Biotransformation of Furanic and Phenolic Compounds with Hydrogen Gas Production in a Microbial Electrolysis Cell

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ABSTRACT: Furanic and phenolic compounds are problematic byproducts resulting from the decomposition of lignocellulosic biomass during biofuel production. This study assessed the capacity of a microbial electrolysis cell (MEC) to produce hydrogen gas (H_2) using a mixture of two furanic (furfural, FF; 5-hydroxymethyl furfural, HMF) and three phenolic (syringic acid, SA; vanillic acid, VA; and 4-hydroxybenzoic acid, HBA) compounds as the sole carbon and energy source in the bioanode. The rate and extent of biotransformation of the five compounds, efficiency of H_2 production, as well as the anode microbial community structure were investigated. The five compounds were completely transformed within 7-day batch runs and their biotransformation rate increased with increasing initial concentration. At an initial concentration of 1,200 mg/L (8.7 mM) of the mixture of the five compounds, their biotransformation rate ranged from 0.85 to 2.34 mM/d. The anode coulombic efficiency was 44-69%, which is comparable to wastewater-fed MECs. The H_2 yield varied from 0.26 to 0.42 g H_2-COD/g COD removed in the anode, and the bioanode volume-normalized H_2 production rate was 0.07-0.1 L/L-d. The major identified fermentation products that did not transform further were catechol and phenol. Acetate was the direct substrate for exoelectrogenesis. Current and H_2 production were inhibited at an initial substrate concentration of 1,200 mg/L, resulting in acetate accumulation at a much higher level than that measured in other batch runs conducted with a lower initial concentration of the five compounds. The anode microbial community consisted of exoelectrogens, putative degraders of the five compounds, and syntrophic partners of exoelectrogens. The H_2 production route demonstrated in this study has proven to be an alternative to the currently used process of reforming natural gas to supply H_2 needed to upgrade bio-oils to stable hydrocarbon fuels.

INTRODUCTION

Lignocellulosic biomass is a promising feedstock for the production of biofuels using thermal, chemical or biological processes. Decomposition of lignocellulosic biomass, regardless of the process used, typically results in furanic and phenolic byproducts, which are inhibitory, problematic compounds. For instance, furanic and phenolic compounds are highly inhibitory to H_2 and ethanol producing, fermentative microorganisms at a concentration greater than 1 g/L.[1, 2] In pyrolysis, a thermal process reforming biomass to bio-oil, the presence of polar and oxygen-rich compounds (e.g., furan aldehydes and phenolic acids) makes the bio-oil acidic, unstable, requiring H_2 in the downstream hydrogenation process to upgrade the bio-oil to a stable fuel. Methods employed for the removal of furanic and phenolic byproducts include solvent extraction, developing inhibitor-tolerant microorganisms, and improving microbial conversion of the inhibitors to less toxic compounds.[3] However, these methods have the trade-offs of lowering biofuel yield and do not lessen the challenge of downstream wastewater treatment.[3] Thus, a process to directly utilize furanic and phenolic compounds to produce biofuels would be an improvement over the existing methods.

Microbial electrolysis cell (MEC) technology is a bioelectrochemical process, which produces H_2. Exoelectrogenic bacteria in the anode oxidize organic substrates by transferring electrons to the electrode and then to the cathode. With a voltage input (> 0.3 V), protons transferred from the anode to the cathode via a cation exchange membrane are reduced to H_2.[4] Unlike other H_2 producing bioprocesses, the MEC produces H_2 through an abiotic half-reaction in the cathode, with the supply of electrons from the microbially-assisted half-reaction in the anode. This feature of MEC eliminates the need for H_2-producing bacteria, which are highly susceptible to furanic and phenolic compounds.[1, 5] Thus, the MEC is potentially able to convert

these problematic compounds to H_2, which in turn can be used in the hydrogenation process and thus minimize the external H_2 supply currently generated by reforming natural gas.

Given the advantage of the MEC technology, this study investigated a MEC for H_2 production from two furanic and three phenolic compounds. This is the first attempt to utilize specific inhibitory compounds to produce H_2 using bioelectrochemical technology. Although the merit of bioelectrochemical systems has been demonstrated in terms of power generation, nutrient recovery, and H_2 production from acetate and various wastewaters,[6-8] the ability of a MEC to use furanic and phenolic compounds as the sole carbon and energy source remains unexplored. Acetate is a favorable organic substrate for exoelectrogens,[9, 10] whereas furanic and phenolic compounds are less biodegradable and have not been reported as direct substrates for exoelectrogens. On the other hand, wastewater streams usually have poorly defined components, and the contribution from individual components has not been delineated. Two previous studies used furanic and phenolic compounds as the substrate in the anode of a microbial fuel cell (MFC), but had mixed results. Catal et al. reported that, with the exception of HMF, the other nine furanic and phenolic compounds were unable to generate voltage.[11] In contrast, Borole et al. demonstrated that an anode microbial consortium was able to convert furfural, HMF, 4-hydroxybezaldehyde, hydroxyacetophenone, and vanillic acid to electricity.[12] Thus, the question remains whether a MEC anode microbial community can use furanic and phenolic compounds as the sole energy and carbon source to produce H_2 in the cathode.

The objective of this study was to assess the capacity of a MEC to use furanic and phenolic compounds for H_2 production. Biotransformation of five furanic and phenolic compounds, formation of metabolites, production of current and H_2, as well as the structure of the anode microbial community were investigated.

