The Effects of Furfural on Biological Hydrogen Production in Batch and Microbial Electrolysis Cell

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Graduate Program in Chemical and Biochemical Engineering

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Abstract

Furfural inhibits biohydrogen production and numerous studies has been carried out on detoxification of furfural to enhance biofuel production. Electrochemical detoxification of furfural is a new approach to remove furfural without the degradation of sugars. However, there is limited information on the effects of the degradation products of furfural on biohydrogen production in batch tests and the impact of furfural on anode-respiring bacteria (ARB) in microbial electrolysis cells (MECs).

This study investigated the effect of furfural and its derivatives, furfuryl alcohol (FFA) and 2-furoic acid (FA), on biohydrogen production. The impact of furfural on hydrogen production in MECs was also evaluated. The hydrogen yield increased from 259±11 mLH₂/gCOD<sub>added</sub> to 276±8 mLH₂/gCOD<sub>added</sub> by the addition of 1 g/L FA, but slightly decreased to 250±14 mLH₂/gCOD<sub>added</sub> by the addition of 1 g/L FFA. More than 90% of furfural was electrochemically removed and the hydrogen yield was 196±4 mLH₂/gCOD<sub>added</sub> from a mixture of glucose and electrochemically treated furfural. While 1 g/L furfural inhibited hydrogen production from glucose at a substrate-to-biomass (S°/X°) of 1, hydrogen yields using synthetic hydrolysate increased by up to 19% at 0.5 and 1 g/L furfural at various S°/X°. In continuous-flow systems, the inhibitory threshold level of furfural and furfural-to-substrate were 0.56 g furfural/L and 0.056 g furfural/g substrate. The threshold furfural concentrations in biohydrogen batch tests were 0 and 1 g/L for glucose and hydrolysate, respectively, and 2 ~ 4 g/L for a continuous-flow system. The results from acetate- and glucose-fed MECs with furfural were entirely different. The addition of 2 g/L furfural to the acetate-fed MEC did not affect the activity of ARB. In contrast, the glucose-fed MEC was inhibited by 0.7 g/L furfural and ARB were not revived.

Keywords

Biohydrogen, furfural, furfuryl alcohol, 2-furoic acid, batch, microbial electrolysis cell, two-stage AD
Co-Authorship Statement

Chapter 3: Impact of Furfural on Fermentative Biohydrogen Cultures and Anode Respiring Bacteria

Hyeongu Yeo, Nael Yasri, Basem Haroun, Chinaza Akobi, Hisham Hafez, and George Nakhla

A version of this chapter has been submitted to Environmental Science and Technology, 2016 (es-2016-041544).

My contributions are as follows:

- Design and conduction of experiments
- Analysis and interpretation of the results
- Writing the paper

Nael Yasri participated in the operation of the acetate-fed MEC.

Basem Haroun setup the continuous-flow reactors.

Chinaza Akobi assisted in the setup of the batch experiments.

Dr. Hisham Hafez reviewed the paper.

Dr. George Nakhla supervised the experiments and data interpretation. He also reviewed and corrected the paper.
I would like to thank God for allowing me to complete this study. I wish to thank all the
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Table of Contents

Abstract ................................................................................................................................. ii
Co-Authorship Statement .................................................................................................... iii
Acknowledgments ................................................................................................................ iv
List of Tables ........................................................................................................................ viii
List of Figures ...................................................................................................................... x
Abbreviations ..................................................................................................................... xii

Chapter 1 ................................................................................................................................. 1
  1 Introduction ......................................................................................................................... 1
    1.1 Background .................................................................................................................... 1
    1.2 Problem Statement ....................................................................................................... 2
    1.3 Research Objectives ................................................................................................... 3
    1.4 Research Contributions ............................................................................................... 3
    1.5 Thesis Organization ..................................................................................................... 4
    1.6 References .................................................................................................................. 4

Chapter 2 ................................................................................................................................. 7
  2 Literature Review ............................................................................................................... 7
    2.1 Introduction .................................................................................................................... 7
    2.2 Hydrogen Production Methods .................................................................................. 7
      2.2.1 Thermochemical Hydrogen Production ............................................................... 7
      2.2.2 Water Electrolysis ............................................................................................... 8
      2.2.3 Biohydrogen Production .................................................................................... 9
    2.3 Microbial Electrolysis Cell (MEC) ............................................................................. 13
      2.3.1 Hydrogen Production in MEC .......................................................................... 13
      2.3.2 Operational Parameters .................................................................................... 15
Chapter 2

2.3.3 Limitations of MEC ................................................................. 24
2.3.4 Thermodynamics of MEC ....................................................... 25
2.3.5 Performance Parameters of MEC .......................................... 31

2.5 Inhibition of Biohydrogen Production ........................................... 33
2.5.1 H₂ consuming Bacteria .......................................................... 33
2.5.2 Ammonia .............................................................................. 33
2.5.3 Metal Ions ............................................................................ 34
2.5.4 Volatile Fatty Acids (VFAs) .................................................... 34
2.5.5 Inhibitors from Pretreatment of Biomass .................................. 36
2.5.6 Methods for Reducing Inhibition ............................................. 39

2.6 Conclusions .............................................................................. 42
2.7 References .............................................................................. 44

Chapter 3 ......................................................................................... 65

3 Impact of Furfural on Fermentative Biohydrogen Cultures and Anode Respiring Bacteria .......................................................................................................................... 65

3.1 Introduction .............................................................................. 65

3.2 Materials and Methods ............................................................. 67
3.2.1 Seed Sludge .......................................................................... 67
3.2.2 Electrochemical Treatment of Furfural .................................... 68
3.2.3 Batch Hydrogen Tests ............................................................. 68
3.2.4 Continuous-Flow Biohydrogen Reactors ................................ 69
3.2.5 Microbial Electrolysis Cells (MECs) ....................................... 70
3.2.6 Analytical Methods ............................................................... 73
3.2.7 Modified Gompertz Model .................................................... 74
3.2.8 Calculations .......................................................................... 74

3.3 Results ..................................................................................... 75
3.3.1 Electrochemical Degradation of Furfural ............................................ 75
3.3.2 Batch Hydrogen Experiments ................................................................. 76
3.3.3 Biohydrogen Production in Continuous-flow Systems .......................... 80
3.3.4 Effects of Furfural on Current Density and Hydrogen Production in MECs ................................................................. 82
3.3.5 Statistical Analysis .................................................................................. 88
3.4 Discussion ................................................................................................... 88
3.4.1 Electrochemical Treatment of Furfural ................................................... 88
3.4.2 Hydrogen potential of furfural degradation products .............................. 89
3.4.3 Effects of Furfural on Biohydrogen Production ...................................... 89
3.4.4 Effects of Furfural on ARB ................................................................... 93
3.5 Conclusions ............................................................................................... 97
3.6 References .................................................................................................. 99

Chapter 4 .......................................................................................................... 105

4 Conclusions and Recommendations .............................................................. 105
4.1 Conclusions ............................................................................................... 105
4.2 Recommendations ...................................................................................... 106

Appendix A ........................................................................................................ 107
A1. Pretreatment Methods for MEC ................................................................. 107

Appendix B ........................................................................................................ 109

Appendix C ........................................................................................................ 111
C1. Copyright for Figure 2-3. ............................................................................ 111

Curriculum Vitae ............................................................................................... 112
List of Tables

Table 2-1. Hydrogen production methods in industry.5–7 ................................................. 8

Table 2-2. Internal resistances in BES and their affecting factors. ........................................ 20

Table 2-3. Terminologies for microorganisms associated with bioelectrochemical systems.65 .......................................................................................................................... 22

Table 2-4. Standard potential (E°) and theoretical potential for typical conditions at pH 7 and 298 K in BES.52 ........................................................................................................ 29

Table 2-5. Standard potential of selected important half reactions at pH 7 and 298 K.52,99 ............................................................................................................................... 30

Table 2-6. Inhibitory threshold levels of selected metal ions .................................................... 35

Table 2-7. Inhibitory threshold levels of selected VFAs .......................................................... 35

Table 2-8. Inhibitory threshold concentrations of furan derivatives and phenolic compounds on biohydrogen production in batch experiments ........................................... 38

Table 2-9. Selected pretreatments to enrich H2 producing bacteria in mixed cultures .... 39

Table 3-1. The composition of synthetic hydrolysate.29 .......................................................... 69

Table 3-2. The concentrations of acetate or glucose and furfural and the corresponding SCOD fed to the MECs ........................................................................................................ 72

Table 3-3. Hydrogen yields and production rates manually calculated and Gompertz parameters from batch experiments at F/M 1 (Average ± standard deviation). .......... 78

Table 3-4. Gompertz and calculated parameters from hydrogen batches at various S°/X°. ............................................................................................................................... 79

Table 3-5. Summary of end-products in the effluent from continuous-flow systems: R1 and R2 ......................................................................................................................... 81
Table 3-6. Calculated parameters for the consecutive batch cycles using the acetate-fed MEC .......................................................... 84

Table 3-7. Calculated parameters for the consecutive batch cycles using the glucose-fed MEC ........................................................................ 87
List of Figures

Figure 2-1. Biological hydrogen production methods .................................................. 9

Figure 2-2. A schematic diagram of a microbial electrolysis cell for biohydrogen production .................................................................................................................................................. 14

Figure 2-3. (a) The current-voltage polarization curve of MFC and (b) the current-voltage polarization curves of anode and cathode versus reference electrode. (Adapted from Zhao et al. 2009 53) ..................................................................................................................................................... 19

Figure 2-4. Extracelluar electron transfer mechanisms: (a) direct electron transfer, (b) electron transfer by a mediator, and (c) electron transfer via nano-wire. ............................................. 23

Figure 2-5. Inhibitors of fermentative biohydrogen production ...................................... 34

Figure 2-6. Formation of inhibitors from lignocellulosic biomass (modified Jonsson et al.137) ......................................................................................................................................................... 37

Figure 3-1. Degradation of furfural to 2-furoic acid by electrochemical treatment. Voltage of 1.0 V was applied to furfural solution of 700 mL for 3 hours. Data represent average concentrations of triplicate ..................................................................................................................................................... 75

Figure 3-2. Hydrogen production from batch experiments using different substrates. (FF: furfural; FFA: furfuryl alcohol; FA: 2-furoic acid; Glu+FF: glucose with furfural; Glu+FFA: glucose with furfuryl alcohol; Glu+FA: glucose with 2-furoic acid; Glu+ETF: glucose with electrochemically treated furfural.) ......................................................................................................................................................... 76

Figure 3-3. Current densities during six consecutive acetate fed-batches over time recorded from MEC (SA: sodium acetate). ............................................................................................................................ 83

Figure 3-4. The trends of current density with different composition of substrate: glucose fed cycles (1-3), glucose and furfural fed cycles (4-6), furfural fed cycles (7-9) and glucose fed cycles (10-12). The influent TCOD of all cycles was 2417±180 mg/L. ....... 86
Figure 3-5. Hydrogen production inhibition (%) plotted against (a) furfural concentration (g/L), (b) furfural-to-substrate, (c) furfural concentration and furfural-to-substrate in continuous-flow systems (R1 and R2). The negative $H_2$ production inhibition means that $H_2$ production increased. .......................................................... 92

Figure 3-6. Concentration of volatile fatty acids and 2-furoic acid in MEC effluent...... 94

Figure 3-7. The degradation of furfural in the glucose-fed MEC........................................ 95
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADS</td>
<td>Anaerobic digested sludge</td>
</tr>
<tr>
<td>AEM</td>
<td>Anion exchange membrane</td>
</tr>
<tr>
<td>ARB</td>
<td>Anodic respiring bacteria</td>
</tr>
<tr>
<td>BES</td>
<td>Bioelectrochemical systems</td>
</tr>
<tr>
<td>CEM</td>
<td>Cation exchange membrane</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>EAB</td>
<td>Electrochemically active bacteria</td>
</tr>
<tr>
<td>EET</td>
<td>Extracellular electron transfer</td>
</tr>
<tr>
<td>emf</td>
<td>Electromotive force</td>
</tr>
<tr>
<td>FA</td>
<td>2-Furoic acid</td>
</tr>
<tr>
<td>FF</td>
<td>Furfural</td>
</tr>
<tr>
<td>FFA</td>
<td>Furfuryl alcohol</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HMF</td>
<td>5-Hydroxymethyl furfural</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>IC</td>
<td>Ion chromatography</td>
</tr>
<tr>
<td>MEC</td>
<td>Microbial electrolysis cell</td>
</tr>
<tr>
<td>MFC</td>
<td>Microbial fuel cell</td>
</tr>
<tr>
<td>NHE</td>
<td>Normal hydrogen electrode</td>
</tr>
<tr>
<td>NRB</td>
<td>Nitrate-reducing bacteria</td>
</tr>
<tr>
<td>PEM</td>
<td>Proton exchange membrane</td>
</tr>
<tr>
<td>RID</td>
<td>Refractive index detector</td>
</tr>
<tr>
<td>SCOD</td>
<td>Soluble chemical oxygen demand</td>
</tr>
<tr>
<td>SHE</td>
<td>Standard hydrogen electrode</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulfate-reducing bacteria</td>
</tr>
<tr>
<td>TCD</td>
<td>Thermal conductivity detector</td>
</tr>
<tr>
<td>TCOD</td>
<td>Total chemical oxygen demand</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
</tbody>
</table>
Chapter 1

1 Introduction

1.1 Background

The price of fossil fuels on the global markets has been rising as the demand for energy has increased due to the growth of world population and limited fossil resources. Moreover, most industries are highly dependent on natural resources such as petroleum and gas. In the United States, over 80% of the energy requirement in 2010 was from fossil fuels and the energy consumption has been projected to grow on average at 0.3% per year from 2013 to 2040. Unexpected disruption in oil supply results in severe economic crisis. The combustion of fossil fuels causes environmental problems such as air pollution and greenhouse gas emissions.

