Hydrogen production from switchgrass via an integrated pyrolysis-microbial electrolysis process

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Abstract

A new approach to hydrogen production using an integrated pyrolysis-microbial electrolysis process is described. The aqueous stream generated during pyrolysis of switchgrass was used as a substrate for hydrogen production in a microbial electrolysis cell, achieving a maximum hydrogen production rate of 4.3 L H₂/L (anode)-day at a loading of 10 g COD/L-anode-day. Hydrogen yields ranged from 50 ± 3.2% to 76 ± 0.5% while anode Coulombic efficiency ranged from 54 ± 6.5% to 96 ± 0.21%, respectively. Significant conversion of furfural, organic acids and phenolic molecules was observed under both batch and continuous conditions. The electrical and overall energy efficiency ranged from 149-175% and 48-63%, respectively. The results demonstrate the potential of the pyrolysis-microbial electrolysis process as a sustainable and efficient route for production of renewable hydrogen with significant implications for hydrocarbon production from biomass.

Keywords

Microbial electrolysis cell (MEC); Integrated pyrolysis-MEC; Bio-oil aqueous phase; Integrated hydrogen production

1.0 Introduction

Sustainable hydrogen supply and water management are the two most significant sustainability issues facing biorefinery development. Production of transportation fuels from biomass via pyrolysis enables production of hydrocarbon fuels, making it a high priority alternative for biorefineries. However, due to the high oxygen content of biomass, significant amount of hydrogen is needed for deoxygenation and generation of fuel hydrocarbons. The ability to produce hydrogen from biomass or biomass-derived streams can significantly reduce the greenhouse gas emissions released by use of natural gas as a hydrogen source. Production of hydrogen from natural gas contributes 18% to capital costs via the thermochemical route converting biomass to fuels (Jones et al., 2013). Biomass used for pyrolysis typically contains 20% moisture or more resulting in a water-rich bio-oil from the pyrolysis process. Fast pyrolysis and intermediate pyrolysis generate a bio-oil product which is essentially a mixture of aqueous and organic phases. Separation of the bio-oil for fuel production produces large amounts of aqueous phase containing organic carbon from biomass. A bio-oil derived from switchgrass was reported to contain over 70% aqueous phase (Imam & Capareda, 2012). If the carbon from the aqueous phase is not recovered, a significant fraction of biomass and energy is lost to the water phase, decreasing the overall efficiency of the process.

Production of renewable hydrogen from biomass is a long-sought technology for moving away from fossil fuels and towards a low-carbon economy. Microbial electrolysis is a versatile technology capable of addressing the issue of hydrogen production as well as water management

(Logan et al., 2008). By extracting energy from the aqueous stream in the form of hydrogen and treating water, the efficiency of biomass to fuel production can be increased by minimizing external hydrogen requirement and enabling water recycling (Borole, 2011). Previous work on bioanode development has resulted in a highly enriched anode consortium using the waste stream from ethanol fermentation, capable of converting furan aldehydes, phenolics, and other normally inhibitory compounds (Borole et al., 2013; Borole et al., 2009d). Optimization of electroactive biofilms to handle complex wastewater streams requires a multipronged approach that includes process, system design and biology parameters (Borole et al., 2011b; Sleutels et al., 2012). Recent advancements in understanding of the electrochemical performance of anode biofilms and the dependence of the enrichment process on organic loading, continuous vs. batch substrate delivery and other parameters have led to current densities reaching above 20 A/m² (Ichihashi et al., 2014; Sleutels et al., 2012).

In this study, we report conversion of the complex mixture of organics present in the bio-oil aqueous phase (boap) derived from biomass pyrolysis containing phenolic compounds, furan aldehydes, organic acids and sugar derivatives in an MEC to produce hydrogen. This mixture is overall more recalcitrant than the fermentation stream previously utilized in bioanode (Borole et al., 2013). The development of an electroactive microbial community capable of handling the toxic compounds and generating hydrogen using a switchgrass pyrolysis-derived aqueous phase is reported. The composition of the microbial community developed for treatment of boap is described. The efficiency of conversion in the anode as well as cathode, and overall hydrogen production efficiency and productivity and their reproducibility in replicate experiments in two different MECs is reported.

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2.0 Methods.

2.1 Biomass pyrolysis

The feedstock used in the study was air-dried switchgrass with particle sizes less than 2 mm and a moisture content of 7–8 wt%, which was obtained from Genera Energy Inc. (Vonore, TN). A semi pilot auger pyrolysis system was used in this study to carry out **intermediate pyrolysis**. The system was equipped with a feeding system, an auger pyrolysis reactor, a biochar collector, a particle-precipitating chamber, and a condensation section (Supplemental Figure S1). **A detailed description of the pyrolysis system used in this study, (located at the Center for Renewable Carbon, University of Tennessee)**, is provided elsewhere (Kim et al., 2011). In brief, the bio-oil used in this study was produced under the following operation conditions. The feedstock was transferred from the feeding hopper to the pyrolysis reactor by a single auger with feeding rate of approximately 8.5 kg/h. The **pyrolysis reactor** (10 W×10 H×250 L cm) contains internal dual augers. The auger speed controlled the residence time of feedstock at 72 seconds. The heated zone comprised of a 200 cm long electrical resistance furnace operating at 500 °C. The sweeping gas (nitrogen gas, 20 L/min) was introduced into the front of the auger reactor and moved with the evolved vapors to the condensation section. Before the vapors enter the condensers from the auger reactor, the particle chamber (20 cm in diameter and 100 cm long) precipitated fine particles from the vapors. The biochar produced from the feedstock was collected into the biochar drum. The condensation section comprised of three condensers in series (10 cm in diameter and 200 cm long, each). Before the pyrolysis operation, all condensers were cooled to 10 - 15 °C using a circulation water cooling system. The bio-oils collected from three condensers