MATERIALS AND METHODS

Chemicals. Furfural (FF, 99%), 5-hydroxymethyl furfural (HMF, ≥ 99%), syringic acid (SA, ≥ 95%) and 4-hydroxybenzoic acid (HBA, ≥ 99%) were purchased from Sigma-Aldrich (St. Louis, MO). Vanillic acid (VA, ≥ 99%) was purchased from Alfa Aesar (Ward Hill, MA). The five compounds are soluble in water (1.5-7 $\times 10^8$ g/L at 25^{o}C), not volatile (Henry's law constant = 10^{-14} - 10^{-6} atm-m^3/mol), and have a low hydrophobicity (log K_{ow} = -0.09 - 1.58) (Table S1). The standard potential at pH 7.0 ($E^{0\prime}$) of the five compounds is from -0.388 to -0.303 V (Table S2) compared to an $E^{0\prime}$value of -0.414 V for proton reduction to H_2.

Inoculum. The bioanode inoculum was a piece of carbon felt with biofilm developed at the Oak Ridge National Laboratory (Oak Ridge, TN), which had been enriched with fermentation inhibitors in a MFC anode. The original inoculum was a sample collected from a municipal anaerobic digester.[13] The bioanode inoculum was further enriched in the present study in a MFC anode, fed with a mixture of the five compounds, prior to being transferred to a MEC anode.

MFC Setup and Operation. An air-cathode MFC was set up to enrich the inoculum. The anode electrode was porous carbon felt (5 stripes, 1 cm × 1 cm × 10 cm each; Alfa Aesar, Ward Hill, MA) tied to a stainless steel rod. The anode chamber was a modified square glass bottle with an open channel on one side. The empty bed volume was 250 mL, and the liquid volume was 200 mL due to electrode displacement. The cathode was a membrane-electrode assembly with a surface area of 5.7 cm^2 purchased from Fuel Cells Etc (College Station, TX), which was made of a cation exchange membrane and carbon cloth containing 0.5 mg/cm^2 Pt. The cathode was clamped to the side channel extended from the anode chamber and exposed to air on one side.

A piece of biofilm-attached carbon felt (approximately 1 cm ×1cm × 3 cm) was placed in the center of the anode carbon felt electrode. The anode medium consisted of (in g/L): NH_4Cl, 0.31; KCl, 0.13; $NaH_2PO_4 \cdot H_2O$, 2.45; Na_2HPO_4, 4.58, along with trace metals and vitamins.[14] The pH of the medium was 7.0. The anolyte was deoxygenated by bubbling N_2 through the liquid phase prior to use, and was continuously mixed magnetically. The MFC was maintained at room temperature (20-22°C). A mixture of the five compounds at equal electron equivalents (each at 62.5 mg COD/L) and a total concentration of 200 mg/L (312 mg COD/L) was fed to the MFC anode once a week (7-day fed-batch). During the first ten feeding cycles (~70 days), glucose (200 mg/L) was fed along with the five compounds to enhance microbial growth. A variable resistor was placed between the anode and cathode, and its resistance was gradually reduced from 500 to 250 and then to 100 Ω, in order to promote the growth of exoelectrogenic bacteria. The voltage was recorded by a potentiostat (Interface 1000™, Gamry Instruments, Warminster, PA) and enrichment lasted for 6 months. The MFC activity stabilized with a mean maximum current of 1.25 mA, soluble COD (sCOD) removal of 50-60% and coulombic efficiency (CE) of 40-60%, measured over 20 feeding cycles.

MEC Setup and Operation. An H-type MEC was developed with two square glass bottles separated by a cation exchange membrane (Nafion 117, 5.7 cm^2; Dupont, Wilmington, DE). Both chambers and the anode electrode had the same configuration as the above-described MFC anode. The cathode electrode was a carbon cloth containing 0.5 mg/cm^2 of Pt (5 cm × 6 cm; Fuel Cell Etc, College Station, TX). A gas collection burette using displacement of an acid brine solution (10% NaCl w/v, 2% H_2SO_4 v/v) was connected to each chamber headspace for gas volume measurement.

125 The inoculation procedure and anolyte of the MEC were the same as for the above-
126 described MFC. The MEC catholyte was a 100 mM phosphate buffer (pH 7.0), deoxygenated by
127 bubbling N_2 prior to use. Both the anolyte and catholyte were replaced at the beginning of each
128 feeding cycle. During the startup, the anode chamber was amended with 200 mg/L of the five
129 compounds mixture (same composition as the MFC feed). After the startup, which lasted for 9
130 weeks, the total initial concentration of the substrate mixture was increased from 200 to 400,
131 800, and then to 1,200 mg/L. A voltage of +0.6 V was set to the MEC anode relative to the
132 cathode, and the current was recorded every 4 hours by the potentiostat. The MEC was
133 maintained at room temperature (20-22^{o}C). The duration of each feeding cycle was 6-7 days
134 until the current dropped below 0.2 mA. The anolyte and catholyte were continuously mixed
135 magnetically and the anode and cathode headspaces were initially filled with N_2.
136 Two controls were evaluated. Control 1 was used to investigate the stability of the five
137 compounds in the presence of the porous carbon felt and anolyte. Four serum bottles containing
138 100 mL anolyte and 200 mg/L compound mixture were kept under a N_2 headspace. Two of the
139 bottles contained carbon felt with equivalent quantity (v/v) as in the MEC anode and the
140 concentration of the five compounds was monitored for 7 days. Control 2, setup with a biomass-
141 free anode electrode in the MEC, was used to evaluate the potential contribution of the applied
142 voltage on current production and transformation of the five compounds in the absence of
143 microbial activity.
144 **Microbial Community Analysis.** Microbial community analysis of the MEC anode was
145 performed after 9 weeks (9 feedings) from the startup. A piece of anode electrode with attached
146 biofilm (approximately 1 cm ×1cm × 3 cm) was washed several times with the anolyte and then
147 cut into small pieces (< 0.5 cm). The genomic DNA was extracted with the PowerSoil DNA

isolation Kit (MO BIO Laboratories, Carlsbad, CA), according to the manufacturer's instructions. The concentration and purity of the DNA sample were determined with a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The 16S rRNA gene was sequenced using Illumina technology (LC Science, Houston, TX). Bacterial primers 319F and 806R were used to amplify the V3-V4 hypervariable regions of the 16S rRNA gene. The obtained sequences were clustered into Operational Taxonomic Units (OTUs) with 97% similarity. The longest read in each OTU was chosen as the representative sequence for taxonomic classification using the RDP classifier Version 2.7. The sequence-based phylogenetic tree of the abundant bacteria (>1% abundance) was constructed by applying the neighbor-joining algorithm using the program MEGA 6.06. The tree topology was evaluated by bootstrap resampling analysis of 1000 data sets. The representative sequences of the abundant species have been submitted to the GenBank, National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/) with sequence accession numbers from xxxxx to xxxxx (pending).