Many countries have been trying to develop technologies to utilize renewable energy sources such as solar, wind, and biomass to replace fossil fuels. Hydrogen is a promising energy resource due to its high energy content and clean emission upon combustion. The high heat of hydrogen is 142 kJ/g and the end-product from the combustion of hydrogen is water while gasoline and ethanol generate 47 and 30 kJ/g of heat energy respectively and produce carbon dioxide which is a greenhouse gas. The main concern with hydrogen as a renewable energy source is the cost and safety of production and storage. Among current methods for hydrogen production, steam reforming of natural gas is the most cost effective process, but it generates greenhouse gases.

Water electrolysis and biological processes (e.g. photo or dark fermentation) are renewable hydrogen production methods. To produce hydrogen from water electrolysis, electrical power is required and the energy efficiency is 56%-73%. Dark fermentation among biological hydrogen production processes is environmentally friendly since it requires less energy and can utilize various types of biomass including lignocellulosic biomass. Besides, the hydrogen production yields and rates of dark fermentation are higher than other biological methods. However, hydrogen production via dark fermentation is not economically commercialized. Hydrogen yield from dark fermentation is approximately 25% of the hydrogen content in the substrate and it
produces volatile fatty acids (VFAs, e.g. acetate and butyrate) which still contain hydrogen and can be utilized to produce more hydrogen.

Microbial electrolysis cell (MEC) is a novel technology to produce hydrogen from organic substances. Anode-respiring bacteria (ARB) oxidize the organic substrate and produce electrons on the anode. These electrons move to the cathode and react with protons to generate hydrogen. Hydrogen can be produced from various types of substrates including VFAs, wastewater, and lignocellulosic biomass in MECs. Thus, an MEC is a promising technology to produce hydrogen and treat wastewater.

Lignocellulosic biomass is the most abundant feedstock for renewable energy production as it contains high concentrations of carbohydrates and exists in agricultural residues (e.g. corn stalk and rice straw), municipal solid wastes (e.g. pulp and activated sludge) and forest residues (e.g. wood chips and leaves). The major issue in hydrogen production from lignocellulosic biomass is its low degradability and production yield resulting from its complex crystalline structure and the presence of lignin which requires pretreatment. Unfortunately, inhibitors (e.g. furfural and 5-hydroxymethyl furfural) can be produced during the pretreatment of lignocellulosic biomass. These inhibitors decrease the hydrogen production yield and rate by deactivating bacteria. To develop practical hydrogen production from lignocellulosic biomass, it is required to remove or detoxify these inhibitors.

1.2 Problem Statement

Many research groups have been studying biological hydrogen production using lignocellulosic biomass because of its abundance. However, it is necessary to pretreat lignocellulosic biomass to improve biohydrogen production due to its complex structure and the presence of lignin. Furfural is generated during the pretreatment of lignocellulosic biomass and it inhibits biohydrogen production. Therefore, it is important to understand the inhibitory threshold level of furfural on fermentative hydrogen producing bacteria in batch and continuous-flow reactors and ARB in MECs as well as the interaction between furfural, substrate, and biohydrogen inhibition.
The conventional furfural detoxification methods showed low removal efficiency and the degradation of sugars. Electrochemical detoxification is a novel approach to remove furfural with no loss of sugars. Furfural is electrochemically degraded to several products including 2-furoic acid or furfuryl alcohol and their toxicity on biohydrogen production should be addressed for practical application of the electrochemical detoxification. However, there is no information on the effects of electrochemically degraded furfural by products on biohydrogen production.

1.3 Research Objectives

This thesis addresses the impact of furfural and its derivatives (furfuryl alcohol and 2-furoic acid) on biohydrogen production in batch experiments and MECs. The specific objectives are as follow:

· To evaluate the effects of furfural on fermentative biohydrogen-producing bacteria.

· To explore the biohydrogen potential from furfuryl alcohol and 2-furoic acid in batch experiments.

· To assess the efficacy of electrochemical treatment of furfural on biohydrogen production in batch experiments

· To investigate the influence of furfural on ARB performance in MECs.

1.4 Research Contributions

This study investigated hydrogen production potentials of furan compounds (furfural, furfuryl alcohol, and 2-furoic acid) in batch experiments. An electrochemical method to detoxify furfural was carried out and the hydrogen potential was assessed. Furthermore, the effects of furfuryl alcohol and 2-furoic acid which are reduced and oxidized furfural degradation intermediates on hydrogen production in batch experiments and MECs are addressed.
1.5 Thesis Organization

This thesis is arranged in four chapters and conforms to the “integrated articles” format as outlined in the Thesis Regulation Guide by the School of Graduate and Postdoctoral Studies (SGPS) of Western University. The four chapters are:

Chapter 1 includes a general introduction, research objectives, and contributions of this thesis.

Chapter 2 presents the literature review of hydrogen production from lignocellulosic biomass, the inhibition of hydrogen production, and microbial electrolysis cells.

Chapter 3 describes the impact of furfural on fermentative bacteria and anode-respiring bacteria.

Chapter 4 summarizes the major conclusions of this research and the recommendations for further works based on the results of this study.

1.6 References


Chapter 2

2 Literature Review

2.1 Introduction

The demand for developing renewable energy instead of petroleum has been increasing because the price of crude oil has dramatically increased and existing fossil fuels have been rapidly depleted.\textsuperscript{1,2} Many research groups have been studying alternative energy sources and novel technologies for renewable energy production. Hydrogen is one of the alternative energy sources, because it is abundant and does not generate greenhouse gases upon combustion.\textsuperscript{3-6}

2.2 Hydrogen Production Methods

Hydrogen can be produced by different technologies: 1) thermo-chemical methods, 2) water electrolysis, and 3) biological methods. Among these methods, 92% of hydrogen is produced by the thermo-chemical methods from fossil fuels and water electrolysis.\textsuperscript{3,4} Table 2-1 describes a list of hydrogen production methods.

2.2.1 Thermochemical Hydrogen Production

Thermochemical hydrogen production consists of thermochemical reactions to separate hydrogen from the feedstocks in the presence of oxidants (e.g. O\textsubscript{2}, air, or steam).\textsuperscript{5,6} At high temperature (> 700ºC), hydrocarbons are converted to hydrogen through the following general reaction:

\[ C_mH_n + [O_x] \rightarrow xH_2 + yCO + zCO_2 \] \hspace{1cm} 2-1

where, C\textsubscript{m}H\textsubscript{n} (m \geq 1, n \geq m) is a hydrocarbon and [O\textsubscript{x}] is an oxidant. Oxidative processes such as steam reforming, autothermal reforming, and partial oxidation are well established for hydrogen production.\textsuperscript{5,7} Steam reforming is a common industrial process for hydrogen production from methane and the maximum yield is about 85\%.\textsuperscript{8} However,
these processes generate a high amount of CO₂ and the system is complex and sensitive to the quality of the feedstock.⁵,⁶

2.2.2 Water Electrolysis

Electrolysis is the electrochemical process in which electrical energy is the driving force of hydrogen production.⁹ Water is broken into hydrogen and oxygen by passing current between two electrodes. Turner et al. (2008) reported that the system efficiency for water electrolysis at 1 atm and 25°C varied from 53% to 73%.¹⁰ This method is the simplest and cleanest process for hydrogen production, but is very expensive and energy-intensive.⁹,¹¹

Table 2-1. Hydrogen production methods in industry.⁵⁻⁷

<table>
<thead>
<tr>
<th>Methods</th>
<th>Process</th>
<th>Feedstock</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermochemical</td>
<td>Oxidation</td>
<td>Liquid and gaseous hydrocarbons</td>
<td>Steam reformation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autothermal reformation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Partial oxidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Combined reformation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coal</td>
<td>Steam-ion process</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma reformation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Photocatalytic conversion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gasification</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Electrolysis</td>
<td>Water</td>
<td>Electrochemical reaction</td>
</tr>
<tr>
<td>Biological</td>
<td>Photolysis</td>
<td>Water and algae</td>
<td>Direct sunlight</td>
</tr>
<tr>
<td></td>
<td>Fermentation</td>
<td>Biomass</td>
<td>Fermentative bacteria</td>
</tr>
</tbody>
</table>
2.2.3 Biohydrogen Production

Biohydrogen production takes place at ambient temperatures and pressures and consumes less energy than chemical production of hydrogen which generally relies on chemical methods by purifying oil or natural gas at high temperatures and pressures.\textsuperscript{9,10} Biological hydrogen production is a method which employs biological processes, including: photosynthesis and fermentation (Figure 2-1).\textsuperscript{11} In general, the yield of a fermentative biohydrogen production is better than the yield of photosynthesis.\textsuperscript{12,13} Facultative anaerobic bacteria such as \textit{Escherichia coli} (\textit{E. coli}) and \textit{Clostridium sp.} use fermentation to generate hydrogen.\textsuperscript{10,14}

Biological hydrogen production is related to various bacteria containing hydrogen-producing enzymes.\textsuperscript{7,8} These hydrogen producing enzymes are known as hydrogenase, nitrogenase, and formate hydrogen-lyase (FHL).\textsuperscript{8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{biological_hydrogen_production.png}
\caption{Biological hydrogen production methods.}
\end{figure}
2.2.3.1 Direct Photolysis

Green algae convert water to hydrogen by capturing light in the photosynthetic apparatus (photosystem I, II), as presented by the following general reaction:

\[ 2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \]  

2-2

The generated hydrogen ions are converted into hydrogen gas or the generated hydrogen gas is used by hydrogenase present in the cells. Photosystems I and II consequently absorb light energy, and generate electrons which are transferred to ferredoxin, and then a reversible hydrogenase (Fe-hydrogenase) accepts electrons directly from the reduced ferredoxin to generate hydrogen. The overall reaction of this process is

\[ \text{Pyruvate} + \text{CoA} + 2 \text{Fd(ox)} \rightarrow \text{acetyl-CoA} + 2\text{Fd(red)} + \text{CO}_2 \]  

2-3

\[ 2H^+ + \text{Fd(red)} \rightarrow H_2 + \text{Fd(ox)} \]  

2-4

However, the problem is that [Fe]-hydrogenase is extremely sensitive to oxygen. For simultaneous \( H_2 \) and \( O_2 \) production, \( O_2 \) should be less than 1 \( \mu \text{M} \) in liquid phase. It is very difficult to maintain such low oxygen partial pressure. In order to overcome this problem, \( O_2 \) scavengers are utilized, but the regeneration of \( O_2 \) scavengers limited process scale-up.
2.2.3.2 Indirect Photolysis

Cyanobacteria (also known as blue-green algae) possess several enzymes directly involved in hydrogen metabolism and synthesis of molecular hydrogen through photosynthesis via the following reactions\(^8,15\):

\[
12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \tag{2-5}
\]

\[
C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \tag{2-6}
\]

Cyanobacteria have nitrogenases which catalyze the hydrogen production as a by-product of nitrogen reduction to ammonia, uptake hydrogenases which catalyze the oxidation of H\(_2\) synthesized by the nitrogenase, and bi-directional hydrogenases which have the ability to both oxidize and synthesize hydrogen.\(^{15,16}\) Nitrogenases play an important role in hydrogen generation, but their activity is inhibited by oxygen also. Thus, hydrogen production is achieved under oxygen and nitrogen free conditions. Also the nitrogenases are less effective than hydrogenases since nitrogenases need ATP and hydrogenases to produce hydrogen.

2.2.3.3 Photo Fermentation

Purple non-sulfur bacteria produce molecular hydrogen catalyzed by nitrogenase under nitrogen-deficient conditions using light energy and reduced compounds.\(^{15,17}\)

\[
C_6H_{12}O_6 + 12 H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \tag{2-7}
\]

Purple non-sulfur bacteria only use photosystem I, but lack photosystem II, which produces organics. These bacteria are capable of converting light energy into hydrogen using organic compounds as substrate. *Rhodopseudomonas capsulate*\(^{17}\) and *Rhodobacter spheroides*\(^{18}\) are well known photo fermentative bacteria.

2.2.3.4 Dark Fermentation

Hydrogen is produced by anaerobic bacteria which degrade carbohydrate-rich substrates without light. The major hydrogen production pathway in dark fermentation
is driven by the pyruvate metabolism. The pyruvate degradation is catalyzed by two enzyme systems: pyruvate formate lyase (PFL) and pyruvate ferredoxin oxidoreductase (PFOR). Dark fermentation produces a mixed biogas containing hydrogen, carbon dioxide, methane and hydrogen sulfide even though the aforementioned processes produces pure hydrogen. Enterobacter, Bacillus, and Clostridium are well known anaerobic hydrogen producing bacteria.