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4 96 were immediately combined and mixed for homogeneity and stored in the walk-in freezer until
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7 97 used for characterization. The pyrolysis operation was performed in duplicate.
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10 11 12 99 *2.2 Bio-oil separation and characterization* 13

14 100 The crude bio-oil obtained from the pyrolysis reactor was mixed with distilled water in a ratio of
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16 101 1:4 (wt.%) to separate bio-oil aqueous phase (boap: water soluble fraction) and an organic phase
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19 102 (water insoluble fraction). The mixture was shaken vigorously and placed at 4°C overnight. Then
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21 103 the mixture was centrifuged in an IEC Clinical centrifuge (model 120) at 5000 rpm for 30
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23 104 minutes to ensure the phase separation. The boap was collected and weighed to determine the
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26 105 amount of bio-oil dissolved in the water. The separated aqueous phase (boap) was used for
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29 106 hydrogen production in the MEC system.
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33 108 The chemical compounds in boap were identified by gas chromatography-mass spectrometry
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36 109 (GC-MS) and quantified by GC-flame ionization detector and high performance liquid
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38 110 chromatography (HPLC). A Shimadzu GC-MS (QP2010S) with a Restek Rtx-5MS capillary
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41 111 column was used. The column was programmed at 45°C for 3 min, then at 5°C/min to 150°C
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43 112 without holding, then 10°C/min to 260°C and a hold time of 7 min. The inlet was set at 240°C,
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46 113 and sample injection was made in split mode (1:20). The compounds were identified by
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48 114 comparing their mass spectra with those from the National Institute of Standards and Technology
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50 115 (NIST) mass spectral data library. Due to the very high water content in the boap, organic
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53 116 compounds were first extracted by ethyl lactate and chloroform and then analyzed by GC-MS.
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55 117 The crude bio-oil was diluted a factor 20 times before being injected into the GC-MS.
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GC-FID with a HP-5 column was used for quantifying volatile compounds in boap. The same program with the GC-MS was used in GC-FID. A HPLC system (Jasco 2000Plus, Jasco analytical instruments) equipped with PU-2089S Plus pump, a MD-2018 Plus Photodiode Array detector (PDA), a RI-2031 Plus intelligent RI detector, and an AS-2055 Plus auto sampler was used to analyze boap for non-volatile compounds. The liquid chromatography was conducted at 50.0°C using Bio-rad HPX-87H (300 x 8 mm) column. The volume of injected sample was 20 μ l. The mobile phase was 5mM H₂SO₄ with or without 15 % acetonitrile (v/v) with a flow rate of 0.6 mL/min. The method using 15% acetonitrile was used primarily to ascertain elution of all peaks from the column and to determine appropriate run time. The use of acetonitrile posed a problem with quantification of organic acids; therefore a method without acetonitrile was used with a longer run time of 120 min.

2.3 MFC and MEC construction

The bioelectrochemical system used in this study consisted of a two-chamber cell that was convertible between MFC and MEC. The anode consisted of a carbon felt (projected surface area = 12.56 cm², specific surface area = 454 cm²cm⁻³, anode volume = 15.96 cm³) that was plasma treated to increase hydrophilicity (Borole et al., 2008). The anode and cathode chambers are separated by a membrane electrode assembly (MEA) consisting of Nafion and platinum-deposited carbon (0.5 mg/cm²; 10% Pt; E-TEC, FuelCellsEtc., Bryan, TX) on the cathode side. The cathode chamber was 16 ml and included a 12 cm² piece of stainless steel mesh as a current collector in contact with the Pt-deposited carbon cathode. The cathode inlet/outlet was open to air during operation of the system as MFC but was sealed and made oxygen-free by sparging with N₂ for MEC operation. The two chambers were connected via an external resistor attached

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4 142 to a carbon rod current collector inserted into the anode and stainless steel wire at the cathode.

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7 143 All enrichment and experiments were carried out at room temperature (22°C).

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11 145 *2.4 Inoculation and operation*