Analytical Methods. The furanic and phenolic compounds were quantified using a high performance liquid chromatography (HPLC) unit equipped with a UV-Vis detector (Agilent 1100, Santa Clara, CA). An HPX-87H column (BioRad, Hercules, CA) was used with an eluent of 15% acetonitrile in 5 mM H_2SO_4 (v/v) at a flow rate of 0.6 mL/min.[12] The wavelength of 280 nm and 210 nm was used for the furanic and phenolic compounds, respectively. Acetate and other volatile fatty acids were quantified by the same HPLC method at the wavelength of 210 nm, except that the eluent was 5 mM H_2SO_4 without any organic solvent. Metabolites of the five compounds were identified using an LC/MS/MS unit (Agilent 1260 Infinity LC system, 6410 Triple Quad MSD) equipped with a Kinetex biphenyl column (3×150 mm, 5 μm; Phenomenex, Torrance, CA). The eluent consisted of (A) 5 mM ammonium acetate with 0.5% acetic acid in

acetonitrile (v/v) and (B) 5 mM ammonium acetate in 0.5% acetic acid (v/v) at a flow rate of 0.5 mL/min, using gradient elution as follows: eluent A was increased from 2% to 30% in 2.3 min and to 90% in 1.2 min, and then was maintained at 90% for 2.5 min. The MS/MS was operated in both positive and negative modes at 100 eV in an m/z range of 50-250. The product ions of the same molecular weight as hypothesized metabolites were fragmented, and the fragmentation patterns were compared with those of purchased pure chemicals. Soluble chemical oxygen demand (sCOD) and pH were measured following procedures outlined in Standard Methods.[15] Total gas production was measured by the acid brine solution displacement in the burettes, equilibrated to 1 atm. Headspace gas composition (i.e., H_2, CO_2, and CH_4) was determined with a gas chromatography unit equipped with two columns and two thermal conductivity detectors.[16]

Calculations. Coulombic efficiency and H_2 yield were calculated as previously described.[17] Current density was normalized to either the empty bed volume of the anode chamber (250 mL) for comparison with single-chamber MECs, or the projected surface area of the Nafion membrane (5.7 cm^2), assuming the membrane surface area was limiting, due to the narrow channel of the H-type reactor.[18]

RESULTS AND DISCUSSION

MEC Startup. During the MEC startup period, fed-batch addition of 200 mg/L compound mixture was conducted in repetitive 7-day feeding cycles. Stable maximum current and H_2 production was observed by day 28 (Figure S1) and the startup period continued for another 35 days (5 feeding cycles) to confirm stable performance. During the MEC operation, the anolyte and catholyte pH was in the range of 6.7-7.0 and 7.0-7.3, respectively. The maximum current density (I_{max}) was 0.16 ± 0.04 mA/cm^2 or 3.6 ± 0.9 A/m^3, cumulative H_2 production was 19.3 ± 1.2 mL (20°C, 1 atm), and coulombic efficiency was 44 ± 12% over the last 5 feeding

cycles. The five compounds were completely transformed, with sCOD removal of 57 ± 10% during each feeding cycle. An abiotic control experiment (Control 2), conducted under the same MEC conditions, with the exception that the anode was not inoculated, resulted in negligible current (<0.12 mA) and H_2 production (< 3 mL) over 7 days, confirming that the current and H_2 production in the inoculated MEC was due to the bioanode activity.

Effect of Initial Substrate Concentration on Current and H_2 Production. After demonstrating H_2 production occurred during the MEC startup period, four consecutive feeding cycles were carried out with increasing initial anode substrate concentrations, in order to assess the capacity of the bioanode to transform the five compounds at higher concentrations, and any potential inhibitory effect of these compounds or their transformation products on current and H_2 production (Figure 1). When the initial anode substrate concentration was increased from 200 to 400 mg/L, the I_{max} almost doubled from 1.36 mA to 2.36 mA. As a result, the maximum H_2 production rate increased from 18.3 to 26.5 mL/d. Electric current of 1 mA corresponds to a maximum H_2 production rate of 11 mL/d (20ºC, 1 atm) or 0.45 mmol/d, based on the fact that production of 1 mole of H_2 requires at least 2 moles of electrons (2 × 96585 coulombs). The current magnitude is also indicative of the rate of exoelectrogenesis. Thus, increasing the initial substrate concentration from 200 to 400 mg/L allowed faster exoelectrogenesis, probably by promoting the growth of the exoelectrogenic bacteria and/or enhancing the substrate mass transfer from the bulk solution through the porous electrode to the biofilm. When the substrate concentration was further increased to 800 mg/L, although the I_{max} did not increase, high current was sustained for a longer time resulting in a higher cumulative H_2 production than in the 400 mg/L run (49 mL vs. 35 mL). However, when the initial substrate concentration was increased to 1,200 mg/L, both the current and H_2 production were severely inhibited (Figure 1). The 1,200

mg/L concentration is comparable to what has been reported for the inhibition of H_2 and ethanol fermentation by mixed cultures, typically around 1,000 mg/L.[1-3] Previous studies also suggested that a combination of inhibitors acted synergistically, resulting in higher inhibition.[3] Little information is available regarding the inhibitory effect of furanic and phenolic compounds on exoelectrogens. Whether the parent compounds or transformation products are responsible for the observed inhibition is currently under investigation. The anode pH was 6.7 on day 3, which ruled out the possibility that low pH inhibited the bioanode activity. In order to determine whether the observed inhibition was reversible or not, the anolyte was replaced with fresh medium and the compound mixture at 200 mg/L was added. Both current and H_2 production were restored to the previous levels within 2 days (Figure S2). Although inhibition was observed at 1,200 mg/L total substrate concentration, the MEC in the present study demonstrated its capacity to use furanic and phenolic compounds (up to 800 mg/L) as the sole carbon and energy source to produce H_2. For comparison, a mixed fermentative culture produced only 0.58 mL/L of H_2 from HMF and no H_2 from furfural at 10-1000 mg/L.[19]