2.2.3.5 Challenges of Biohydrogen Production

Biohydrogen production technologies appear to be impractical, particularly due to their low hydrogen yields, production rates, and the requirement for extra gas cleaning due to diverse gaseous impurities. Most biologically produced H₂ evolves in microbial dark fermentation processes converting organic matter to CO₂ and H₂. Theoretically, 12 moles of H₂ can be produced from 1 mole of glucose through dark fermentation (Eq. 2-8). However, the production of hydrogen via current anaerobic dark fermentation is limited to a maximum of 4 moles of hydrogen per mole of glucose, with a fermentation end product that cannot be further converted to hydrogen by bacteria due to its endothermic nature. In particular, a theoretical maximum of 4 moles of hydrogen per mole of glucose is obtained when acetic acid is the end-product (Eq. 2-9); whereas 2 moles H₂ are only obtained in the case of butyrate as a final end-product (Eq. 2-10).

\[
C_6H_{12}O_6 + 6H_2O \leftrightarrow 12H_2 + 6CO_2 \quad 2-8
\]

\[
C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad 2-9
\]

\[
C_6H_{12}O_6 \leftrightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad 2-10
\]

However, this thermodynamic barrier can be overcome by generating hydrogen from acetate using MEC technology. Through this new approach, almost three times higher hydrogen yield can be achieved compared to current technologies, thus enhancing H₂ practically.
2.3 Microbial Electrolysis Cell (MEC)

2.3.1 Hydrogen Production in MEC

MECs are recognized as a novel green wastewater treatment technology that can generate value-added products (e.g. electricity, hydrogen and hydrogen peroxide). Figure 2-2 shows a typical configuration of a dual-chamber MEC using a proton exchange membrane (PEM) for hydrogen production in the cathode chamber. Anode respiring bacteria (ARB) attach on the anode and utilize biodegradable organic substances as an electron donor. Electrons and protons are generated on the anode by ARB. However, an external power boost is required to overcome thermodynamic barriers since the production of hydrogen and carbon dioxide from acetate hydrolysis is not thermodynamically spontaneous. For example, the potentials for the oxidation of acetate (1M) at the anode and the reduction of protons to hydrogen at the cathode are -0.28 and -0.42 V (vs. normal hydrogen electrode, NHE), respectively. Therefore, hydrogen can theoretically be produced at the cathode by applying a voltage > 0.14 V (i.e., -0.42 - (-0.28) V).\(^{19,20}\) The required voltage is relatively lower than that needed to produce hydrogen from direct water electrolysis (1.23 V at pH 7). Practically, higher external power than 0.14 V is applied due to high internal resistance in MEC system, nevertheless the potential is lower than that of water electrolysis. Liu et al.\(^ {19}\) reported 8.7 mol H\(_2\)/mol glucose of overall hydrogen yield with 72.5% hydrogen conversion efficiency. Moreover, Rozendal et al.\(^ {20}\) observed high hydrogen production of 6.36 mol H\(_2\)/mol glucose by applying 0.6 V.
Figure 2-2. A schematic diagram of a microbial electrolysis cell for biohydrogen production.

Biohydrogen production from MEC has advantages over other technologies (photo fermentation, dark fermentation, and photolysis). First, the high conversion efficiency to hydrogen is achievable. Cheng and Logan\textsuperscript{21} reported high efficiency of 8.55 mol H\textsubscript{2}/mol glucose at 0.6 V compared with the typical 4 mol H\textsubscript{2}/mol glucose by dark fermentation. Hydrogen production by various renewable technologies, e.g. wind turbines, photovoltaic cells has low conversion efficiencies of about 65%\textsuperscript{22}.

Second, the high purity of hydrogen produced at the cathode chamber, eliminates the need for expensive hydrogen purification processes. In the case of direct photolysis, hydrogen purification processes are necessary since hydrogen and oxygen are generated together by the light-driven dissociation of water (2H\textsubscript{2}O + light → 2H\textsubscript{2} + O\textsubscript{2}).
The biocatalyst in the anode of the MEC is the same as electrochemically active microorganisms (or anode respiring bacteria).\textsuperscript{23-26} There are few reports on the phylogenetic community analysis of the anodic biocatalyst, which reveal that \textit{Pelobacter propionicus} was the most dominant population in the MEC.\textsuperscript{27,28} The external power boost to the anode influences the activity or viability of the electrochemically active bacteria (EAB). Recently, the effect of the anode potential on ARB growth and MFC performance was studied, however, the effect of the external potential on the MEC anode electrode is still not clear.\textsuperscript{29}

Recently, a pilot-scale MEC fed with wastewater has been studied. The first pilot system (1000 L) contained 144 electrode pairs in 24 modules without membrane to treat winery wastewater at 1 day of hydraulic retention time (HRT).\textsuperscript{30} Hydrogen production was detected only during 20 - 40 days over the 100 day-operation period. The average soluble chemical oxygen demand (SCOD) removal was 62\% and hydrogen production rate was 0.2 m\textsuperscript{3} H\textsubscript{2}/m\textsuperscript{3}-d. Heidrich et al.\textsuperscript{31} operated 120 L of MEC using domestic wastewater. The aforementioned study reported that the average chemical oxygen demand (COD) removal and hydrogen production rate were 34\% and 0.015 m\textsuperscript{3} H\textsubscript{2}/m\textsuperscript{3}-d, respectively. Moreover, the energy recovery was approximately 100\%. Low hydrogen yields and current densities were observed and all studies suggested that the most crucial factors for successful scale-up system are inoculation, enrichment procedures, and hydrogen recovery.\textsuperscript{30-33}

\subsection*{2.3.2 Operational Parameters}

The performance of MFC is affected by several different factors such as electrode materials, reactor configurations, types of seed culture, substrate types, and electrolyte condition. Practically, the power output cannot reach the theoretical value due to overvoltage relating to activation, ohmic, and concentration losses. These losses can be reduced by modifying the reactor configuration such as minimizing electrode spacing, using different membranes, increasing the electrode surface area, and using a catalyst (e.g. Pt).\textsuperscript{34}
2.3.2.1 Electrode Materials

Materials for the anode should be noncorrosive, conductive, biocompatible, and chemically stable in the electrolyte. Anode materials should have larger surface area relative to the cathode surface area to decrease activation loss.\textsuperscript{23,35} For instance, approximately threefold higher current was generated with graphite felt than graphite rods, due to the differences in electrode surface area (graphite rod, $6.5 \times 10^{-3}$ m$^2$; graphite felt, $20.0 \times 10^{-3}$ m$^2$).\textsuperscript{35} Beside, brush-type anodes were beneficial producing 2400 mW/m$^2$ compared with 1070 mW/m$^2$ for carbon cloth anode, due to reducing internal resistance from 31 to 8 $\Omega$.\textsuperscript{36} Other approaches to enhance anode performance include chemical and physical modification of electrode materials. It has been reported that electrical energy produced by MFC increased 1000-fold by adapting Mn$^{4+}$-graphite anode and a Fe$^{3+}$-graphite cathode (788 mW/m$^2$) compared to the woven graphite anode (0.17 mW/m$^2$).\textsuperscript{36} The polypyrrole modified anode or poly(2-fluoroaniline) modified anodes showed higher current generation capacity than the unmodified platinum electrode (530 $\mu$A/cm$^2$, polypyrrole; 455 $\mu$A/cm$^2$, poly(2-fluoroaniline); 90 $\mu$A/cm$^2$, platinum).\textsuperscript{37} Anodes modified with anthraquinone-1,6-disulfonic acid (AQDS) or a graphite-ceramic composite containing Mn$^{2+}$ and Ni$^{2+}$ had between 1.7- to 2.2-fold greater kinetic activity and 5 times more power density than plain graphite.\textsuperscript{38} Anode materials modified with inorganic or organic charge transfer mediators allowed better kinetics, quicker transfer of electrons, or the mediator directly reduced extracytoplasmic electron carriers.\textsuperscript{37,38}

For the cathode, platinum is the most preferred material, but it is not cost-competitive. Additionally, in order to use platinum for in-situ electricity generation, it is critical to prevent electrode poisoning by fermentation by-products resulting in deactivation of platinum.\textsuperscript{37,39} Although cathode materials are similar to anode materials, catalysts are necessary to reduce oxygen. Using a plain carbon cathode recorded 0.02 mW of maximum power, whereas 0.097 mW was marked with Pt-coated (0.5 mg/cm$^2$) carbon electrode.\textsuperscript{40} As mentioned, one of the most effective catalysts is platinum, but it is expensive so cheaper and efficient alternatives are being actively investigated. The performance of iron(II) phthalocyanine (FePc) increased a power density of 634 mW/m$^2$ compared to 593 mW/m$^2$ using Pt cathode.\textsuperscript{41} Freguia et al.\textsuperscript{42} reported that a non-
catalyzed cathode for MFC was as effective as catalyzed cathode due to its large surface area.

2.3.2.2 Membrane

One of the factors affecting bioelectrochemical system (BES) performance is the ion exchange membrane such as Nafion and Ultex. Nafion is the most preferred one due to its high selectivity but it is not an economical choice. Although Nafion is known as proton exchange membrane (PEM), these are observations of high concentrations of cations (\(\text{Na}^+, \text{K}^+, \text{NH}_4^+, \text{Ca}^{2+}, \text{Mg}^{2+}\)) migrating to the cathode through PEM\(^{43,44}\) causing inhibition of proton transport through Nafion leading to pH decrease of the anode chamber and pH increase of the cathode chamber.\(^{45}\) Cation exchange membrane (CEM) size is also one of the factors determining the performance of BES. CEM affects internal resistance and mass transport. Clear observation of increased power density as the CEM size increases have been noted (45 mW/m\(^2\), \(A_{CEM}\) 3.5 cm\(^2\); 68 mW/m\(^2\), \(A_{CEM}\) 6.2 cm\(^2\); 190 mW/m\(^2\), \(A_{CEM}\) 30.6 cm\(^2\)).\(^{40}\)

Anion exchange membrane (AEM) is used to avert pH decrease in the cathode chamber. Protons transfer a form of phosphate thorough AEM and phosphate ions maintain pH in the cathode chamber. An MFC using AEM generated power density up to 610 mW/m\(^2\) and the coulombic efficiency was 72%.\(^{46}\) Liu et al.\(^{47}\) used carbon paper as a separator instead of membrane and observed that the internal resistance significantly decreased.

2.3.2.3 Temperature

Temperature is a minor factor in MFC, as they are typically operated at around 30 ºC since an MFC is related to biological process. Liu et al.\(^{48}\) reported the effect of temperature on MFC performances. Power density diminished by 9% and coulombic efficiency (CE) was almost same (17-45% at 20ºC and 25-46% at 32ºC) when temperature was changed from 32 to 20ºC. Moreover, an MEC pilot was operated for a year with a wide range of temperatures from 1ºC to 22ºC and the authors observed that temperature did not significantly affect the performance of MEC.\(^{49}\) While the energy recovery and coulombic efficiency in July were 37.5% and 29.3%, those in February were 66.8% and 51.3%, respectively. Patil et al.\(^{50}\) suggested that maximum
bioelectrocatalytic activity of the biofilm and high current generation were achieved at a temperature 35°C. It was assumed that the temperature during the initial growth phase of the biofilm determines the abundance of the different microbial species as well as their distribution within the biofilm matrix.

2.3.2.4 Internal Resistance
Although the power or current density from BES are still low due to high internal resistance, BES are attractive technologies for energy generation using organic matter. Power density indicates the performance of MFCs using a cell voltage and current. The cell voltage of an MFC is expressed as shown in Eq. 2-11:

\[ V = E - \eta_{\text{act, }c} - \eta_{\text{act, }a} - \eta_{\text{ohmic}} - \eta_{\text{conc, }c} - \eta_{\text{conc, }a} \]  

where, \( E \) is the reversible open circuit potential, \( \eta_{\text{act, }c} \) is the activation resistance of the cathode, \( \eta_{\text{act, }a} \) is the activation resistance of the anode, \( \eta_{\text{ohmic}} \) is the ohmic resistance, \( \eta_{\text{conc, }c} \) is the mass transport resistance of the cathode, and \( \eta_{\text{conc, }a} \) is the mass transport resistance of the anode. Each over-potential is obtained from current-voltage (I-V) curve as shown in Figure 2-3. OCV is open circuit voltage and OCP is open circuit potential. OCP (OCV) is the difference of electrical potential (voltage) between two electrodes when the circuit is disconnected.

There are three types of internal resistances: activation resistance, ohmic resistance, and mass transport (or concentration) resistance. The ohmic resistance is caused by the resistance of ion conductance due to the solution and membrane and should be overcome for practical application of BES. Table 2-2 shows the factors affecting each resistance.
Figure 2-3. (a) The current-voltage polarization curve of MFC and (b) the current-voltage polarization curves of anode and cathode versus reference electrode. (Adapted from Zhao et al. 2009 [53])
Table 2-2. Internal resistances in BES and their affecting factors.

<table>
<thead>
<tr>
<th></th>
<th>Anode</th>
<th>Membrane</th>
<th>Cathode</th>
</tr>
</thead>
</table>
| **Activation resistance** | - Amount of activity of EAB  
- Specific surface area of electrode  
- Intrinsic electron transfer rate of the rate determining enzyme/redox system  
- Temperature  
- Substrate and nutrient supply  
- Buffer strength |                                                                 | - Amount and activity of catalyst  
- Specific surface area of electrode  
- Temperature  
- Oxidant supply  
- Buffer strength |
| **Ohmic resistance** | - Electrode conductivity  
- Resistance of electrical contacts and wire  
- Anolyte conductivity  
- Electrode-spacing |                                                                 | - Electrode conductivity  
- Resistance of electrical contacts and wire  
- Catholyte conductivity |
| **Mass transport resistance** | - Substrate and nutrients supply  
- Effective diffusivity of reactants and products  
- Biofilm structure  
- Anode design  
- Hydrodynamic condition |                                                                 | - Oxidant supply  
- Buffer strength  
- Effective diffusivity of reactants and products  
- Cathode design  
- Hydrodynamic condition |
| **Others** | - Undesired reactions | - Substrate crossover  
- O$_2$ crossover | - Undesired reactions |
2.3.2.5 Electron Acceptor

The concentration of dissolved oxygen (DO) in the cathode chamber affects the performance of MFC since oxygen is an electron acceptor in the cathode and inhibits anode respiring bacteria (ARB) in the anode chamber. Unsaturated DO in the cathode chamber is a major limiting factor of the MFC operation.45,55 The power output in a two-chamber MFC caused by the DO concentration follows the Monod equation with a half-saturation constant, \( K_{DO} \), of 1.74 mg O\(_2\)/L 40, and there was no further increase in power density above a DO concentration of 6.6 mg/L.56 However, excess DO can adversely decrease the power density and coulombic efficiency due to the undesirable growth of heterotrophic bacteria that compete with ARB as a result of oxygen diffusion from the cathode to the anode. Oxygen is the most favorable electron acceptor due to its abundance and non-toxicity and ferricyanide (K\(_3\)[Fe(CN)\(_6\)]) is popular as well as because of its low over-potential.36 A comparison of the power density and coulombic efficiency between oxygen- and ferricyanide-cathode MFCs showed no significant differences. Several studies reported remarkable power outputs with ferricyanide of 3600 mW/m\(^2\) 57, 4310 mW/m\(^2\) 58, and 7200 mW/m\(^2\) 59 compared with those obtained with oxygen (e.g., 496 mW/m\(^2\) 47 and 788 mW/m\(^2\) 36). However, ferricyanide should be replaced regularly due to the insufficient re-oxidation of hexacyanoferrate (II).56,60 Moreover, ferricyanide can diffuse to the anode chamber through the CEM23 and inhibit ARB. Kim et al.61 reported that the cathodic reaction is a serious limiting factor in an MFC, and proton mass transfer is the main constraint in a MFC, increasing the internal resistance. This suggests that the electron acceptor is a critical factor for improving the performance of MFCs.