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14 146 The MFC was inoculated using previously enriched consortia from two different anodes. The
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16 147 first was from an anode pre-enriched on boap, which used an inoculum from a bioanode grown
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19 148 on a corn stover-derived fermentation process stream (Borole et al., 2013). The second came
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21 149 from an anode fed with furan aldehydes and phenolic model compounds as the substrate (Borole
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24 150 et al., 2009d). Multiple consortia were used to enable broad specificity in the new community to
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26 151 handle the different class of compounds present in boap. The inoculum consisted of a total of
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29 152 two cores (5 mm diameter x 12.5 mm length) cut out of the anode carbon felt along with anodic
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31 153 biofilm grown on the felt from the previously mentioned anodes. The inoculum was immediately
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34 154 transplanted into a similar-sized hole in new MFC anode felt. Two duplicate MFCs were
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36 155 developed in parallel and followed an enrichment procedure similar to that reported previously
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38 156 (Borole, 2010; Borole et al., 2009b). The operation of the MFC prior to testing included
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41 157 continuous supply of boap added directly in the flow line via syringe pump as shown in Figure 1.
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43 158 The starting load on the MFC was 220 Ω , at which the MFCs reached their peak voltage of 0.36
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46 159 V after 14 days. The resistance was then dropped to 100 ohms on day 15 and to 51 ohms on day
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48 160 17. Glucose was supplemented in batches of 0.2 g/l approximately once a month over the first 6
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51 161 months of operation to facilitate growth of boap converting microbes. A minimal nutrient
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53 162 medium consisting of mineral salts and vitamin solution was used as reported previously (Borole
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55 163 et al., 2009b). The medium was placed in an external reservoir and circulated through the anode
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4 164 chamber at a flow rate of 5 mL min⁻¹. Media and planktonic cells were replaced intermittently to
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6 165 maintain an optical density (600 nm) of the medium below 0.05 units.
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9 166 10 11 167 *2.5 Chronoamperometry - Hydrogen production* 12 13

14 168 The air-cathode was converted to an oxygen-free cathode by sparging with N₂ to enable
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16 169 operation of the system as MEC. Additionally, the cathode chamber was filled with 12 ml of 100
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19 170 mM phosphate buffer followed by additional sparging with N₂. A viton tube was attached to the
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21 171 cathode outlet and was inserted into an inverted 100 or 250 ml graduated cylinder containing DI
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24 172 water for gas collection. The cathode inlet was then sealed and the nitrogen sparging stopped to
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26 173 allow collection of hydrogen gas. Chronoamperometric measurements were conducted by
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29 174 poisoning the anode electrode at a potential of -0.2 V relative to a Ag/AgCl reference electrode
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31 175 (Nam et al., 2011). The cell potential was also recorded to determine the total energy applied to
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33 176 the cell. The method to operate MEC by poisoning the anode electrode was used instead of poisoning
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36 177 the cathode or applying a fixed potential across the cell since it offers the advantage of
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38 178 maintaining the anode potential at an optimal value and preventing damage to the anode
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41 179 community from excessively positive potentials .The cathode gas outlet line included a T-
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43 180 junction to allow gas sampling via a sealed septa sampling port. Boap was tested in batch at
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46 181 concentrations of 0.3, 0.2 and 0.1 g/L as well as in continuous addition mode at an organic
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48 182 loading of 2, 4, 10 g/L-day. The concentration and loading refer to COD of the boap supplied to
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51 183 the anode. The boap was diluted to 50 g/L and filtered using a 0.45 µm nylon filter before use.
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53 184 During batch tests with boap, time zero samples were taken before poisoning of the anode or
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55 185 circulation of the aqueous medium. Between experiments, the anode medium was replaced and
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58 186 the inlet and outlet lines flushed with fresh medium. The cathode buffer was also replaced
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4 187 between experiments. The hydrogen production measurements were initiated immediately after
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6 188 starting chronoamperometry. A Reference 3000 potentiostat/galvanostat/zero resistance ammeter
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9 189 (Gamry Instruments, Warminster PA) was used for all MEC experiments. Depending on the
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12 190 substrate level used, each experiment was allowed to run between 8-24 hours. At the end of each
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14 191 run, the volume displaced by hydrogen production was measured, gas samples for GC analysis
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16 192 were taken from the cathode outlet to confirm hydrogen production, and samples for HPLC
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19 193 analysis were taken from the external anode reservoir to measure the extent of conversion of the
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21 194 substrates in boap. HPLC samples from MEC were centrifuged for 10 min at 4500 rpm, and then
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24 195 filtered with 0.2 μ m nylon filter and stored at 4 °C for analysis. HPLC samples were analyzed as
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26 196 described in section 2.0.

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33 199 Samples prepared for HPLC as outlined above in section 2.5 were also analyzed via the Hach
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36 200 COD method. A 2 ml sample was added to a Hach COD digestion vial and inverted several times
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38 201 for mixing. The samples were then placed in a Hach DRB 200 reactor at 150 °C for 2 hours.
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41 202 Upon completion, samples were allowed to cool to room temperature before spectroscopic
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43 203 analysis. A calibration curve was generated prior to running samples, utilizing 10 concentrations
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45 204 from 25 mg to 1000 mg of COD standard solution (Potassium Acid Phthalate). Absorbance
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48 205 readings were taken at 620 nm on Spectronic 20 Genesys and converted to concentration based
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50 206 on the calibration curve.