MEC Efficiency. Efficiency parameters were calculated for the MEC feeding cycles performed with 200-800 mg/L initial substrate concentrations (Table 1). Between 39 and 51% of the electron equivalents (COD) in the feed remained by the end of the feeding cycles. Details of the observed biotransformation products contributing to the residual COD are discussed below. From the electron equivalents removed, 44-69% were converted to current (i.e., coulombic efficiency), and 26-42% were captured as H_2 (H_2 yield). The H_2 yield in the MEC was higher than reported values for dark fermentation of glucose using mixed cultures (~2 mol H_2/mol glucose or 17% based on electron equivalence).[20, 21] The H_2 yield, sCOD removal and coulombic efficiency achieved in the present study were comparable to those achieved with wastewater-fed

MECs. However, the maximum H_2 production rate was lower than that of domestic wastewater-fed MECs (0.1 vs. 0.3 L/L-d).[22-24] To improve the H_2 production rate, the key is to achieve more effective exoelectrogenesis, because the H_2 production rate is proportional to current density (A/m^3). In the present study, as discussed below, not all of the transformation products were used by the exoelectrogens. Therefore, a robust anode microbial community needs to be further developed to improve the H_2 production rate of the MEC.

Effective collection of H_2 was a challenge in the present study, which negatively impacted the H_2 production rate. The cumulative H_2 volume declined noticeably during the latter part of each batch run when anode bioactivity was lower (Figure 1). The difference between the maximum cumulative H_2 on day 3 (day 2 for the 200 mg/L run) and the total H_2 collected at the end of each run was considered to be the minimum H_2 loss. Thus, at least 3.5- 18.6 mL (15-35%) of the produced H_2 was not captured during the 200-800 mg/L runs, and 93% was lost during the 1200 mg/L run, when overall H_2 production was very low. According to the Henry's law constant of H_2 at 20^{o}C (8.03×10^{-4} mol/L/atm)[25] and the maximum partial pressure of H_2 in the cathode headspace (0.34 atm on day 3 in the 800 mg/L run), the dissolved H_2 in 250 mL catholyte at equilibrium was 0.017 mL at 20^{o}C. The displacement solution of acid brine should have even less dissolved H_2 than the catholyte. Therefore, H_2 dissolution was negligible in the cathode.

Cathode H_2 diffusion through the cation exchange membrane to the anode has been widely acknowledged in previous studies,[26, 27] as in the present study. A control experiment revealed that 6% of H_2 added to the cathode headspace diffused through the cation exchange membrane to the anode headspace of an un-inoculated MEC with an open circuit tested for 7 days (Figure S3). Under MEC conditions with an active bioanode and in the absence of external

electron acceptors (e.g., O_2, NO_3^-, etc.), the H_2 diffused to the anode can readily be used by exoelectrogens as an electron donor (Equation 1) or by homoacetogens to form acetate[9, 28, 29] (Equation 2). Thus, the loss of H_2 from the cathode due to diffusion to the anode could be higher than 6% during the normal 7-day MEC operation.

$$H_2 = 2H^+ + 2e^- \quad \text{(Equation 1)}$$

$$4H_2 + 2CO_2 = CH_3COOH + 2H_2O \quad \text{(Equation 2)}$$

Another effect caused by H_2 diffusion and utilization in the anode can be pseudo-current production by recycling H_2 between cathode and anode. In addition to the current generation, according to Equation 1, acetate generated as shown in Equation 2 can also be used as electron donor by exoelectrogens[9, 28] (Equation 3).

$$CH_3COOH + 2H_2O = 2CO_2 + 8H^+ + 8e^- \quad \text{(Equation 3)}$$

Adding Equation 2 and 3 results in Equation 1. Therefore, regardless of the pathways (direct exoelectrogenesis using H_2 or homoacetogenesis), every 1 mole of H_2 utilized in the anode will contribute at most 2 moles of e^- to the current. Taking this effect into consideration, the corrected coulombic efficiency was 35-52%, about 10% lower than the values reported in Table 1.

Biotransformation of the Furanic and Phenolic Compounds. The concentration of the five compounds was monitored during the MEC feeding cycles with increased initial substrate concentration from 200 to 1,200 mg/L (Figure 2). All five compounds were completely transformed within 7 days at all initial concentrations tested. The results from Control 1 (biomass free, non-electrochemical condition) showed no detectable adsorption of the five compounds to the carbon felt (Figure S4), which was expected because the five compounds have log $K_{ow} < 2.0$ (Table S1). In addition, analysis of the catholyte showed no detectable compounds, confirming the selectivity of the cation exchange membrane. Thus, the disappearance of the five compounds

was attributed to electrochemical reactions and/or microbial metabolism in the anode. To distinguish the possible contribution of each of these two mechanisms, Control 2 was conducted under the same operating conditions as the MEC, except that the anode was biomass-free (i.e., un-inoculated carbon felt). Within 7 days, the concentration of the phenolic compounds decreased by about 20% (Figure 2). It is not clear what products were formed, but none of the identified biotransformation products from the three phenolic compounds (discussed below) was detected. The two furanic compounds were completely transformed in Control 2. Furoic acid (FA) and 2,5-Bis(hydroxymethyl)furan (HMF-OH) were observed as the oxidized product of furfural and reduced product of HMF, respectively. It has been reported that a mixture of products could be generated from electrolysis of furfural and 5-HMF, including oxidized and reduced furan derivatives, open-ring products, and dimers, depending upon the type of electrode and electrolysis condition.[30, 31] However, it is important to note that the transformation rate of all five compounds substantially increased with a bioactive anode (Figure 2).