2.3.2.6 Bacterial Community

The electrochemically active bacteria (EAB) on the anode are named as electicigens, anodophiles, exoelectrogens and anode respiring bacteria 24,62–64, based on their capability of electron transport to the electrodes (Table 2-3). Electron flows are inherent to the microbial metabolism, and are derived from an electron donor (lower potential) to an electron acceptor (higher potential). These EAB can oxidize organic compounds (an electron donor) to carbon dioxide and then transfer electrons to the external electrode (a terminal electron acceptor).
The EAB will attempt to maximize their energy gain by selecting the electron acceptor with the highest potential and the electron donor with the lowest potential available facilitating metabolism. When soluble electron acceptors are depleted, microorganisms use non-soluble electron acceptors. In the case of the BES, the anode serves as an insoluble electron acceptor. These extracellular electron transfer (EET) can occur through membrane-associated processes, soluble electron shuttles generated by specific bacteria, or highly conductive nanowires. There are three mechanisms that were proposed to explain EET to the anode in BES as shown in Figure 2-4: a) the direct electron transfer via outer membrane cytochrome C, b) mediated electron transfer by electron shuttles, and c) conductive filamentous pili (nano-wire).

a. Direct electron transfer: related with membrane bound or associated enzyme complexes

<table>
<thead>
<tr>
<th>Name</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anodophile</td>
<td>Microorganisms capable of reducing electrodes</td>
<td>62</td>
</tr>
<tr>
<td>Electrochemical active bacteria (EAB)</td>
<td>Microorganisms capable of transporting electrons to and from extracellular environment</td>
<td>27</td>
</tr>
<tr>
<td>Electrocigens</td>
<td>Microorganisms capable of reducing electrodes</td>
<td>24</td>
</tr>
<tr>
<td>Exoelectrogens</td>
<td>Microorganisms capable of transporting electrons to and from extracellular environment</td>
<td>66</td>
</tr>
<tr>
<td>Electrode reducing bacteria</td>
<td>Bacterial using an anode as electron acceptor</td>
<td>67</td>
</tr>
<tr>
<td>Electrode oxidizing bacteria</td>
<td>Bacteria using a cathode as electron donor</td>
<td>67</td>
</tr>
<tr>
<td>Anode respiring bacteria (ARB)</td>
<td>Microorganisms capable of reducing electrodes</td>
<td>63</td>
</tr>
</tbody>
</table>
b. Indirect electron transfer: involves a reduction or oxidation of an organic or inorganic shuttle (pyocyanin and humic acids), soluble compound shuttle (sulfur compounds and hydrogen)

c. Conductive pili or pilus-like structures (nanowires)

![Diagram of Extracellular Electron Transfer Mechanisms]

Figure 2-4. Extracellular electron transfer mechanisms: (a) direct electron transfer, (b) electron transfer by a mediator, and (c) electron transfer via nano-wire.

In general, a mixed culture such as anaerobic sludge and wastewater is utilized for inoculation, and then only the EAB and other synergetic bacteria are expected to proliferate. However, there is no single dominant bacterial species. According to phylogenetic community analysis of anode biofilms, Proteobacteria and Firmicutes exist with high abundances in BES anodic biofilm. Out of them, Geobacter and Shewanella sp., are extensively studied, which contribute to current generation through EET mechanisms. Nevertheless, the abundance of various facultative anaerobic bacteria, for example, Alcaligenes faecalis, Enterococcus gallinarum, Pseudomonas aeruginosa, Rhodopseudomonas palustris, Clostridium butyricum, Rhodobacter sphaeroides, Aeromonas hydrophila and Therminocola sp. have been reported. These diverse communities indicate that current generation is associated with syntrophic interactions between fermentative organisms, archaea and EAB like a food chain. The existence of archaea, mainly methanogens, diminishes electron transfer to electricity
since they compete with EAB for substrate utilization. Chae et al. studied the various approaches to inhibit the methanogenic activity and noted that an inhibitor (2-bromoethanesulfonate) was the most potent strategy for the selective inhibition of methanogens without damaging EAB. While EET mechanisms of Geobacter sp. and Shewanella sp. have been extensively studied, further study is still required to reveal EET of other EAB and EET mechanisms in mixed cultures.

2.3.3 Limitations of MEC

MECs requires external power to overcome the thermodynamically nonspontaneous reaction of organic hydrolysis and hydrogen production. This external power is derived from the combustion of fossil fuel producing greenhouse gases. Thus, a main challenge of the MEC is the requirements of external energy. Lee and Rittmann suggested that the applied voltage to achieve a viable hydrogen production with a positive energy balance from the MEC is lower than 0.6 V. Thus, a MEC should be optimized, to have the lowest energy losses related to the biocatalyst metabolism, membrane, and conductive materials. Recently, a dye-sensitized solar cell (DSSC) was introduced as the external energy source for the MEC, however, the energy conversion efficiency of DSSC is still very low (~7%).

Secondly, Pt on the cathode is necessary to overcome the over-potentials and improve hydrogen production. Recently, alternatives to platinum for the cathodic catalyst such as tungsten carbide, nickel based alloy, carbon based nano materials, palladium nanoparticles and biocatalysts were suggested. These catalysts showed comparable catalytic performance in terms of hydrogen evolution, but H₂ evolution rates were still lower than that with Pt.

In order to maintain a high purity of hydrogen, the installation of a membrane is essential. However, the membrane is easily biofouled by the inoculums of the ARB causing inhibition of proton transport. Furthermore, cation transport rather than proton from the anode to the cathode via CEM causes pH gradients, with the anode pH decreasing and cathode pH increasing. A low pH results in inhibition of microbial metabolism and an increase in the substrate oxidation potential, consequently leading to a decrease in current
In addition, the main hydrogen loss was estimated hydrogenotrophic methanogenesis, thus it is clear that the MEC cell design have to be modified to better isolate the cathode from the substrate solution and the enriched anode electrode.

2.3.4 Thermodynamics of MEC

Energy generation from bioelectrochemical reactions can be calculated in terms of Gibbs free energy, which is a thermodynamic potential that measures the capacity of a system to do useful work. The Gibbs free energy of a reaction is regarded as:

\[ \Delta G = \Delta G^0 + RT \ln(\Pi) \]  \hspace{1cm} 2-12

where \( \Delta G \) is the Gibbs free energy of a reaction at specific conditions, \( \Delta G^0 \) is the Gibbs free energy of a reaction at standard conditions (298.15 K, 1 bar, 1 M concentration for all species), \( R \) is the gas constant (8.3145 J/mol·K), \( T \) (K) is the absolute temperature, and \( \Pi \) is the reaction equilibrium quotient. For a reaction \( aA + bB \rightarrow cC + dD \), the reaction quotient is defined as Bard and Faulkner:

\[ \Pi = \left( \frac{[C]^c[D]^d}{[A]^a[B]^b} \right)^2 \]  \hspace{1cm} 2-13

In the reaction quotient \( \Pi \), the terms in the brackets \{ \} are the activities of the various reactants and products. The activity, \{A\}, of an ion can be found by multiplying its concentration, \([A]\), by an activity coefficient (\( \gamma \)): \{A\} = \gamma [A]. In dilute systems, calculations can be conveniently simplified by replacing the activities in the reaction quotient \( \Pi \) with concentrations.

\[ \Pi = \left( \frac{[C]^c[D]^d}{[A]^a[B]^b} \right)^2 \]  \hspace{1cm} 2-14

In order to evaluate the reaction for bioelectrochemical conversion processes, the electromotive force (emf) is the difference between the redox potential of the cathodic
half reaction and that of the anodic half reaction, which is expressed in Volts (V) instead of Joules (J). The emf of a reaction is related to the Gibbs free energy of a reaction, according to:

$$-\Delta G = QE_{emf} = nFE_{emf}$$

where $Q$ (C) the charge transferred in the reaction, $E_{emf}$ (V) the emf of a reaction at specific conditions, $n$ (mol) the number of electrons per reaction, and $F$ Faraday’s constant (96485 C/mol). Thus,

$$E_{emf} = -\frac{\Delta G^0}{nF}$$

If all reactions are evaluated at standard conditions (at 298.15 K, 1 bar or 1 M for all species), i.e., $\Pi=1$, then Eq. 2-16 converts to Eq. 2-17 with $E_{emf}^0$ (V) the emf of a reaction at standard conditions.

$$E_{emf}^0 = -\frac{\Delta G^0}{nF}$$

From Eq. 2-12, 2-16, and 2-17, the emf is rewritten as

$$E_{emf} = E_{emf}^0 - \frac{RT}{nF} \ln(\Pi)$$

Galvanic process has a positive emf value, while electrolytic processes have a negative emf value. Here two types of bioelectrochemical conversion technologies are currently being studied (a) MFC technology for electricity production represents galvanic cells, and (b) MEC for hydrogen production exemplifies electrolysis cells.
Another way to evaluate bioelectrochemical systems is utilization of the potentials of the half-cell reactions, i.e., the separate anode and cathode potentials. According to the International Union of Pure and Applied Chemistry (IUPAC) convention, standard electrode potentials (at 298.15 K, 1 bar or 1 M for all species) are reported as a reduction potential, so that the reaction is written as consuming electrons. The theoretical potential at specific conditions is calculated in the similar with the electromotive force (Eq. 2-18).

\[ E = E^0 - \frac{RT}{nF} \ln(\Pi) \]  

For instance, acetate oxidized by ARB at the anode is written as:

\[ 2\text{HCO}_3^- + 9\text{H}^+ + 8e^- \leftrightarrow \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \]

Thus, the theoretical anode potential at specific conditions (\(E_{An}\)) can be estimated regarding to Eq.2-19:

\[ E_{An} = E_{An}^0 - \frac{RT}{8F} \ln \left( \frac{[\text{CH}_3\text{COO}^-]}{[\text{HCO}_3^-]^2[H^+]^9} \right) \]

In MFC, oxygen reduced in the cathode is written as (Table 2-4):

\[ \text{O}_2 + 4\text{H}^+ + 4e^- \leftrightarrow 2\text{H}_2\text{O} \]

The theoretical cathode potential at specific conditions (\(E_{Ca,O2}\)) can be estimated regarding to Eq.2-17:
\[ E_{Ca,O_2} = E_{Ca,O_2}^0 - \frac{RT}{4F} \ln \left( \frac{1}{pO_2[H^+]^4} \right) \]  

For a cathode that produces hydrogen via biocatalyzed electrolysis, the theoretical electrode potential at specific conditions \((E_{Ca,H2})\) can be calculated according to (Table 2-4):

\[ 2H_2O + 2e^- \leftrightarrow 2H_2 \]  

\[ E_{Ca} = E_{Ca}^0 - \frac{RT}{4F} \ln \left( \frac{pH_2}{[H^+]^2} \right) \]  

The electromotive force of the cell can be calculated from the separate anode and cathode potential according to:

\[ E_{emf} = E_{Ca} - E_{An} \]  

When the pH in the anode and cathode are equal, the result of Eq. 2-25 equals that of Eq. 2-18.