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MFC's were disconnected and moved into an anaerobic glove bag for sampling of the microbial community from the anode felt. The MFC's anode chamber was opened up and a coring tool was used to remove a 5 mm diameter x 12.5 mm long core of felt from above the inlet area in the anode, which was replaced by a fresh piece of matching felt. Cores were stored in either sterile MilliQ water or 25% glycerol in freezer vials for storage at -80°C. Genomic DNA was isolated using the standard freeze-thaw procedure, followed by phenol-chloroform extraction (Borole et al., 2009a; Borole et al., 2009b). Extracted and purified DNA samples were submitted to Hudson Alpha Genomic Services Lab (Huntsville, AL) for library prep and 16s rRNA analysis. Their methods included PCR reactions were run using 10ng of input template, V3-V4 amplicon primers, and Kapa Master mix (20 cycles). Products were purified using 0.75X beads, and were then put into a second, indexing PCR reaction (Kapa master mix, 12 cycles of PCR, GSL3.7 indexes for I7). Final libraries were purified and quantified by Qubit. Products were pooled using the Biomek liquid handler and profiled using the Agilent Bioanalyzer. Kapa qPCR was also done for another quality control. Sequencing was then carried out with MiSeq 250 bp PE run and sequence data was analyzed on BaseSpace using 16S Metagenomics v1.0.1 (ILLUMINA, INC).

2.8 Calculation of Coulombic efficiency and other conversion efficiencies.

Performance was characterized by anodic Coulombic efficiency (CE), cathodic conversion efficiency, percent hydrogen yield, electrical efficiency and overall energy efficiency. CE was calculated from the produced current I_{obs} , the moles of substrate n_s used and the number of electrons available per mol of substrate (based on complete conversion to CO₂) n_e , using the following equation:

$$CE_{anode} = \frac{I_{obs}t}{n_s n_e F}$$

F is Faraday constant and t is the duration of the experiment. The boap concentration was measured as COD as well as using HPLC analysis. However, all compounds were not identified by HPLC and therefore the total concentration of organics in boap was not available. Therefore, COD measurements before and after MEC treatment were used to determine the substrate concentration and loading. The total COD of the boap before dilution for MEC treatment was 130 g/L. The compounds identified by HPLC are being reported separately (Ren et al., 2015, manuscript in preparation) and were used to understand the conversion of the individual compounds in the anode. Percent hydrogen yield was calculated as grams of hydrogen produced per gram of hydrogen expected from conversion of substrate utilized in the anode. Since absolute boap concentration was not available, COD was used as a measure of substrate concentration to calculate the percent yield:

$$Y_{H_2} = \frac{PV_{H_2}/RT}{2\Delta nCOD}$$

where P is the atmospheric pressure, V_{H_2} is the volume of hydrogen produced, R is the gas constant, T is the temperature in Kelvin and $\Delta nCOD$ is the moles of COD consumed during the experiment. Hydrogen productivity or rate of hydrogen production was calculated directly from the volume of hydrogen produced per unit anode volume per unit time:

$$Q_{H_2} = \frac{V_{H_2}/V_{anode}}{t}$$

Cathodic conversion efficiency was calculated as the ratio of the amount of hydrogen produced and the maximum amount of hydrogen that could be produced based on the average current generated over the experiment, I_{obs} . This was calculated by the following equation:

$$CE_{cat} = \left(\frac{V_{H_2}}{\frac{I_{obs} t R T}{2 F P}} \right)$$

Energy efficiency calculations were done using the method described by Logan et al., 2008 defined by the overall (η_{E+S}), which is calculated from the combustion energy of hydrogen (W_{H_2}), substrate input (W_s), and electrical energy input (W_E):

$$\eta_{E+S} = \frac{-W_{H_2}}{W_E - W_s}$$

The energy content used for boap was based on COD and estimated to be 14.955 kJ/gCOD (Lide, 1995). Similarly, electrical efficiency (η_E) is the ratio of total energy recovered as hydrogen (W_{H_2}) to the electrical energy input (W_E)

$$\eta_E = \frac{-W_{H_2}}{W_E}$$

3. Results and Discussion

3.1 Bio-oil production and pyrolysis yields

Pyrolysis of switchgrass using a pilot-scale auger reactor yielded 50-54% bio-oil, 29% biochar, and 17-21% non-condensable gas. Two separate pyrolysis runs were carried out, but only the bio-oil from the first run was used in the experiments described in this report. The compounds identified in the two bio-oils were similar as described in Section 3.2.

3.2 Bio-oil and aqueous phase characterization

After separation of the aqueous phase from the bio-oil via addition of deionized water, about 35.1 wt% organic compounds in the crude bio-oil were extracted to the aqueous phase (boap) to form a solution of about 8.3 wt% of organics in the aqueous phase. This corresponds to a COD

of 130 g/L. The aqueous fraction contained about 19.1 wt% of the original biomass as dissolved organics. Figure S2 shows a distribution of the pyrolysis products and the organic and aqueous fractions resulting from fractionation of bio-oil after water addition. Compounds identified by GC-MS in the crude bio-oil and aqueous phase are provided in Table S1 in Supplemental information. The identified compounds were mainly categorized to nine groups: acids, alcohols, aldehydes, esters, furans, ketones, phenolics, sugars, and PAHs (poly aromatic hydrocarbons). The compounds extracted into the aqueous phase based on GC-MS analysis indicated that the boap contained alcohols, furans, ketones, and phenolics. Organic acids and levoglucosan were detected in crude bio-oil at high concentration and were extracted into the aqueous phase. According to the GC-MS analysis for crude bio-oil and boap, a total of 17 compounds including 3 acids, 3 furans, 2 alcohols, 6 phenolics, 2 ketones and 1 anhydrosugar were quantified by GC-FID and HPLC-PDA. Among these compounds eight major compounds were quantified by HPLC-PDA and another 9 compounds were quantified by GC-FID using external standards. The results for quantification of the compounds in boap are shown in Table 2. Levoglucosan and acetic acid were detected in boap at concentration of 15.33 and 11.96 g/L. Other compounds were present at relatively lower concentrations, generally below 3 g/L. The total amount of compounds quantified by HPLC in boap was about 37g/L. GC-FID analysis quantified 9 volatile compounds in boap. GC-FID analysis showed 1,3-propanediol, 2(5H)-furanone, and 1-hydroxybutanone at relatively higher concentrations (> 1 g/L) with additional 6 other compounds which were below 0.6g/L in boap. Total amount of compounds quantified in boap by GC-FID was about 6.02 g/L. The total concentration of compounds quantified together by HPLC-PDA and GC-FID was 43.01g/L.