There was a clear trend for the transformation rate of the five compounds, which increased with increasing initial substrate concentrations from 200 to 1,200 mg/L (Figure 2; Table S3). Increasing the initial concentration of the substrate mixture from 200 to 1,200 mg/L resulted in the following range of volumetric transformation rates (mM/d): 0.333-2.343 for FF; 0.197-1.029 for HMF; 0.047-0.917 for SA; 0.052-0.854 for VA; and 0.085-0.965 for HBA. The increase of the transformation rates, with increasing initial substrate concentration, implies that the five compounds were not inhibitory to the initial biotransformation step(s) even at the 1,200 mg/L run. In contrast, as discussed above, the current and H_2 production during the 1,200 mg/L run were severely inhibited (Figure 1). The observation that the transformation rate of the five compounds increased, while current and H_2 production decreased, indicates that the five

309 compounds were not the direct substrates for exoelectrogens and current generation. Previous
310 studies have suggested that electricity generation was carried out primarily via fermentation
311 products, like acetate and H_2, when fermentable substrates were used in bioelectrochemical
312 systems.[9, 29] Because there was no external electron acceptor available in the anode medium in
313 the present study, the initial biotransformation process of the five compounds in the MEC
314 bioanode is assumed to be fermentation.

315 Transformation products identified using LC/MS/MS include furoic acid (FA), 2,5-
316 Bis(hydroxymethyl)furan (HMF-OH), 3,4-dihydroxybenzoic acid (diHBA), catechol, phenol and
317 acetate. These identified transformation products accounted for ca. 50% of the residual sCOD
318 measured during the four feeding cycles. Thus, half of the transformation products on the basis
319 of electron equivalence have not been identified, which is also indicated by the unknown peaks
320 in the UV and TIC chromatograms at the end of the four feeding cycles (Figure S5).
321 Nevertheless, ethanol, pyruvate, lactate and propionate were not detected at the end of the batch
322 runs. Among the identified transformation products, catechol and phenol accumulated during all
323 feeding cycles (Figure 3). Acetate accumulated only during the 1,200 mg/L run (maximum
324 acetate at 326 mg/L), while other compounds were transient (Figure S6). The transformation
325 products detected in Control 2 (biomass-free anode) include HMF-OH and FA, but no phenolic
326 products (Figure 3 and Figure S6). Thus, FA and HMF-OH could be produced from
327 electrochemical reactions in the absence of bioactivity, whereas the formation of the detected
328 phenolic products (catechol, phenol and diHBA) was the result of biotransformation.

329 It has been widely accepted that fermentation is an important metabolic process, in
330 addition to exoelectrogenesis, when fermentable substrates are used in bioanodes. Fermentation
331 first transforms relatively complex substrates to lower molecular weight molecules (e.g., acetate

and H_2) which are then used as electron donors for exoelectrogenesis.[9, 10, 29] This syntrophic interaction was also observed in the present study, in which fermentable substrates were applied. Acetate accumulated at 326 mg/L (5.4 mM) in the 1,200 mg/L run, but was not detected in the 200-800 mg/L runs. In the 1,200 mg/L run, fast transformation of the five parent compounds resulted in fast production of acetate, but exoelectrogenesis was inhibited by either the five compounds or their transformation products (as discussed above), and thus contributed to a very low acetate utilization (Figure 1 and 2). In contrast, in the 200-800 mg/L runs, active exoelectrogenesis occurred (Figure 1), resulting in rapid consumption of acetate produced by fermentation.

Stoichiometrically, 1,200 mg/L of the five compounds could result in a maximum acetate level of 29.3 mM. It is possible that the acetate was partially produced by homoacetogenesis using CO_2 resulting from fermentation and H_2 diffused from the cathode. Yet, as discussed above, homoacetogenesis could contribute 2 mM acetate at the most, if the current production during the 1,200 mg/L was fully converted to H_2 (reverse of Equation 1) and then to acetate (Equation 2). In addition, the accumulated acetate at the end of the 1200 mg/L run could have resulted in the production of 105 mL of H_2 (1 mol Acetate = 4 mol H_2)[4] if inhibition did not occur. Compared to the cumulative H_2 production during the 200, 400 and 800 mg/L runs (21, 35 and 49 mL, respectively), 105 mL would have been proportional to the initial substrate concentration. Therefore, acetate is considered to be the direct substrate for exoelectrogenesis in the present study.

Other significant fermentation products detected and accumulated were catechol and phenol. These two compounds are both reduced alcohols, with higher electron equivalents (0.24 and 0.30 eeq/g) than the five parent compounds (0.18-0.21 eeq/g; Table S1). Therefore, catechol

and phenol could be electron sinks of fermentation as opposed to the oxidized products resulting from exoelectrogenesis. Previous studies have reported catechol and phenol as biotransformation products from phenolic compounds under fermentative, anaerobic conditions.[32, 33] To understand how catechol and phenol were not rapidly transformed, as was the case of the other identified intermediates (i.e., FA, HMF-OH and diHBA), the thermodynamics of putative fermentation reactions were analyzed. It is assumed that catechol and phenol undergo fermentation, instead of exoelectrogenesis, because they have not been reported as suitable electron donors for exoelectrogens. The standard Gibbs free energy ($\Delta G^{0\prime}$) values of catechol and phenol fermentation are -69.12 and 8.46 kJ/mol, much more positive than those of FA, HMF-OH and diHBA (-763.66, -215.76, and -257.66 kJ/mol, respectively), as shown in Table S4. Thus, fermentation of phenol and catechol is expected to be less favorable than that of the other detected transformation products.