A series of redox reactions in the BECs and biological systems is listed in Table 2-5. The redox potentials are referred to the standard hydrogen electrode (SHE) at pH 7 and 25 °C indicated by the symbol \(E^{0'}\).
Table 2-4. Standard potential (E°) and theoretical potential for typical conditions at pH 7 and 298 K in BES.\textsuperscript{52}

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Reaction</th>
<th>(E^0) (V vs. NHE)</th>
<th>Conditions</th>
<th>(E^0) (V) at pH 7 (V vs. NHE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anode</td>
<td>(2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- \leftrightarrow \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O})</td>
<td>0.187\textsuperscript{a}</td>
<td>([\text{HCO}_3^-] = 5\ \text{mM}) [(\text{CH}_3\text{COO}^-) = 5 mM pH = 7]</td>
<td>-0.296\textsuperscript{b}</td>
</tr>
<tr>
<td>Cathode</td>
<td>(\text{O}_2 + 4\text{H}^+ + 4\text{e}^- \leftrightarrow 2\text{H}_2\text{O})</td>
<td>1.229</td>
<td>(p\text{O}_2 = 0.2\ \text{bar}) pH = 7</td>
<td>0.805\textsuperscript{b,c}</td>
</tr>
<tr>
<td></td>
<td>(2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2)</td>
<td>0</td>
<td>(p\text{H}_2 = 1\ \text{bar})</td>
<td>-0.414\textsuperscript{b,d}</td>
</tr>
<tr>
<td>Cell emf</td>
<td>(\text{CH}_3\text{COO}^- + \text{O}_2 \leftrightarrow 2\text{HCO}_3^- + \text{H}^+) (\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \leftrightarrow 2\text{HCO}_3^- + \text{H}^+ + 4\text{H}_2)</td>
<td>1.101\textsuperscript{c}</td>
<td></td>
<td>-0.118\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Calculated from Gibbs free energy data\textsuperscript{99}
\textsuperscript{b} Calculated using Eq. 2-15
\textsuperscript{c} Microbial fuel cell
\textsuperscript{d} Microbial electrolysis cell
\textsuperscript{e} Cell emf calculated using Eq. 2-23
Table 2-5. Standard potential of selected important half reactions at pH 7 and 298 K.\textsuperscript{52,99}

<table>
<thead>
<tr>
<th>Oxidant/reductant</th>
<th>n\textsuperscript{a}</th>
<th>E\textsuperscript{0'}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO\textsubscript{2}/glucose</td>
<td>24</td>
<td>-0.43</td>
</tr>
<tr>
<td>2H\textsuperscript{+}/H\textsubscript{2}</td>
<td>2</td>
<td>-0.42</td>
</tr>
<tr>
<td>NAD\textsuperscript{+}/NADH</td>
<td>2</td>
<td>-0.32</td>
</tr>
<tr>
<td>CO\textsubscript{2}/acetate</td>
<td>8</td>
<td>-0.28</td>
</tr>
<tr>
<td>Acetoadlyde/ethanol</td>
<td>2</td>
<td>-0.20</td>
</tr>
<tr>
<td>Pyruvate/lactate</td>
<td>2</td>
<td>-0.19</td>
</tr>
<tr>
<td>2H\textsuperscript{+}/H\textsubscript{2}</td>
<td>2</td>
<td>+0.00\textsuperscript{b}</td>
</tr>
<tr>
<td>Fumarate/succinate</td>
<td>2</td>
<td>+0.03</td>
</tr>
<tr>
<td>Cytochrome c Fe\textsuperscript{3+}/Fe\textsuperscript{2+}</td>
<td>1</td>
<td>+0.25</td>
</tr>
<tr>
<td>O\textsubscript{2}/H\textsubscript{2}O\textsubscript{2}</td>
<td>2</td>
<td>+0.30</td>
</tr>
<tr>
<td>Fe(CN)\textsubscript{6}\textsuperscript{3-}/Fe(CN)\textsubscript{6}\textsuperscript{4-}</td>
<td>1</td>
<td>+0.37</td>
</tr>
<tr>
<td>NO\textsubscript{3}/NO\textsubscript{2}\textsuperscript{-}</td>
<td>2</td>
<td>+0.42</td>
</tr>
<tr>
<td>NO\textsubscript{3}/\textfrac{1}{2}N\textsubscript{2}</td>
<td>5</td>
<td>+0.74</td>
</tr>
<tr>
<td>\textfrac{1}{2}O\textsubscript{2}/H\textsubscript{2}O</td>
<td>2</td>
<td>+0.82</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The number of electrons in the half reaction
\textsuperscript{b} Standard potential at STP and pH 0
2.3.5 Performance Parameters of MEC

The current density (CD) is calculated as\(^{100}\):

\[
CD = \frac{I}{A_{\text{mem}}}
\]  \hspace{1cm} 2-27

where, \(I\) is measured current and \(A_{\text{mem}}\) is the geometric surface area of anion exchange membrane (18 cm\(^2\)).

The theoretical hydrogen production \((n_{th})\) is calculated based on SCOD removal in MEC\(^{100}\):

\[
n_{th} = \frac{\Delta \text{SCOD} \times V_R \times 2}{\text{MW}_{O_2}}
\]  \hspace{1cm} 2-28

where, \(\Delta \text{SCOD}\) is the removal of SCOD, \(V_R\) is the volume of anode chamber, \(\text{MW}_{O_2}\) is a molecular weight of oxygen.

The mole of hydrogen generated from current \((n_{CE})\) is calculated as\(^{52}\):

\[
n_{CE} = \frac{\int_0^t \text{Id}t}{2F}
\]  \hspace{1cm} 2-29

where, \(t\) is the operating time, and \(F\) is the Faraday constant (96485 C/mol \(e^-\)).

Coulombic efficiency \((C_E)\) is computed based on the ratio of mole of hydrogen produced from current \((n_{CE})\) to the theoretical hydrogen production \((n_{th})\).\(^{52}\)

\[
C_E = \frac{n_{CE}}{n_{th}}
\]  \hspace{1cm} 2-30

The cathodic hydrogen recovery \((r_{cat})\) means how much of hydrogen is recovered from the current generated from MEC.\(^{52}\) It is calculated at the ratio of mole of hydrogen produced from MEC \((n_{H2})\) to the mole of hydrogen from current \((n_{CE})\).

\[
r_{cat} = \frac{n_{H2}}{n_{CE}}
\]  \hspace{1cm} 2-31
The overall hydrogen recovery is the efficiency of hydrogen production based on the total hydrogen moles generated from MEC ($n_{H2}$) versus the theoretical hydrogen production ($n_{th}$).\(^5^2\)

$$r_{H2} = r_{cat} C_E = \frac{n_{H2}}{n_{th}} \quad 2-33$$

The maximum value of the overall hydrogen recovery is 1 mol/mol.\(^5^2\)

The electrical energy input ($W_{in}$) is calculated as\(^1^0^0\):

$$W_{in} = IE_{ap} t - I^2 R_{ext} t \quad 2-34$$

where, $E_{ap}$ is the applied voltage (1.0 V) and $R_{ext}$ is an external resistor (10 Ω).

The overall energy recovery ($\eta_{E+S}$) indicates energy production from MEC based on the electrical energy and substrate inputs and hydrogen production and is calculated\(^5^2\):

$$\eta_{E+S} = \frac{-(n_{H2} \times \Delta H_{H2})}{W_{in} - (n_{substrate} \times \Delta H_{substrate})} \quad 2-35$$

where, $n_{H2}$ is the mole of hydrogen produced from MEC, $\Delta H_{H2}$ is the heat energy of hydrogen (-286 kJ/mol)\(^5^2\), $n_{substrate}$ is the mole of substrate added to MEC and $\Delta H_{substrate}$ is the heat energy of substrate: $\Delta H_{glucose}$=-2805 kJ/mol\(^1^0^1\), $\Delta H_{furfural}$=-2339 kJ/mol\(^1^0^2\), $\Delta H_{furfuryl alcohol}$=-2549 kJ/mol\(^1^0^3\), and $\Delta H_{furoic acid}$=-2041 kJ/mol\(^1^0^3\).
2.5 Inhibition of Biohydrogen Production

There are many factors which inhibit fermentative biohydrogen production. Figure 2-5 illustrates typical inhibitors of dark fermentation, including hydrogen-consuming bacteria, metal ions, ammonia, volatile fatty acids (VFAs), and inhibitors from pretreatment of substrates.

2.5.1 H₂ consuming Bacteria

Among mixed cultures for fermentative biohydrogen production, there is a specific group of H₂ consuming bacteria: hydrogenotrophic methanogens, sulfate-reducing bacteria and nitrate-reducing bacteria. Hydrogenotrophic methanogens are dominant methanogens in mixed cultures and consume H₂. They utilize H₂ and CO₂ as an electron donor and acceptor respectively to produce CH₄. Sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) require a variety of electron donor to reduce sulfate and nitrate. Not all SRB and NRB consume H₂, but hydrogenotrophic SRB and autotrophic NRB need to use H₂ as an electron donor for sulfate and nitrate reduction. Consequently, the presence of these bacteria consumes hydrogen as an electron donor and decreases hydrogen yield during dark fermentation. Moreover, autotrophic NRB release ammonia, which inhibits biohydrogen production.

2.5.2 Ammonia

Ammonia can provide nitrogen which is a key nutrient for bacterial growth and is produced through the degradation of amino acids, proteins, and urea or the reduction of nitrate by NRB. A high concentration of ammonia changes pH which causes the suppression of bacterial or enzyme activity or the switch of metabolic pathways during dark fermentation. Wang et al. reported that concentrations of ammonia higher than 0.1 gN/L inhibited dark fermentation while Sterling Jr. et al. observed that ammonia concentration of 0.6 and 1.5 gN/L promoted and 3.0 gN/L suppressed biohydrogen production, respectively. Salerno et al. observed that the inhibitory threshold concentration of ammonia at pH of 5.2 was 7 g N/L, but it decreased to 5 g N/L at pH of 6.2.
Figure 2-5. Inhibitors of fermentative biohydrogen production.

2.5.3 Metal Ions

Metal ions (e.g. Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{2+}\), Ni\(^{2+}\), etc.) are very important for bacterial growth, metabolism, and enzymatic activity.\(^{112-114}\) However, they are trace elements and also hinder biohydrogen production by changing the metabolic pathway if their concentrations are above the threshold levels.\(^{112,114}\) Table 2-6 shows the threshold concentrations of metal ions inhibitory to hydrogen production.

2.5.4 Volatile Fatty Acids (VFAs)

VFAs (e.g. formate, acetate, propionate, butyrate, etc.) are also produced via dark fermentation by acetogenic bacteria. Table 2-7 presents the threshold concentrations of VFAs. The formation of VFAs depends on the metabolic pathway of microorganisms\(^{115}\) and the pathway is affected by various factors including pH, substrate concentration, temperature, and a partial pressure of hydrogen.\(^{116-119}\) Low concentrations of VFAs are favorable for hydrogen production.\(^{120,121}\)
Table 2-6. Inhibitory threshold levels of selected metal ions.

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Inoculum</th>
<th>Substrate</th>
<th>Concentrations (mg/L)</th>
<th>Inhibitory threshold (mg/L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>Anaerobic sludge</td>
<td>Hexose</td>
<td>270 – 21000</td>
<td>270</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Sucrose</td>
<td>4 – 393</td>
<td>393</td>
<td>123</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Clostridium acetobutylicum</td>
<td>Glucose</td>
<td>0 – 272</td>
<td>0</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Sucrose</td>
<td>0 – 300</td>
<td>150</td>
<td>125</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>Clostridium beijerinckii RZF-1108</td>
<td>Glucose</td>
<td>0 – 118</td>
<td>12</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Sucrose</td>
<td>0 – 24</td>
<td>14</td>
<td>123</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>Clostridium beijerinckii RZF-1108</td>
<td>Glucose</td>
<td>0 – 60</td>
<td>40</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Clostridium butyricum EB6</td>
<td>Glucose</td>
<td>0 – 221</td>
<td>144</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0.5 – 100</td>
<td>50</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>1 – 8</td>
<td>4</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Sucrose</td>
<td>0.04 – 1.01</td>
<td>0.6</td>
<td>123</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0.5 – 50</td>
<td>25</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0 – 50</td>
<td>0.1</td>
<td>130</td>
</tr>
</tbody>
</table>

Table 2-7. Inhibitory threshold levels of selected VFAs.

<table>
<thead>
<tr>
<th>VFAs</th>
<th>Inoculum</th>
<th>Substrate</th>
<th>Concentrations (g/L)</th>
<th>Inhibitory threshold (g/L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0 – 18</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Sucrose</td>
<td>0 – 50</td>
<td>0</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Clostridium bifermentans 3AT-ma</td>
<td>Glucose</td>
<td>0 – 30</td>
<td>0</td>
<td>133</td>
</tr>
<tr>
<td>Propionate</td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0 – 22</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td>Butyrate</td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0 – 26</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td>Glucose</td>
<td>0 – 25</td>
<td>4.2</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Clostridium bifermentans 3AT-ma</td>
<td>Glucose</td>
<td>0 – 22</td>
<td>0</td>
<td>133</td>
</tr>
<tr>
<td>Formate</td>
<td>Caloramator celer strain</td>
<td>Glucose</td>
<td>0 – 16</td>
<td>1.8</td>
<td>134</td>
</tr>
</tbody>
</table>
2.5.5 Inhibitors from Pretreatment of Biomass

Lignocellulosic biomass is an abundant energy resource to produce biofuels including biohydrogen in the world. However, lignocellulosic biomass has a high fraction of hemicellulose and lignin and it is difficult to digest for biohydrogen production. Thus, appropriate pretreatment processes are required to improve its biodegradability and biohydrogen production.\textsuperscript{135,136} As shown in Figure 2-6, unexpected chemicals such as furan derivatives (e.g. furfural and 5-hydroxymethyl furfural) and phenolic compounds (e.g. syringaldehyde and vanillin) are generated and they inhibit fermentative hydrogen production.\textsuperscript{137}

In the furan derivatives, there are two main inhibitors which are produced during pretreatment of lignocellulosic biomass: furfural and 5-hydroxymethyl furfural (HMF). Xylose and arabinose from hemicellulose are transformed to furfural under high temperature and pressure of pretreatments while HMF is formed from the degradation of glucose, mannose, and galactose.\textsuperscript{137–139} Furfural and HMF can be degraded to levulinic acid or formate which are inhibitors to dark fermentation as well.\textsuperscript{137} DNA damage or mutation in bacteria occurs and fermentation pathways are changed by furan derivatives.\textsuperscript{140,141} In addition, they inhibit enzyme activity and bacterial cell growth.