3.3 Microbial anode development

The duplicate MFCs developed using the methods described in section 2.4 were operated for 8 months prior to this study with boap as the substrate and reached a stable output of 1 mA at a loading of 2 g/L-day. The development of an anode microbial consortium capable of utilizing the complex compounds in boap that are also inhibitory to microbial growth was possible due to addition of glucose as a co-substrate. Previous trials without inclusion of glucose did not yield sufficient current production (data not shown). Acclimation and enrichment of a variety of microbes with different roles is critical to enhance the overall ability of the community to collectively breakdown a wide spectrum of compounds and harvest the electrons (Borole et al., 2013; Borole et al., 2011a; Borole et al., 2011b).

3.4 16s rRNA analysis:

Figure 2 shows characterization of the anode community based on taxonomical classification of 16S rRNA genes, identified to the family level. As expected, Proteobacterium dominated the community potentially due to its diverse metabolic capabilities and utilization of the molecules in the complex boap. The duplicate bioanode showed significant similarity in community structure among the major families identified, sharing 5 of the top 8 families, including Geobacteraceae, and Rhodocyclaceae. The relative proportions of the members did vary slightly, with the most significant difference between duplicates being the presence of Enterococcaceae (16%) and Comamonadaceae (10%) occurring at high proportions in the first and second duplicate, respectively. While these duplicate MFC's were treated identically, natural variation in the starting inoculum, which was a biofilm growing on carbon felt electrode core removed from other bioanodes, could be a reason for the differences. In addition to the common

Geobacteraceae family known for electrogenic activity, Rhodocyclaceae has also been previously found in acetate-fed bioanodes and have also been linked to versatile metabolic capabilities including fatty acid and aromatic conversion (Borole et al., 2009a; Hesselsoe et al., 2009). The family Comamonadaceae has been found in cellulose-fed bioanodes and can also convert short-chain fatty acids and has been shown to be capable of electricity generation (Rismani-Yazdi et al., 2007; Xing et al., 2010). The family Enterococcaceae also contains known electrogens such as *Citrobacter freundii* and *Klebsiella oxytoca* (Sun et al., 2010). The presence of a variety of microbes with differing capabilities and unclassified bacteria is likely the result of the complex substrate used in this study, since multiple studies have indicated the effect of carbon source on community development and the establishment of syntrophic interactions (Borole et al., 2011b; Pant et al., 2010).

3.5 Microbial electrolysis

3.5.1 Batch operation

Investigations into hydrogen production with batch addition of boap resulted in an average hydrogen production rate of 1.28 ± 0.17 L H₂/L-anode-day (referred to as L/L-day henceforth) at a boap concentration of 0.1 g/L. Increasing the boap concentration increased the hydrogen productivity to 1.91 ± 0.47 L/L-day. This rate was an average obtained over a period of 8 -20 hours, depending on the boap concentration used. The current production during the MEC operation is shown in Figure 3. An average of four replicates is shown in Figure S5 in the Supplemental Information to demonstrate reproducibility. The initial current produced in the MEC during the first hour was in the range of 5-10 mA, depending on the boap concentration, which dropped to less than 1 mA over the duration of the experiment. This shows that the initial

rate of hydrogen production was much higher than the average. At a concentration of 0.3 g/L, the rate of hydrogen production in the first two hours was 9.3 L/L-day, but the average over the duration of the experiment was less than a fifth of the initial rate. Hydrogen collection was halted after the current dropped below 1 mA in all experiments. A replicate MEC (MEC B), operated under the same conditions resulted in a similar hydrogen production profile. The results are shown in Supplemental Figure S3. The differences in the two replicates can be attributed to small differences in inoculum and unequal biocatalyst density during growth in the anode.

The anode Coulombic efficiency (CE) for the MEC A ranged from $93 \pm 1.7\%$ at boap concentration of 0.1 g/L to $76 \pm 3.1\%$ at a concentration of 0.3 g/L. The results for the replicate (MEC B) as well as MEC A are shown in Figure 4a. The anode CE was calculated using COD of the samples before and after MEC treatment. The cathode conversion efficiency ranged from $77 \pm 3.4\%$ to $81 \pm 2.4\%$ for MEC A and $81 \pm 2.99\%$ to $86 \pm 7.6\%$ for the duplicate, MEC B (Figure 4b). Thus, the boap concentration appears to have a negligible effect on the anode CE and cathode conversion efficiency. The percent hydrogen yield obtained at the three batch concentrations ranged between $56 \pm 8.2\%$ and $72 \pm 1.8\%$ (Figure 4c). Thus, the percent hydrogen yield also appears to be negligibly affected by boap concentration under batch conditions.