Anode Microbial Community. The 16S rRNA gene sequencing analysis revealed that after 9 weeks of MEC operation, the anode microbial community was dominated by *Proteobacteria* phylum, representing 68% of the population (Figure S7). The abundant genera belonging to this phylum were *Desulfovibrio* (39% of the total 16S rRNA gene sequences), *Pleomorphomonas* (11%), and *Geobacter* (5%). The second abundant phylum was *Bacteroidetes* (17%), comprised of *Petrimonas* (10%) and *Dysgonomonas* (7%) genera, followed by *Firmicutes* (12%) with the major genera of *Anaerovorax* (3%), *Phascolarctobacterium* (3%), and *Clostridium XIVa* (3%). Other phyla present with abundance less than 2% were *Synergistetes* and *Actinobacteria*. At the phylum level, the structure of the anode microbial community in the present study was similar to that of the original inoculum.[13]

The phylogenetic relationships of the abundant microorganisms (>1% abundance) detected in the MEC bioanode are summarized in Table S5 and the positions of the phylotypes in the phylogenetic tree are shown in Figure 4. The detected species in the bioanode are mainly related to exoelectrogens, putative degraders of the furanic and phenolic compounds, and potential syntrophic partners with exoelectrogens. *Desulfovibrio desulfuricans* is a sulfate-reducing bacterium, which is able to perform exoelectrogenesis through cytochrome c.[34] In addition, the major known degraders of furfural and 5-HMF under anaerobic conditions belong to *Desulfovibrio* genus.[35] *Geobacter spp.* are well studied exoelectrogens, using acetate and H_2 as primary electron donors.[28, 36] *Eubacterium limosum* is known to grow on methoxylated aromatic compounds, such as syringic acid and vanillic acid.[37] *E. limosum* is also a homoacetogen, which could consume H_2 formed during fermentation and produce acetate for exoelectrogens.[38] *Pelobacter propionicus* is not known to perform exoelectrogenesis or to use acetate as the electron donor, but is thought to be involved in syntrophic interactions with exoelectrogens fermenting initial substrates to acetate.[10, 39] *Clostridium populeti* and *Clostridium aminobutyricum* are known mixed-acids fermenters, and the latter has been reported in acetate- or glucose-fed MFC anodes.[40, 41] *Phascolarctobacterium faecium* can convert succinate to propionate.[42] Therefore, *E. limosum*, *P. propionicus*, *P. faecium* and the *Clostrium spp*. could be syntrophic partners with exoelectrogens by converting the furanic and phenolic compounds or their biotransformation products to readily available substrate (e.g., acetate) for exoelectrogenesis. Several detected species are closely related to bacteria, which have been reported in bioelectrochemical systems, but with unclear functions, such as *Dysgonomonas spp.*,[13] *Pleomorphomonas oryzae*, [43] and the uncultured bacterium clones (JX462549.1 and GU083415.1).[39, 44] The other related species have been reported in anaerobic sludge digesters,

such as *Cloacibacillus evryensis*, an amino-acid degrading bacterium,[45] and the bacterium isolated from cellulose and xylan-pectin enrichments of cow feces.[46] These species may have been carried over from the original inoculum, which came from a municipal anaerobic digester.

The present study demonstrated the potential of MEC as a waste-to-resource process to convert the problematic components in lignocellulosic hydrolysate and pyrolysate to H_2, which can be used for the hydrogenation of bio-oils, thus eliminating the need to reform natural gas to H_2. The effective conversion of the furanic and phenolic compounds also demonstrates the advantage of MEC as a bioprocess for H_2 production, utilizing compounds which are known inhibitors in the dark fermentation process. However, the H_2 production rate achieved in this study needs to be further improved. The observed inhibition of exoelectrogenesis at 1,200 mg/L must also be addressed. On-going research is investigating continuous-flow MEC operation to find the means to mitigate the observed inhibitory effect during the batch MEC operation as well as increase the H_2 production rate and yield.

ASSOCIATED CONTENT

Supporting Information

Tables S1-S5, and Figures S1−S7 are available free of charge via the Internet at http://pubs.acs.org.

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553 **Table 1.** Efficiency parameters of the MEC fed with the mixture of the five compounds.

Parameter	Initial substrate concentration (mg/L)		
	200	400	800
H_2 yield			
(mol/mol) [a]	2.9	2.5	1.7
(%) [b]	38	42	26
sCOD removal (%)	49	49	61
CE (%)	58	69	44
H_2 production rate (L/L-d) [c]	0.07	0.10	0.10

554

555 **[a]** Moles of H_2 collected per mole of the compound mixture transformed

556 [b] H_2 COD per COD removed during each batch run

557 [c] Maximum production rate, observed on day 1 during each feeding cycle (20ºC and 1

558 atm) normalized to the empty bed volume of the anode chamber (0.25 L)