Phenolic compounds are produced from the degradation of lignin during pretreatment of lignocellulosic biomass.\textsuperscript{142,143} Vanillin and syringaldehyde are the major compounds found in hydrolysates. Phenolic compounds also inhibit cell growth and shift the fermentation pathway by damaging the cell membrane.\textsuperscript{139,144,145}

Table 2-8 shows the threshold concentrations of furan derivatives and phenolic compounds. Most studies reported that the inhibitory threshold concentration of furan derivatives and phenolic compounds to fermentative biohydrogen production is 0 g/L. However, the inhibition effects of furan derivatives and phenolic compounds are dependent on the operational conditions including pH, temperature and pressure of pretreatment and biomass used.\textsuperscript{146,147}
Figure 2-6. Formation of inhibitors from lignocellulosic biomass (modified Jonsson et al.\textsuperscript{137})
Table 2-8. Inhibitory threshold concentrations of furan derivatives and phenolic compounds on biohydrogen production in batch experiments.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inoculum</th>
<th>Substrates</th>
<th>Concentration (g/L)</th>
<th>Inhibitory threshold (g/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furfural</td>
<td>Pure</td>
<td>Hydrolysate (corn stover)</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>Glucose</td>
<td>0.0-1.44</td>
<td>0.0</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrolysate (corn stalk)</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose</td>
<td>0.0-1.0</td>
<td>0.0</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>149</td>
</tr>
<tr>
<td>HMF</td>
<td>Pure</td>
<td>Hydrolysate (corn stover)</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>Glucose</td>
<td>0.0-1.89</td>
<td>0.0</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrolysate (corn stalk)</td>
<td>0.0-2.0</td>
<td>0.5</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galactose</td>
<td>0.0-2.0</td>
<td>1.0</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>0.0-1.0</td>
<td>0.0</td>
<td>149</td>
</tr>
<tr>
<td>Vanillin</td>
<td>Pure</td>
<td>Hydrolysate (corn stover)</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>Glucose</td>
<td>0.0-2.28</td>
<td>0.0</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose</td>
<td>0.0-1.0</td>
<td>0.0</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>149</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>Pure</td>
<td>Hydrolysate (corn stover)</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>Glucose</td>
<td>0.0-2.73</td>
<td>0.0</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose</td>
<td>0.0-1.0</td>
<td>0.0</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>0.0-2.0</td>
<td>0.0</td>
<td>149</td>
</tr>
</tbody>
</table>
2.5.6 Methods for Reducing Inhibition

2.5.6.1 H\textsubscript{2} Consuming Bacteria

The major method to decrease inhibition of H\textsubscript{2} consuming bacteria is to pretreat inoculum prior to dark fermentation to suppress H\textsubscript{2} consuming bacteria and to enrich H\textsubscript{2} producing bacteria.\textsuperscript{104,106,150} Table 2-9 summarizes selected pretreatments to enrich H\textsubscript{2} producing bacteria. Some H\textsubscript{2} producing bacteria (e.g. *Clostridium sp.* ) forms spores to survive under severe conditions of temperature, pH, and sonication while H\textsubscript{2} consuming bacteria are not able to form spores.\textsuperscript{106,150} However, there are many H\textsubscript{2} producing bacteria which cannot form spores and are inhibited by pretreatment while H\textsubscript{2} consuming spore forming bacteria can survive.\textsuperscript{151} Pendyala et al.\textsuperscript{152} observed that methanogen (*Methylophilus methylotrophus*) survived after heat, acid, and base pretreatment. Moreover, Oh et al.\textsuperscript{153} and Ren et al.\textsuperscript{154} reported survival of acetogens (*Clostridium thermoautotrophicum* and *Clostridium acetocum*) and *Propionibacterium propionicus* after acid pretreatment which decreased hydrogen yields due to the production of acetic and propionic acids.

### Table 2-9. Selected pretreatments to enrich H\textsubscript{2} producing bacteria in mixed cultures.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Inoculum</th>
<th>H\textsubscript{2} producing bacteria enriched</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock</td>
<td>Secondary sludge</td>
<td><em>Clostridium sp.</em></td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td><em>Thermoanaerobacterium thermosaccharolyticum</em></td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td><em>Escherichia vulneris</em> and <em>Clostridium bifermentans</em></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Marine intertidal sludge</td>
<td><em>Clostridium sp.</em> and <em>Enterococcus sp.</em></td>
<td>157</td>
</tr>
<tr>
<td>Acid</td>
<td>Anaerobic sludge</td>
<td><em>Escherichia vulneris</em></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Anaerobic sludge</td>
<td><em>Clostridium sp.</em></td>
<td>155,158</td>
</tr>
<tr>
<td></td>
<td>Marine intertidal sludge</td>
<td><em>Bacillus sp.</em> and <em>Clostridium sp.</em></td>
<td>157</td>
</tr>
<tr>
<td>Base</td>
<td>Anaerobic sludge</td>
<td><em>Clostridium sp.</em></td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Marine intertidal sludge</td>
<td><em>Clostridium sp.</em></td>
<td>157</td>
</tr>
<tr>
<td>Sonication</td>
<td>Anaerobic sludge</td>
<td><em>Enterobacter aerogenes</em></td>
<td>156</td>
</tr>
</tbody>
</table>
2.5.6.2 Ammonia

Few studies have been investigated to reduce the inhibition of ammonia to dark fermentation whereas there are many studies to reduce ammonia inhibition to anaerobic digestion: dilution of substrate\textsuperscript{159}, acclimation of the inoculum\textsuperscript{160}, and adjustment of pH\textsuperscript{161}. These approaches can be adopted for mitigating ammonia inhibition to hydrogen production. Salerno et al.\textsuperscript{111} reported that the hydrogen yield using acclimatized inoculum to 0.8 g N/L produced showed a similar hydrogen yield compared to control, but hydrogen production decreased at ammonia concentration over 0.8 g N/L from 1.9 to 1.1 mol H\textsubscript{2}/mol substrate.

2.5.6.3 Metal Ions

Since inhibitory threshold concentrations of metal ions are varied as shown in Table 2-6, it is hard to reduce the inhibition of metal ions to hydrogen production. The threshold level of each metal may be affected by several factors including types and concentrations of inoculum and substrate and pH.\textsuperscript{112,162} Precipitation using sulfide and chelation using ligands remove heavy metal ions prior to dark fermentation\textsuperscript{163}, but the effectiveness of precipitation and chelation still remains debatable since sulfide and ligands also inhibit dark fermentation. Kim et al.\textsuperscript{122} operated a continuous-flow reactor using acclimatized inoculum to sodium ion ranging from 0.3, 2.4, 5.5, and 10.1 g Na\textsuperscript{+}/L. The volumetric hydrogen production rate increased from 4.1 LH\textsubscript{2}/L-d at 0.3 g Na\textsuperscript{+}/L to 4.4 and 4.3 LH\textsubscript{2}/L-d at 2.4 and 5.4 g Na\textsuperscript{+}/L, respectively, but decreased to 0.7 LH\textsubscript{2}/L-d at 10.1 g Na\textsuperscript{+}/L.

2.5.6.4 VFAs

As shown in Table 2-7, VFAs considerably affect fermentative hydrogen production and the inhibitory threshold concentrations in most studies were 0 g/L except for formate, 1.8 g/L. It is important to maintain appropriate range of pH to reduce the formation of VFAs because VFAs production depends on pH.\textsuperscript{164} Propionate and butyrate were dominant at pH of 5.0-6.0 and 4.0-4.5, respectively.\textsuperscript{165} Increasing operating temperature from 35ºC to 45ºC decreased the accumulation of propionate in a continuous-flow system, which
resulted in significant improvement of hydrogen yield and production rate from 1.0 to 1.7 mol H\textsubscript{2}/mol hexose and from 8.6 to 13.6 LH\textsubscript{2}/L-d, respectively.\textsuperscript{118}

2.5.6.5 Inhibitors from Pretreatment of Biomass

The formation of furan derivatives and phenolic compounds during pretreatment of lignocellulosic biomass is unavoidable and the inhibitory threshold levels of these compounds are 0 g/L as observed from Table 2-8. Most researches have focused on reducing these compounds in the hydrolysates via physical-, chemical-, and biological-detoxifications.\textsuperscript{146}

Biological detoxification involves the use of enzymes and microorganisms. Biological detoxification is simple process and generates less amounts of wastes compared to chemical detoxification. Lopez et al.\textsuperscript{166} achieved 80% removal of furfural and HMF from corn stover hydrolysate using \textit{Coniochaeta ligniaria}. Syringealdehyde, ferulic acid, furfural and HMF from sugarcane bagasse were reduced by 67%, 73%, 62%, and 85%, respectively, by \textit{Issatchenkia occidentalis} CCTCC M206097.\textsuperscript{167}

Vacuum evaporation is a physical detoxification approach for volatile inhibitors (e.g. furfural and vanillin), but the concentration of non-volatile inhibitors slightly increased during this process.\textsuperscript{168} Larsson et al.\textsuperscript{169} reported that more than 90% of furfural from wood hydrolysate was removed by the vacuum evaporation, but HMF decreased by only 4%.

Activated carbon is a popular adsorbent to remove furan derivatives and phenolic compounds.\textsuperscript{170,171} Orozco et al.\textsuperscript{170} reported that activated carbon removed 86% of the HMF of starch hydrolysate and enhanced hydrogen yields from 0.17 to 0.37 molH\textsubscript{2}/mol glucose\textsubscript{consumed}. Vanillin, HMF, and furfural from hydrolysate of water hyacinth were removed by 85%, 45%, and 40%, respectively, by activated carbon and hydrogen yield increased from 104 mLH\textsubscript{2}/g TVS to 135 mLH\textsubscript{2}/g TVS, but glucose also decreased by 14% after the detoxification.\textsuperscript{171}

Alkali treatment using Ca(OH)\textsubscript{2}, known as overliming, is a cost-effective method to remove inhibitors. The mechanism for removing inhibitors produced from pretreatment
of lignocellulose by overliming is still unknown, but significant removal efficiencies have been reported. Horvath et al.\textsuperscript{172} observed that 59\% of furfural and 22\% of phenolic compounds were removed by overliming of spruce hydrolysate at 30ºC. In another study, overliming of bagasse hydrolysate at 60ºC achieved 69\% and 35\% removal of furfural and phenolic compounds.\textsuperscript{173} However, the disadvantage of overliming is that not only inhibitors are removed, but sugars decreased. In the aforementioned studies, sugar contents decreased by 14\% and 15\%, respectively. Nilvebrant et al.\textsuperscript{174} found that the degradability of sugars during overliming depended on treatment time, pH, and temperature with xylose slightly more degraded than other sugars.

Electrochemical treatment is a novel technology in chemical detoxification of furan derivative and phenolic compounds without sugar loss. Nilges and Schroder\textsuperscript{175} studied the electrochemical detoxification of furfural and HMF using various electrode materials with applied current of 200 mA. The authors reported that furfural and HMF were completely degraded to less toxic compounds. Lee et al.\textsuperscript{176} electrochemically treated rice straw hydrolysate with applied voltage of 1.15 V (vs. Ag/AgCl) for 10 hours to remove phenolic compounds and reported that 71\% of total phenolic compounds in the hydrolysate were removed without any loss of sugars. Moreover, the cell growth and metabolic pathway of \textit{Clostridium tyrobutyricum} and \textit{Clostridium beijerinckii} in electrochemically treated hydrolysate were recovered by 100\% while those in untreated hydrolysate were completely inhibited.

### 2.6 Conclusions

Biohydrogen production from lignocellulosic biomass has gained a lot of interest in the last decade, but furfural is generated during the pretreatment of lignocellulosic biomass and is considered one of the main barriers to biohydrogen production. Recent studies attempted to mitigate furfural in hydrolysates to improve biohydrogen production. However, the conventional detoxification methods (e.g. adsorption and overliming) showed low removal efficiency or the degradation of sugars during the detoxification. Furthermore, biological detoxification is not suitable for removing all inhibitors due to
the selectivity of enzymes and bacteria. Although electrochemical treatment was introduced to detoxify furfural, the impact of electrochemically degraded furfural byproducts was not assessed. Moreover, the influence of furfural on fermentative hydrogen bacteria and ARB is not clear and there is no information about the inhibitory threshold concentration of furfural. In order to overcome the inhibition of furfural, it is important to understand the threshold level of furfural and the correlation between concentrations of furfural and substrate.

This study was carried out to evaluate the impact of furfural and its electrochemically degraded products on fermentative hydrogen bacteria in batch and continuous-flow reactors, as well as ARB in MECs, and to delineate the threshold concentration of furfural and the correlation between the concentrations of furfural and substrate.
2.7 References


Chapter 3

3 Impact of Furfural on Fermentative Biohydrogen Cultures and Anode Respiring Bacteria*

3.1 Introduction

Among the existing hydrogen production processes, fermentative hydrogen production is a promising method because it produces hydrogen from a variety of carbohydrate-rich feedstocks and wastes at ambient temperature and pressure.\textsuperscript{1,2} Lignocellulosic biomass has received significant attention as a substrate for hydrogen production since it is an abundant organic substance.\textsuperscript{3,4} However, the major challenge in hydrogen production from lignocellulosic biomass is the low degradability and yield resulting from its complex chemical structure which makes it recalcitrant to utilization by microbes. Thus, it is necessary to pretreat lignocellulosic biomass for enhancing hydrogen production. Unfortunately, various types of byproducts, which inhibit hydrogen-producing bacteria, are produced during the pretreatment of lignocellulosic biomass.\textsuperscript{5-7} These inhibitors are furan derivatives, phenolic compounds, and weak acids. Among these compounds, furan derivatives including fufurual and 5-hydroxymethylfurfural (HMF) are found in most hydrolysates.\textsuperscript{6} Furfural is easily formed from the degradation of hemicellulose to xylose and arabinose under harsh pretreatment conditions and it exists at higher concentrations in hydrolysates than HMF.\textsuperscript{5,8,9} Furfural is a strong inhibitor to biohydrogen and bioethanol production since it reduces the activities of bacteria by damaging cell walls and membranes, DNA mutation, and changing fermentation pathways.\textsuperscript{5}

Quemeneur et al.\textsuperscript{10} and Siqueira and Reginatto\textsuperscript{11} reported that hydrogen production yields in batch tests decreased by up to 75 % at 1 g/L of fufurual while Lin et al.\textsuperscript{12} found that the inhibitory effect of 1.44 g/L fufurual on hydrogen production yield was negligible. According to the research conducted by Haroun et al.\textsuperscript{13}, hydrogen yields in long-term

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continuous-flow studies increased by 17% and 6% at low concentrations of furfural (0.25 g/L and 0.5 g/L), but decreased by 21%, 29% and 62% at concentrations of furfural at 1, 2 and 4 g/L in continuous systems.