3.5.2 Continuous substrate addition

Effect of boap loading on MEC performance was assessed by changing the loading from 2 g/L-day to 10 g/L-day. The hydrogen production rate increased proportionally with the boap loading rate from 0.9 ± 0.06 to 4.3 ± 0.05 L/L-day as shown in Figure S4 in Supplemental information.

The corresponding current density was $1.2 \pm 0.1 \text{ A/m}^2$ to $4.5 \pm 0.22 \text{ A/m}^2$, respectively. Compared to the batch run, the current production as well as hydrogen generation was relatively constant during the continuous addition experiment. The anode CE, cathode conversion efficiency and percent hydrogen yield are shown in Figure 4d-f. The anode CE decreased from $96 \pm 0.2\%$ at 2 g/L-day to $54 \pm 6.5\%$ at 10 g/L-day. The cathode conversion efficiency, on the other hand increased from $79 \pm 0.7\%$ at 2 g/L-day to $94 \pm 5.5\%$ at 10 g/L-day. The hydrogen yield, on the other hand, decreased from $76 \pm 0.5\%$ to $50 \pm 3.2\%$, respectively.

3.5.3 Conversion of individual components of boap

In addition to determination of the total COD of boap, the samples were also analyzed by HPLC. This allowed determination of conversion of many of the individual compounds that could be resolved by the HPLC. Figure 5 shows the percent conversion of acetic acid, propionic acid, levoglucosan, 2-furfural and hydroxymethylfurfural (HMF) as well as the total which represented the sum of all compounds that were quantified by HPLC. It is observed that under batch conditions, more than 98% removal of acetic acid and furfural was achieved. Conversion of propionic acid was between 67-100% and that of HMF was between 21-57%. The majority of the phenolic compounds were also removed. Based on all the peaks quantified by HPLC, a total reduction of 92-95% was observed, demonstrating significant conversion of the furan aldehyde and phenolic compounds present in boap as well. Under continuous boap addition experiments, similar results were obtained (Figure 5b). This shows that the anode microbial community was very efficient in conversion of the organic acids, levoglucosan and furfural present in boap. A high level of conversion of the quantified phenolic compounds was also observed as evidenced

by the reduction in the ‘Total’ percent reduction in Figure 5, which includes phenol, 1,2-benzenediol, vanillic acid, guaiacol and 2,6-dimethoxyphenol.

3.5.6 Energy efficiency

The electrical energy efficiency (EE) and overall energy efficiency for the conversion of boap into hydrogen are shown in Figure 6. Under batch conditions, the overall EE decreased from $58 \pm 5.6\%$ at a boap concentration of 0.1 g/L to $51 \pm 3.3\%$ at 0.3 g/L. The efficiency results provided are average of the two replicate MECs. The electrical EE also decreased from $175 \pm 11\%$ to $149 \pm 6\%$, respectively. Under continuous addition conditions, the efficiencies decreased with increasing loading rate as shown in Figure 6b.

3.6 Improvement in treatment of complex feed streams in MEC

In this study, the primary goal was to demonstrate the capability of microbial electrolysis systems to produce hydrogen from carbon/energy rich waste streams such as those resulting from biomass conversion platforms. A bioanode capable of efficiently converting a real-world, complex mixture of organic compounds was developed and its ability to remove major compounds of interest within the mixture including corrosive and acidic compounds such as acetic acid and phenolic acids was demonstrated. The productivity of 4.3 L H₂/L-day achieved using the boap feedstock is the highest reported productivity for a complex substrate that we know of. Table 3 contains H₂ productivities and system information for MEC studies reported in the literature. Lu et al were able to achieve a productivity of 1.41 L/L-day using a post-fermentation effluent at an applied potential of 0.6 V in a single-chamber membraneless MEC (Lu et al., 2009). Escapa et al. were able to achieve a similar productivity of 1.42 L/L-day at

much larger anode volume > 200 ml using a synthetic fermentation effluent by altering the HRT and increasing the applied voltage to 1 V (Escapa et al., 2013). A common occurrence among other studies with lower output could be the lack of sufficient enrichment of the anode biocatalyst. It is our observation that in many other studies use of an anode with un-enriched inoculum or use of a model substrate for enrichment and testing with the substrate of interest is practiced. Our experience has shown this is insufficient for developing a robust community needed for treating complex waste streams, and use of enrichment procedures as that developed in our laboratory for mixtures of various substrates can alter community structure to enhance the functional capabilities of degradation and electron transfer (Borole et al., JPS 2008; ChemElectroChem 2014). Our focus on bioanode enrichment using a patented enrichment process with a previously enriched inoculum over several months resulted in development of a community capable of converting a variety of compounds into electrons. The dominant phyla of Proteobacteria and Firmicutes in the bioanodes have well-established syntrophic interactions for conversion of a variety of carbon compounds, as well as the presence of known electrogenic families such as Geobacteraceae allow for the conversion of a variety of compounds with high CE (Freguia et al., 2010; Kiely et al., 2011). Furthermore, our two-chamber system has been previously optimized relative to many other studies employing different designs such as H-type reactors, minimizing anode dead space and reducing electrode spacing, etc. (Borole et al., 2009b). Additionally, where other studies commonly utilize an applied potential difference between anode and cathode, the use of a method setting anode potential at -0.2 V vs. Ag/AgCl reference electrode used in our study may have contributed to maintaining a steady, high bioanode performance. This method also allows the cell voltage to reach the 1 V range, but prevents the anode from reaching damaging positive potentials that can occur when setting a

strict potential difference (Nam et al., 2011). The bioanode developed for this study also achieved high productivities of 12.4 L/L-day under continuous addition of acetic acid as a substrate (data not shown), even though this community has been enriched for a multi-substrate complex mixture and not pure acetic acid. Further growth of anode biofilm and utilization of alternate cathodes can potentially improve the performance even higher.