LIST OF FIGURES

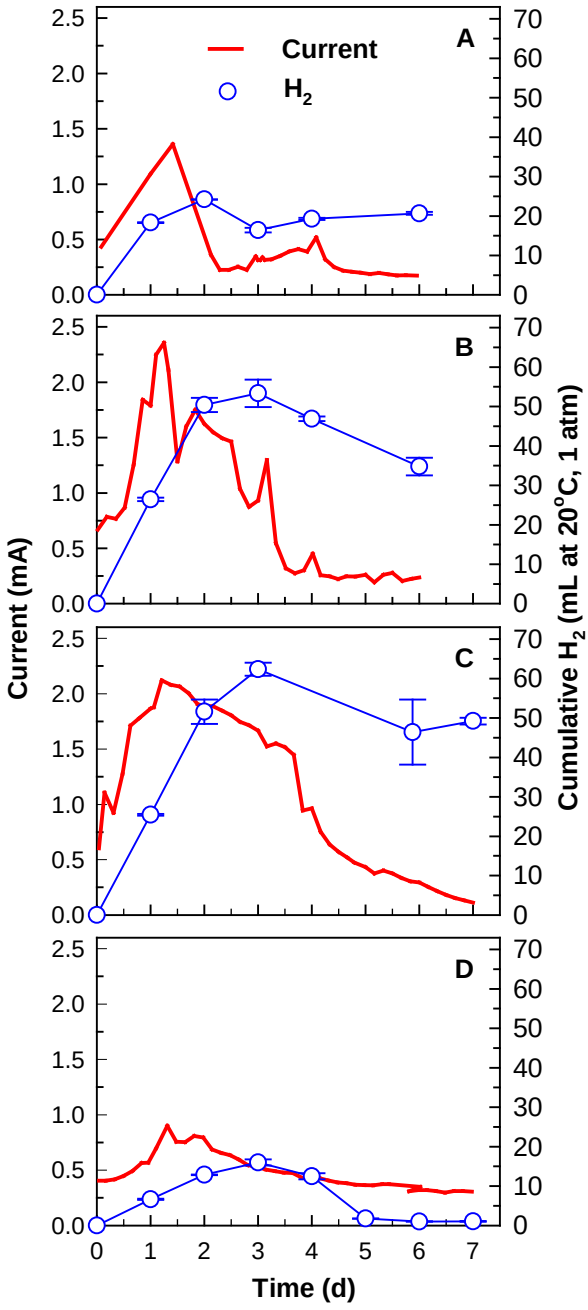

Figure 1. Current and cumulative H_2 production during four feeding cycles conducted at increased initial substrate concentrations in the MEC anode (A, 200 mg/L; B, 400 mg/L; C, 800 mg/L; D, 1,200 mg/L). Error bars represent mean values ± one standard deviation, $n = 3$.

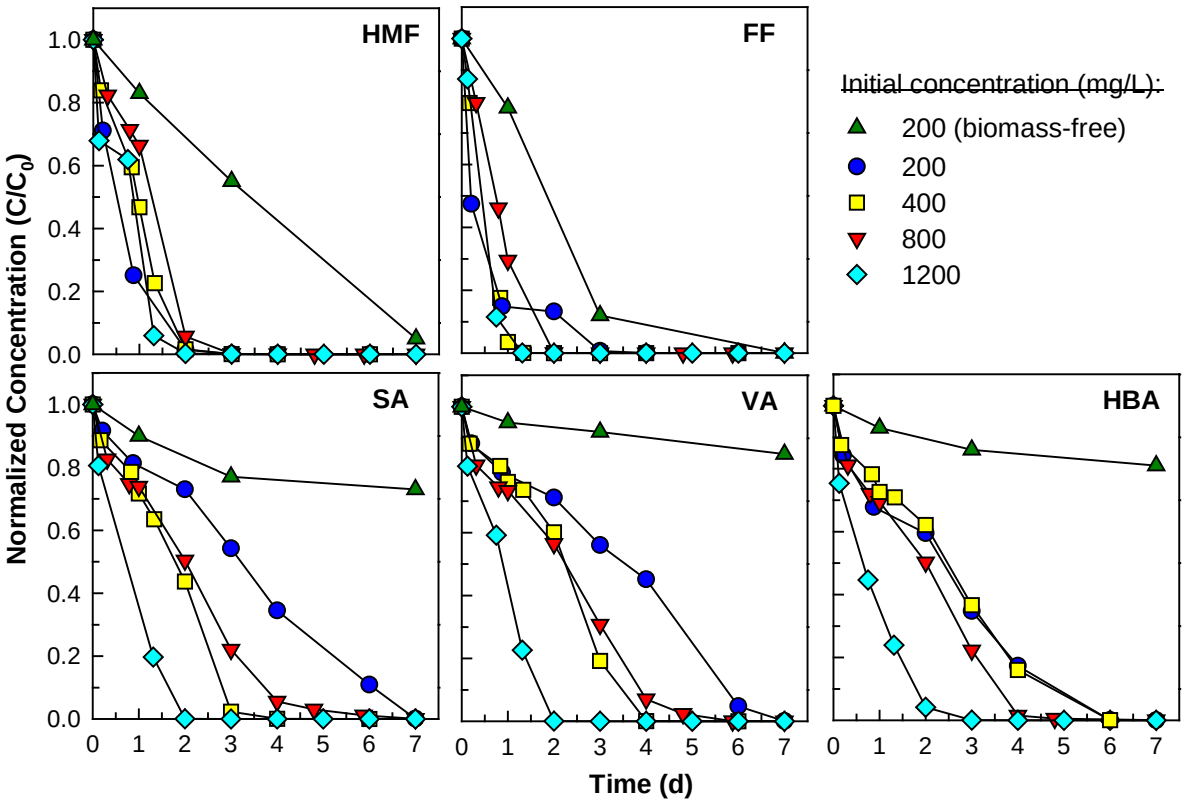

Figure 2. Normalized concentration profiles of the five compounds fed to the MEC anode at various initial concentrations (200 to 1,200 mg/L total concentration).