Biohydrogen production from dark fermentation is not economical on a commercial scale due to low hydrogen yield and production rate. As an alternative method for biohydrogen production, microbial electrolysis cell (MEC) is a promising technology for hydrogen production from organic wastes and wastewater treatment since anode respiring bacteria (ARB) in MECs can utilize volatile fatty acids (VFAs), such as acetate and butyrate, which are the end-products of dark fermentation, to produce hydrogen. Several studies on harvesting energy (e.g. hydrogen or electricity) from lignocellulosic biomass in MECs and microbial fuel cells (MFCs) have been reported recently. Using an MFC, Hassan et al. observed an open circuit voltage of 0.723 and 2.17 V from stackable MFCs in parallel and series with current of 1.5 and 0.5 mA from rice straw. Thygesen et al. reported that the average hydrogen production rate from wheat straw hydrolysate was 0.61 LH_2/L-d and the total energy recovery was 78% in a single-chamber MEC with a cell voltage of 0.7 V. The aforementioned authors also found that the hydrolysate from 1 kg of wheat straw in an MEC was converted to hydrogen (22 g), xylan (8 g) and phenolic compounds (9 g). The hydrogen yield from biomass pyrolysis in a dual-chamber MEC was 4.3 LH_2/L-d and the maximum coulombic efficiency and cathode conversion efficiency were 96% and 94%, respectively with 98% removal of furfural. Gupta and Parkhey obtained coulombic efficiency of 88%, a maximum hydrogen yield of 801 mLH_2/gCOD_removed, overall hydrogen recovery of 51%, and total energy recovery of 74% from rice straw hydrolysate in a single-chamber MEC.

Furfural is reduced to furfuryl alcohol (FFA) by methanogens in anaerobic digestion and it is electrochemically degraded to FFA or 2-furoic acid (FA). The end-products of electrochemical treatment depends on the material of electrode and catalyst. However, the influence of furfural on the activity of exoelectrogenic bacteria in the bioelectrochemical system (BES) is not clear. Catal et al. operated an air-cathode MFC using glucose (1200 mg/L) with furfural ranging from 1 mg/L to 19 mg/L, and found that the voltage generation decreased by 17% at 4.8 mg/L furfural whereas the voltage was
not changed by adding 1 mg/L furfural. There was no output voltage at 19 mg/L furfural and the electricity production was not recovered by glucose without furfural. In contrast, Luo et al.\textsuperscript{26} reported that the maximum voltage generation was not significantly affected by furfural. While the highest voltage of 650 mV from 1 g/L glucose was sustained for 8 hours, the maximum voltages of 660 and 700 mV with 200 mg/L glucose and 0.5 and 0.64 g/L furfural only were sustained for 20 and 30 hours, respectively. Zeng et al.\textsuperscript{24} operated MECs using a mixture of five common biohydrogen production inhibitors: two furans (HMF and furfural) and three phenolic compounds (syringic acid, vanillic acid, and 4-hydroxybenzoic acid) ranging from 200 mg/L to 1200 mg/L without a biodegradable electron source using carbon felt electrodes. The hydrogen yield and coulombic efficiency in the aforementioned study ranged from 1.7 to 2.9 mol H\textsubscript{2}/mol inhibitor\textsubscript{removed} and 44% to 69%, respectively, with no hydrogen produced at 1200 mg/L of a mixture of five inhibitors. The aforementioned authors observed that furfural electrochemically degraded to FA followed by the fermentation of FA to acetate.

It is evident from the literature that the impact of furfural on fermentative biohydrogen bacteria (FBB) and ARB is riddled with controversial results. The overall goal of this work is to shed more light on the impact of furfural, given its abundance in lignocellulosic biomass hydrolysates. The specific objectives of this study are 1) to evaluate batch hydrogen potential using various furfural degradation products, 2) to assess the impact and the threshold level of furfural on batch hydrogen production at different substrate-to-biomass (S\textdegree/X\textdegree) ratios, 3) to investigate the long-term effect of furfural and the recovery of the inhibited biomass in a continuous-flow system, 4) to explore the effects of furfural on ARB in MECs fed with different substrates, i.e. acetate and glucose.

3.2 Materials and Methods

3.2.1 Seed Sludge

Anaerobic digested sludge (ADS), used as an inoculum for fermentative biohydrogen studies, was collected from St. Marys wastewater treatment plant (St. Marys, ON,
Canada). ADS was preheated at 70°C for 30 minutes to suppress hydrogen-consuming bacteria. Total suspended solids (TSS) and volatile suspended solids (VSS) of the seed sludge varied in time: 1) for the batch biohydrogen test using different furfural derivatives, TSS: 28.6 ± 1.9 and VSS: 22.4 ± 1.2 g/L, 2) for the impact of furfural at various S⁰/X⁰, TSS: 16.2 and VSS: 12.2 g/L, 3) for a continuous-flow system, TSS: 10.1 and VSS: 6.9 g/L.

3.2.2 Electrochemical Treatment of Furfural

Furfural (2 g/L) was electrochemically treated in a glass beaker (1 L) using a power supply (B&K Precision Corp., USA) and graphite plate (5 cm x 8 cm x 0.3 cm) as working and counter electrodes. The initial concentration of furfural was 2.13±0.14 g/L in 10 mM of NaCl and 50 mM H₂SO₄ solution as the electrolyte. For the electrochemical treatment of furfural, a voltage of 1.0 V was applied to 700 mL of furfural solution for 3 hours with constant stirring at 70 rpm.

3.2.3 Batch Hydrogen Tests

Two sets of batch tests were conducted: one to assess the biohydrogen potential of FFA, FA and electrochemically treated furfural (ETF) at a constant S⁰/X⁰, and another set tested the impact of furfural at various S⁰/X⁰. Glass bottles (Wheaton, USA) were used for all batch tests and the volumes of liquid and headspace were 200 and 110 mL, respectively. The chemical composition of the nutrient solution is (in mg/L): CaCl₂, 140; MgCl₂.6H₂O, 160; MgSO₄.7H₂O, 160; Urea, 1500; Na₂CO₃, 200; KHCO₃, 200; K₂HPO₄, 15; H₃PO₄, 500; trace metal solution (TMS), 500. All batch tests were conducted in triplicates.

The S⁰/X⁰ for biohydrogen potential tests of FFA, FA, and ETF based on glucose was 1 g COD/g VSS. The effects of furfural, FFA, FA and ETF on hydrogen production were evaluated by separately adding each compound at 1 g/L.

The impact of furfural on batch biohydrogen production at different S⁰/X⁰ was assessed using synthetic hydrolysate as shown in Table 3-1. The volume of seed per bottle was 40 mL and the amount of substrate was calculated based on a gCODsubstrate/gVSSseed.
concentrations of furfural (0.5, 1, 2, and 4 g/L) were tested at each $S^o/X^o$ of 0.5, 1, 2, and 4 gCOD$_{substrate}$/gVSS$_{seed}$.

The initial pH of all batches was adjusted to 5.54±0.05 using 1 M HCl or 1 M NaOH solutions and 5 g/L NaHCO$_3$ was added into all bottles to maintain pH during the tests. After all bottles were capped, nitrogen gas (99.999% N$_2$, PraxAir, Canada) was purged into the head space for 5 minutes to ensure anaerobic condition, and the bottles were placed in a shaker (MaxQ 4000 Benchtop orbital shaker, Thermo-Scientific, USA) operating at 170 rpm and set at 37ºC. Glucose was used as a control test and the blank was composed of seed sludge only. All sugars, furfural, FFA, and FA were purchased from Sigma-Aldrich, Canada.

<table>
<thead>
<tr>
<th></th>
<th>Arabinose (g/L)</th>
<th>Xylose (g/L)</th>
<th>Mannose (g/L)</th>
<th>Galactose (g/L)</th>
<th>Glucose (g/L)</th>
<th>Formate (g/L)</th>
<th>Acetate (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.9</td>
<td>50</td>
<td>0.3</td>
<td>2.5</td>
<td>6.7</td>
<td>1.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

3.2.4 Continuous-Flow Biohydrogen Reactors

Two continuous-flow biohydrogen reactors (R1 and R2) were operated for biohydrogen production for 143 days. The feed contained 10 g/L of glucose (R1) or xylose (R2) and sufficient nutrients as described in Hafez et al.$^{28}$ The organic loading rate (OLR) excluding furfural was 32 gCOD/L-d and hydraulic retention time (HRT) was 8 hours. The working volume was 7 L and the temperature was kept at 37ºC using a water circulation system (VWR Heated Circulating Bath, VWR International, USA). Solids retention time (SRT) was decoupled from HRT by an uncovered gravity settler (8 L) and varied from 1.2 to 2.0 days (R1) and from 1.2 to 2.2 days (R2). To ensure anaerobic conditions, N$_2$ gas was purged in the bioreactor headspace for 10 minutes. A pH controller (HI 21 series, HANNA Instruments, Italy) and chemical feed pumps (BL 1.5, HANNA Instruments, Romania) were installed to control pH. This test comprised of 7 phases in each reactor. The concentrations of furfural in each phase were 0, 0.25, 0.5, 1, 2,
4 g/L in both reactors. The 7th phase of R1 was for testing the recovery of the bacteria after removing furfural from the feed.

3.2.5 Microbial Electrolysis Cells (MECs)

Two-compartment MEC systems were used in this study. The cells were fabricated from plexiglass with working anode and cathode chamber volumes of 350 mL and 180 mL, respectively. The anode was made of stainless steel frames with carbon fiber (2293-B, 24K Carbon Tow, Fiber Glast Development Corp, USA) intertwined through holes drilled on frames. Carbon fiber was bundled by the manufacturer into a rope-like material with a specific surface area of 571,429 m²/m³. Each bundle contained about 24,000 carbon filaments (1.5 m length and 7 µm diameter) with a total surface area of about 7913 cm² for the fabricated carbon fibers anode and a specific surface area of about 2261 m²/m³ of the MEC anode chamber. The cathode was a circular with a diameter of 4.8 cm, and made of stainless steel mesh (Type 304, McMaster-Carr, USA). An anion exchange membrane (AMI-7001, Membrane International Inc., NJ, USA) was used as a separator between the anode and the cathode chambers and its geometric surface area was 18 cm². The membrane was pre-treated at 40°C in 5% NaCl solution for 24 hours. The distance between the anode and cathode was 1 cm. In order to avoid leakage, non-conductive polyethylene mats were placed between the electrodes and the membrane. The electrodes were powered at a voltage of 1.0 V using a direct current power supply (B&K Precision Corp., California, USA), connected in series through a 10 Ω resistor (R) with a data acquisition system (2700, KEITHLEY Multimeter, Cleveland, USA) to record the voltage drop across the external resistor.

An inoculum volume of 50 mL harvested from conventional two-chambers MECs which were selectively enriched from waste activated sludge (Adelaide Wastewater Treatment Plant, London, Canada) operated in batch mode with acetate as a substrate for more than 5 months was used. Sodium acetate or glucose was used as the electron donor at 2 g/L and COD loading is presented in Table 3-2. The inoculum was fed into the anodic chamber (320 mL) as a growth medium and was adjusted at pH 7.2 ± 0.2 using 50 mM phosphate buffered saline (PBS, 2.3 g/L KH₂PO₄ and 4.66 g/L Na₂HPO₄) containing 0.038 g/L NH₄Cl and 0.84 g/L NaHCO₃ and 1 mL/L of a trace element mixture with the
following composition (in mg/L): MgCl₂·6H₂O, 25; MnCl₂·4H₂O, 6; CaCl₂·2H₂O, 1.2; ZnCl₂, 0.5; NiCl₂, 0.11; CuSO₄·5H₂O, 0.1; AlK(SO₄)₂·12H₂O, 0.1; Co(NO₃)₂·6H₂O, 1; H₃BO₃, 0.1; EDTA, 5; Na₂WO₄·2H₂O, 0.1; NaHSeO₃, 0.1; Na₂MoO₄·2H₂O, 0.2. 20 mM FeCl₂·4H₂O and 77 mM Na₂S·9H₂O were also added to the medium (1 mL/L).³⁰,³¹

The effect of furfural on the performance of the acetate-fed MEC was studied in cycles 2, 3, and 4, by spiking 0.5 g/L (cycle 2) and 2.0 g/L (cycles 3 and 4), which correspond to influent furfural COD of 830 mg/L and 3300 mg/L respectively. To assess the recovery of the grown biofilm in the acetate-fed MEC after spiking furfural, only sodium acetate (2 g/L) was used as electron donor in cycles 5 and 6. Throughout the experiments, the cathode chamber of the acetate-fed MEC was maintained at pH of 7.2 ± 0.2 using 50 mM phosphate buffered saline (PBS).

The glucose-fed MEC was operated for 3 cycles with glucose without furfural to maintain stable current generation. From the 4th to the 6th cycles, 1 g/L glucose and 0.7 g/L furfural was introduced to the glucose-fed MEC for 3 cycles followed by 1.4 g/L furfural as sole carbon source for another three cycles. After the 9th cycle, furfural-free glucose medium (2 g/L glucose) was fed to the glucose-fed MEC to test the recovery of ARB. The cathode chamber of the glucose-fed MEC was filled with MilliQ water (18.2 MΩ·cm) which was purged with N₂ for 10 minutes to remove dissolved oxygen. The water in the cathode chamber of the glucose-fed MEC was replaced when a fresh medium was fed to the anode chamber.
Table 3-2. The concentrations of acetate or glucose and furfural and the corresponding SCOD fed to the MECs.