3.7 Advancements in conversion efficiency and hydrogen yield

While there was some variation in values for anode Coulombic efficiency among batch experiments due to experimental run time, maximum CE and percent hydrogen yield reached values of $93\% \pm 1.7\%$ and $81\% \pm 2.35\%$ respectively. The volume of hydrogen produced over the length of the experiments varied from 9-54 mL depending on the type and rate of loading, reaching the highest value during 10 g/L-day of continuous boap addition. A reduction in both H_2 yield and CE was observed at the higher concentrations, but this reduction was much more pronounced for continuous addition experiments, where the average CE and H_2 yield reduced to $54\% \pm 6.5\%$ and $54\% \pm 3.4\%$ at 10 g/L-day, respectively. CE and H_2 yield tend to trend together as H_2 production is a direct function of the electrons and protons harvested from the anode, but H_2 yield values can be slightly lower due to system losses and proton diffusion. Conversely, COD removal increased in opposite fashion, reaching the highest percentage of 52% at 10 g/L-day boap. The increased COD removal and decreased CE and H_2 yield could be explained by higher activity of fermentative organisms in the anode due to increased substrate saturation at higher loading rates, contributing to COD removal without harvesting electrons and protons decreasing CE and H_2 yield. Maximum COD removal for batch studies was $48\% \pm 1.5\%$ at 0.2 g/L boap. COD removal values were somewhat lower than those reported in the literature for

other complex/wastewater streams. This is likely due to the complexity of compounds present in boap, many of which are recalcitrant and commonly inhibitory to many microbes. Additionally, the experiments were stopped when the current production reached below 1 mA, and not continued until the current production ceased, indicating potential for higher COD removal with longer run times. Nonetheless, overall energy efficiency and electrical efficiency values were in the range of those reported for MEC studies, reaching values of $62\% \pm 0.67\%$ and $167\% \pm 2.26\%$ in batch studies.

One of the problems with bioanodes operated at high substrate concentration or loading rate is production of methane (Escapa et al., 2013; Ishii et al., 2008). Absence of methane in the product gas and absence of archaea in the bioanode demonstrate the effective suppression of methanogenic population from bioanode developed in this study. Experiments conducted in continuous and batch mode of substrate addition further illustrate that even when high substrate loading was used, methane production did not occur. The efficiency of conversion of boap to hydrogen was similar in both modes of operation, further illustrating the advantages of the enrichment and MEC operational procedure employed (Borole et al., 2011a; Borole et al., 2009b).

3.8 Implications for biorefinery application.

With continuing improvement in the ability to utilize significant portions of compounds present in many biorefinery waste streams at high efficiencies, microbial electrolysis systems have real potential for commercial integration into multiple biomass conversion platforms (Borole et al., 2013; Borole et al., 2009d). While commercial-scale pyrolysis technology is still being

developed, making it difficult to predict the volume and composition of an aqueous-phase stream, a recent update at the Pacific Northwest National Laboratory (PNNL) estimates that a 2000 ton/day pyrolysis plant would require 1000 m³ water/day, and produce approximately 1000 m³ wastewater/day (Jones et al., 2013) . This high water demand coupled with high GHG emitting natural gas reforming for H₂ production makes microbial electrolysis an attractive alternative for continued development. The PNNL study also alludes to this, stating that alternative, sustainable forms of hydrogen will be necessary for advancing the technology if they can be developed and made economically viable. Besides the pyrolysis platform, biomass to ethanol bioconversion and algal hydrothermal liquefaction are additional platforms that could be integrated with microbial electrolysis systems. A 2000 ton/day lignocellulosic biorefinery has the theoretical potential to generate 7200 m³/hr of hydrogen, based on the assumption of 50% conversion efficiency and a biodegradability factor of 0.5 (Borole & Mielenz, 2011) . In order to continue the rapid improvement of bioelectrochemical systems to meet future commercial demands, expanded efforts in under-investigated areas of metagenomics and transcriptomics of anode communities along with development of sustainable cathode materials or biocathodes, will be required in addition to continued efforts in improving reactor design and performance.

4. Conclusion

An integrated pyrolysis-microbial electrolysis process for hydrogen production was demonstrated. The MEC demonstrated a productivity of 4.3 L H₂/L-day and a percent hydrogen yield of 50.4 ± 3.2% at a loading of 10 g COD/L-anode-day. A maximum anode Coulombic efficiency of 96 ± 0.21% and a maximum cathode conversion efficiency of 94 ± 5.5% were also achieved, albeit at different loading rates. Nearly complete conversion of acetic acid, propionic

acid, levoglucosan and furfural was observed with an overall energy efficiency of 48-63%. The hydrogen produced from the integrated process can be used to hydrodeoxygenate bio-oil to make fuel, while reducing lifecycle greenhouse gas emissions.