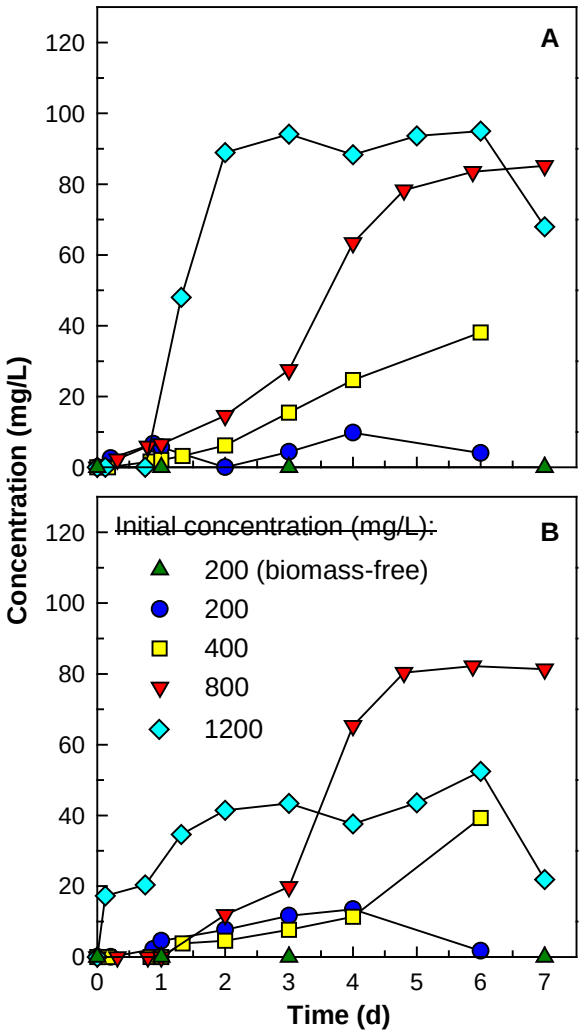

Figure 3. Accumulation of catechol (A) and phenol (B) during the four feeding cycles at increased initial substrate concentrations in the MEC anode (200-1,200 mg/L).

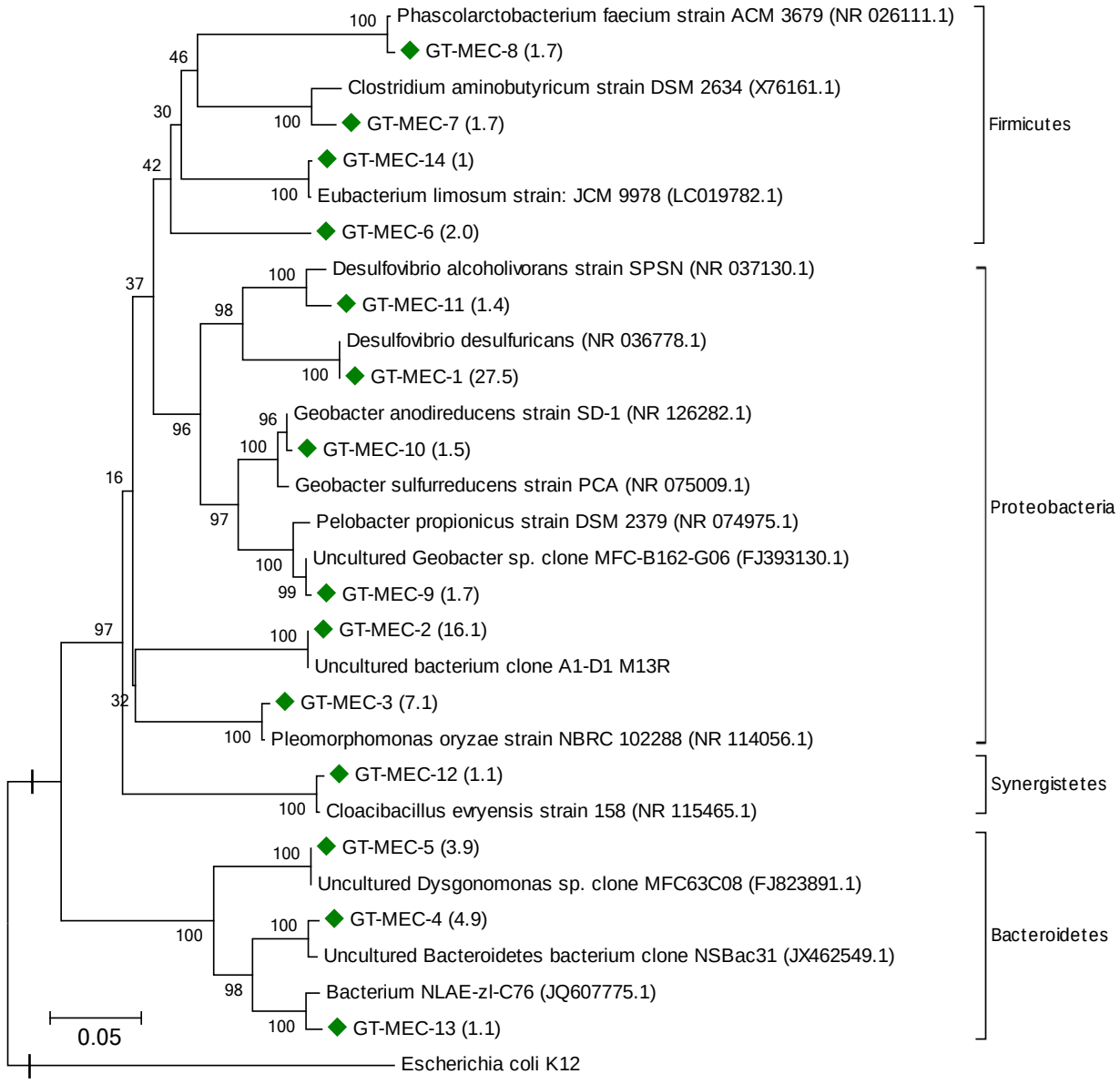

Figure 4. Phylogenetic tree of the dominant bacteria identified in the MEC anode. Fraction (%) of bacterial population and GenBank accession numbers shown in parentheses. *Escherichia coli* K12 was used as the outgroup.