron equivalents distribution to biomass, current and non-exoelectrogenic end products per unit of each parent compound utilized

The proposed framework of substrate utilization and electron distribution can be useful to the development of electron balances in bioanodes, especially when COD measurements and transformation products are not available. The anode efficiency (α) accounts for the persistent transformation products (S_{ne}), and its value can be easily calculated based on current measurements and the concentration of the parent compound. The other two parameters needed in the calculation are $Y_{obs,1}$ and $Y_{obs,2}$. Obtaining values of $Y_{obs,1}$ and $Y_{obs,2}$ can be a challenge as

the total biomass (X_e + X_f) is usually measured. If specific stoichiometric reactions are available, $Y_{obs,1}$ and $Y_{obs,2}$ may be estimated based on bioenergetic principles [15]. Adopting $Y_{obs,1}$ and $Y_{obs,2}$ values from studies based on fermentation and exoelectrogenesis requires cautious examination of the definition and applicability of the observed yield coefficient, because of partial oxidation. To distinguish biomass associated with the biofilm or in suspension, appropriate fractionation factors can further be developed and applied. Although the above-described mass balance-based framework was developed based on individual parent compounds, simulation of a mixture of known compounds used as bioanode feed can be implemented by accounting for the metabolic fate of each individual compound. In the case where a complex, un-defined organic mixture is used as a bioanode feed, the values of α, $Y_{obs,1}$ and $Y_{obs,2}$ will need to be obtained experimentally or from the literature.

An important application of the above-presented, mass-based framework is to simulate bioprocesses in MEC bioanodes, by introducing kinetic rate expressions for the utilization of S_p and S_e, as well as for biomass production. S_p utilization in the fermentation sub-process can be described by Monod kinetics, while S_e utilization in the exoelectrogenic sub-process can be described by Nernst-Monod or Butler-Volmer kinetics [18-20]. Using rate expressions and the above-described distribution of electron equivalents in each sub-process, mass rate balance equations for either batch or CSTR reactors can then be developed for dynamic simulation of the overall process.

CONCLUSIONS

In the MEC bioanode, microbial reactions (fermentation and exoelectrogenesis) were primary processes in the conversion of furanic and phenolic compounds to current or cathodic H_2; the extent and distribution of electrochemical reactions was negligible. A mass-based framework was developed to describe and quantify the electron equivalents flow during the utilization of individual furanic and phenolic compounds. Electron equivalents in current, biomass, persistent transformation products, as well as intermediate exoelectrogenic substrate were estimated based on parameters of anode efficiency and observed biomass yield coefficients. The fraction of electron equivalents used for current production varied significantly among the five compounds, and was negatively related to the electron equivalents remained in persistent products. The findings of the present study advance our understanding of the processes and electron flow in MEC bioanode fed with complex, fermentable organic compounds resulting from the pretreatment of biomass. The proposed mass-based framework of substrate utilization and electron flow can be used for the simulation of bioanode processes to guide the optimization of MECs converting biomass-derived waste streams to renewable H_2.

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