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Figure 1. Experimental setup for investigating hydrogen production from boap using MEC.

Figure 2. Characterization of anode microbial consortia via 16S rRNA analysis for two replicate bioanodes.

Figure 3. Current production profile during batch and continuous operation of MEC generating hydrogen from bio-oil aqueous phase (boap). The concentration of boap was varied from a COD of 0.1 to 0.3 g/L. In continuous experiment, the organic loading rate was increased from 2 to 10 g/L-day resulting in a proportional increase in current production.

Figure 4. Batch substrate addition: (A) Anode Coulombic efficiency (B) cathode conversion efficiency (C) percent hydrogen yield. Continuous substrate addition: (D) Anode Coulombic efficiency (E) cathode conversion efficiency (F) percent hydrogen yield.

Figure 5. Extent of removal of key compounds from bio-oil aqueous phase via MEC. The column represented by 'Total' includes all peaks quantified by HPLC, which contribute 33% to the COD of boap. (A) Continuous addition of boap (B) Batch addition of boap (LG – Levoglucosan, AA – Acetic acid, PA – Propionic acid, HMF -Hydroxymethylfurfural, FF – Furfural)

Figure 6. Electrical and overall energy efficiency (EE) obtained during (A) batch and (B) continuous substrate addition experiments.

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Table 1. Concentrations of major chemical compounds in bio-oil aqueous phase quantified by HPLC-PDA and GC-FID.

Quantification method	Major chemicals	Concentration based on aqueous phase (g/L)
HPLC-PDA	Furfural	1.01
	1,2-benzendiol	1.77
	Phenol	1.8
	Levoglucozan	15.33
	Acetic acid	11.96
	Proponic acid	1.89
	Vanillic acid	2.69
	HMF	0.54
Total		36.99
GC-FID	Phenol, 2-methoxy-	0.25
	2-methyl-4-methyphenol	0.07
	Cyclohexanone	0.07
	3-methyl-1,2-cyclophetandiol	0.46
	2,6-Dimethoxyphenol	0.26
	1,3-propanediol	1.84
	3-ethylphenol	0.56
	2(5H)-Furanone	1.17
	1-hydroxybutanone	1.35
Total		6.02
Sum		43.01

Table 2. Hydrogen production from complex substrates in MECs and key efficiency parameters

Substrate	MEC Volume (ml)	System	Anode electrode	Cathode electrode	H ₂ productivity (LH ₂ /L-day)	Applied voltage Eap (V)	Current Density		Coulombic Efficiency	Overall energy Efficiency	References
							A/m ³	A/m ²			
lignocellulose effluent	28	single	Graphite fiber brush	Carbon cloth/Pt (10% Pt/C)	1.00 ± 0.19	0.5	1.15		110%	61%	(Lalaurette et al., 2009)
Fermentation effluent	72	single	carbon fiber brush	Carbon cloth/Pt 0.5 mg/cm ²	0.48	0.435			58%	23%	(Wang et al., 2011)
Domestic wastewater	584	two	Carbon paper	Carbon paper/Pt 0.5 mg/cm ²	0.154 LH ₂ /g-COD	0.5	< 0.6		23%	9.90%	(Ditzig et al., 2007)
Swine wastewater	28	single	Graphite fiber brush	Carbon cloth/Pt 0.5 mg/cm ²	0.9-1.0	0.5	106		29 ± 17% to 43 ± 2%	190 ± 39% (electrical)	(Wagner et al., 2009)
Fermentation effluent	26	single	Graphite brush	Carbon cloth/Pt 0.5 mg/cm ²	1.41	0.6	135		87%	70%	(Lu et al., 2009)
A de-oiled refinery wastewater	5	single	Graphite plates	Stainless steel mesh (Type 304)	N/A	0.7		2.1			(Ren et al., 2013)
Industrial and Food processing wastewater	28	single	graphite fiber brush	Carbon cloth/Pt (10 wt% on Vulcan XC-72)	0.58 0.35	0.7		2.1 2.4	12% 35%	3.8 ± 0.2 -1.2 ± 0.2 kWh/kg-COD	(Tenca et al., 2013)
Potato wastewater	28	single	graphite fiber brush	Carbon cloth/Pt 0.5 mg/cm ²	0.74	0.9	N/A		80%		(Kiely et al., 2011)
Winery wastewater	1000	single	graphite fiber brush	SS mesh	0.19 ± 0.04	0.9	7.4				(Cusick et al., 2011)
Milk, glycerol, starch	28	single	graphite fiber brush	graphite fiber cloth/Pt, 5.0 mg Pt/cm ²	0.94	0.8	150		50-70%		(Montpart et al., 2015)
Synthetic fermentation effluent	200	single	graphite felt	Ni-based gas diffusion	1.42	1.0	206			97%	(Escapa et al., 2013)
bio-oil aqueous phase	29.3	two	carbon felt	Carbon cloth/Pt 0.5 mg/cm ²	4.3 [‡] 2.5*	0.96 0.8	202 99		54% 79%	48% 60%	This Study

[‡] Continuous substrate addition conditions, * batch conditions.