(a) Acetate and furfural

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Acetate (g/L)</th>
<th>Furfural (g/L)</th>
<th>SCOD added (mg)</th>
<th>SCOD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>2.0</td>
<td>-</td>
<td>560</td>
<td>1600</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>2.0</td>
<td>0.5</td>
<td>840</td>
<td>2400</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>2.0</td>
<td>2.0</td>
<td>1681</td>
<td>4800</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>2.0</td>
<td>2.0</td>
<td>1681</td>
<td>4800</td>
</tr>
<tr>
<td>Cycle 5</td>
<td>2.0</td>
<td>-</td>
<td>560</td>
<td>1600</td>
</tr>
<tr>
<td>Cycle 6</td>
<td>2.0</td>
<td>-</td>
<td>560</td>
<td>1600</td>
</tr>
</tbody>
</table>

(b) Glucose and furfural

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Glucose (g/L)</th>
<th>Furfural (g/L)</th>
<th>SCOD added (mg)</th>
<th>SCOD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1-3</td>
<td>2.0</td>
<td>-</td>
<td>561±9</td>
<td>2339±38</td>
</tr>
<tr>
<td>Cycle 4-6</td>
<td>1.0</td>
<td>0.7</td>
<td>641±13</td>
<td>2674±30</td>
</tr>
<tr>
<td>Cycle 7-9</td>
<td>-</td>
<td>1.4</td>
<td>586±47</td>
<td>2442±36</td>
</tr>
<tr>
<td>Cycle 10-12</td>
<td>2.0</td>
<td>-</td>
<td>531±13</td>
<td>2212±56</td>
</tr>
</tbody>
</table>
3.2.6 Analytical Methods

The biogas volume generated from the batch bottles was determined using appropriately sized glass syringes (5–50 mL, Micro-Mate glass syringes, Cadence Science, VA, USA). The hydrogen production from MECs was measured using the water displacement method. The biogas was analyzed using a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Mole sieve 5A, mesh 80/100, 6 ft x 1/8 in), with argon as carrier gas. The following conditions were used in the gas analysis; a gas flow rate of 30 mL/min; the temperatures of the column and the detector were 90ºC and 105ºC, respectively.

Initial and final samples were analyzed for the following parameters (unless stated): total and soluble chemical oxygen demand (TCOD and SCOD), total nitrogen (TN) and total phosphorus (TP) using standard HACH methods and test kits (HACH Odyssey DR/2500 spectrophotometer manual). Total and volatile suspended solids (TSS and VSS) were quantified according to the standard methods.

Furfural and FA in batch samples were quantified using an ion chromatograph (Dionex IC20, Dionex, USA) with a refractive index detector (PerkinElmer Series 200, PerkinElmer Instruments Inc. USA) and an Aminex HPX-87H column (Bio-Rad Laboratories, USA). The operational conditions were: a mobile phase of 50 mM H₂SO₄ with a flow rate of 0.6 mL/min; the column temperature of 50ºC and the injection volume of 0.5 mL.

The initial and final concentrations of volatile fatty acids (VFAs) in batch samples were measured using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) equipped with a flame ionization detector (FID) and a fused silica column (30 m x 0.32 mm, DB-5, Agilent, USA). The temperatures of the column oven and the detector were 100ºC and 250ºC, respectively, and the carrier gas was helium at a flow rate of 5 mL/min.

The standard t-tests at the 95% confidence level were conducted to evaluate the statistical significance of the differences in hydrogen yields from various furfural derivatives and current densities of the acetate- and the glucose-fed MECs in the presence of furfural.
3.2.7 Modified Gompertz Model

The modified Gompertz equation (Eq. 3-1) was used to determine the kinetic parameters of the hydrogen production from the batch tests: the maximum hydrogen production potential ($P_{max}$), the maximum hydrogen production rate ($R_m$) and the lag phase ($\lambda$).

$$P = P_{max} \cdot e^{\exp\left[-\exp\left(\frac{R_m}{P_{max}} (\lambda - t) + 1\right)\right]} \quad 3-1$$

where, $P$ = the cumulative volume of hydrogen production (mLH$_2$), $P_{max}$ = the maximum potential of hydrogen production (mLH$_2$), $R_m$ = the maximum hydrogen production rate (mLH$_2$/h), $\lambda$ = the lag phase (h) and $t$ = duration of batch tests (h).

3.2.8 Calculations

Hydrogen gas volume produced in batch experiments was calculated according to the Eq. 3-2.

$$V_{H_2,t} = V_{H_2,t-1} + C_{H_2,t} \times V_{g,t} + V_h \times (C_{H_2,t} - C_{H_2,0}) \quad 3-2$$

where $V_{H_2,t}$ is the cumulative volume of hydrogen at time $t$, $V_{H_2,t-1}$ is the cumulative volume of hydrogen at time $t-1$, $V_{g,t}$ is the cumulative volume of biogas at time $t$, $V_h$ is the headspace volume (110 mL) in serum bottles, $C_{H_2,t}$ is the hydrogen percentage of biogas in headspace at time $t$, $C_{H_2,0}$ is the hydrogen percentage of biogas in headspace at time 0.$^{34}$

Hydrogen recovery parameters, namely hydrogen yield ($Y_{H_2}$), moles of hydrogen produced ($n_{H_2}$), moles of substrate utilized ($n_S$), percentage of overall H$_2$ recovery ($R_{H_2}$), CE, theoretical hydrogen production ($n_{\text{th}}$), cathodic hydrogen recovery ($r_{\text{cat}}$), current of 90% of the charge accumulation ($I_{90}$) were all calculated as described elsewhere.$^{30,31}$ The energy content of the hydrogen recovered was compared with the energy input. The pertinent electrical energies input to the system are; 1) the external electrical energy input ($\eta_E$), 2) energy input from the substrate ($\eta_S$), and 3) energy input in both electricity and substrate ($\eta_{E+S}$). Calculation of $\eta_E$, $\eta_S$, and $\eta_{E+S}$ as well as biomass distribution analysis are also described in our previous work.$^{30}$
3.3 Results

3.3.1 Electrochemical Degradation of Furfural

Furfural was degraded to FA by electrochemical treatment as shown in Figure 3-1. The initial furfural concentration of 2.12±0.04 g/L was decreased to 0.22±0.02 g/L after 3 hours while the concentration of FA increased to 2.16±0.06 g/L. Acetate (0.27±0.06 g/L) was detected in ETF. The COD concentration during treatment did not change as evidenced by the close agreement of the initial concentration of 3535±72 mg/L and the final concentration of 3426±60 mg/L and this indicated that a stoichiometric amount of FA was electrochemically produced from furfural and that furfural was not mineralized.

Figure 3-1. Degradation of furfural to 2-furoic acid by electrochemical treatment. Voltage of 1.0 V was applied to furfural solution of 700 mL for 3 hours. Data represent average concentrations of triplicate.
3.3.2 Batch Hydrogen Experiments

Figure 3-2 illustrates the cumulative hydrogen production from different substrates. Minimal hydrogen, i.e. 40±1 mL and 63±1 mL, were produced from FFA and FA without glucose, respectively, while the volume of hydrogen produced from furfural alone was negligible (11±3 mL). In the presence of glucose, the lowest and highest volumes of hydrogen were observed in the bottles with added furfural (399±11 mL) and FA (727±8 mL), respectively. Interestingly, the biogas profiles for glucose with FFA, FA, and ETF with the two-lag phase indicates two distinct stages with different biogas production rates.

**Figure 3-2.** Hydrogen production from batch experiments using different substrates. (FF: furfural; FFA: furfuryl alcohol; FA: 2-furoic acid; Glu+FF: glucose with furfural; Glu+FFA: glucose with furfuryl alcohol; Glu+FA: glucose with 2-furoic acid; Glu+ETF: glucose with electrochemically treated furfural.)
The hydrogen production from ETF with glucose was 486±5 mL. The electrochemical treatment of furfural improved the hydrogen yield and maximum production rate by 34% and 29% compared with 1 g/L raw furfural and glucose. The lag phase (18.1 hours) was shorter than that of untreated furfural. However, the hydrogen yield and maximum hydrogen production rate were 76% and 70% of the control, respectively, and the lag phase was longer than the control (9.7 h). This indicated that ETF still inhibited FBB due to residual furfural (0.22±0.02 g/L) and acetate (0.27±0.07 g/L) after electrical treatment.

Table 3-3 depicts the hydrogen yields and maximum production rates determined both by the Gompertz model and manual calculations since as mentioned above, the two-stage hydrogen production in the case of glucose with FFA, FA, and ETF is not well modeled by the Gompertz. It is apparent from Table 4-3 that there are differences between \( R_m \) and the manually calculated maximum specific hydrogen production rates, for the three aforementioned cases. The addition of FFA and FA of 1 g/L enhanced \( P_{\text{max}} \) by 6% and 12%, but decreased the manually calculated maximum specific hydrogen production rate by 24% and 9%, respectively. Moreover, the addition of FFA and FA caused longer lag phases (\( \lambda \)) of 15.4 and 11.9 hours compared to 9.7 h in the control. \( P_{\text{max}} \) and the maximum specific hydrogen production rate decreased by 36% and 46% while \( \lambda \) increased from 9.7 h in the control to 23.8 h at a furfural concentration of 1 g/L.

However, the second batch tests using synthetic hydrolysate showed a different trend. As shown in Table 3-4, hydrogen yields at \( S'/X^o \) 2 and 4 with 1 g/L furfural were 7% and 19% higher than the control. In addition, the hydrogen production was not inhibited at 0.5 g/L furfural at \( S'/X^o \) 0.5 and 1. The maximum hydrogen production rate was 134.8 mLH\(_2\)/gVSS-d at 1 g/L furfural and \( S'/X^o \) 2. The highest hydrogen yield and the shortest lag phase, 1.18±0.02 molH\(_2\)/mol sugar\(_{\text{initial}}\), were observed at 1 g/L furfural and \( S'/X^o \) 4. The hydrogen yields and maximum hydrogen production rates in each \( S'/X^o \) were not correlated with increasing furfural concentrations and concentrations of furfural greater than 1 g/L inhibited hydrogen production at all \( S'/X^o \).
Table 3-3. Hydrogen yields and production rates manually calculated and Gompertz parameters from batch experiments at F/M 1 (Average ± standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>Manually calculated</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cumulative H₂</td>
<td>Max. HPR</td>
<td>H₂ yield</td>
<td>Pₘₐₓ</td>
<td>Rₘ</td>
<td>λ</td>
</tr>
<tr>
<td></td>
<td>(mL H₂)</td>
<td>(mL H₂/gVSS-d)</td>
<td>(mL H₂/gCODₐd)</td>
<td>(mL H₂)</td>
<td>(mL H₂/gVSS-d)</td>
<td>(h)</td>
</tr>
<tr>
<td>FF</td>
<td>10.9 ± 2.9</td>
<td>1.4±0.2</td>
<td>19 ± 6</td>
<td>10.7 ± 1.8</td>
<td>0.7 ± 0.1</td>
<td>18.5 ± 1.9</td>
</tr>
<tr>
<td>FFA</td>
<td>39.8 ± 1.2</td>
<td>2.1±0.4</td>
<td>87 ± 5</td>
<td>41.6 ± 1.6</td>
<td>2.3 ± 0.5</td>
<td>40.1 ± 3.3</td>
</tr>
<tr>
<td>FA</td>
<td>62.6 ± 0.8</td>
<td>5.7±0.3</td>
<td>103 ± 6</td>
<td>63.1 ± 1.1</td>
<td>4.6 ± 0.4</td>
<td>43.6 ± 0.6</td>
</tr>
<tr>
<td>Glu+FF</td>
<td>399.1 ± 10.6</td>
<td>73.1±4.7</td>
<td>146 ± 13</td>
<td>392.3 ± 19.5</td>
<td>62.1 ± 5.2</td>
<td>23.8 ± 1.1</td>
</tr>
<tr>
<td>Glu+FFA</td>
<td>671.3 ± 4.9</td>
<td>103.4±3.1</td>
<td>250 ± 14</td>
<td>651.6 ± 5.6</td>
<td>76.3 ±0.1</td>
<td>15.4 ± 0.5</td>
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<tr>
<td>Glu+FA</td>
<td>727.2 ± 7.9</td>
<td>124.0±9.1</td>
<td>276 ± 8</td>
<td>690.1 ± 15.1</td>
<td>89.1 ± 3.5</td>
<td>11.9 ± 1.4</td>
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<tr>
<td>Glu+ETF</td>
<td>485.9 ± 4.6</td>
<td>94.5±7.3</td>
<td>196 ± 4</td>
<td>489.8 ± 4.1</td>
<td>50.6 ±2.3</td>
<td>18.1 ± 0.5</td>
</tr>
<tr>
<td>Glu (Control)</td>
<td>643.6 ± 12.3</td>
<td>135.6±11.2</td>
<td>259 ± 11</td>
<td>613.7 ± 13.8</td>
<td>125.0 ± 9.6</td>
<td>9.7 ± 0.2</td>
</tr>
</tbody>
</table>

FF: furfural; FFA: furfuryl alcohol; FA: 2-furoic acid; Glu+FF: glucose with furfural; Glu+FFA: glucose with furfuryl alcohol; Glu+FA: glucose with 2-furoic acid; Glu+ETF: glucose with electrochemically treated furfural; Glu: glucose; Max. HPR: maximum hydrogen production rate.
Table 3-4. Gompertz and calculated parameters from hydrogen batches at various S°/X°.

<table>
<thead>
<tr>
<th>S°/X° (gCOD/gVSS)</th>
<th>Sugar concentration (g/L)</th>
<th>Furfural concentration (g/L)</th>
<th>Cumulative H₂ (mLH₂)</th>
<th>H₂ yield (mol H₂/mol sugars initial)</th>
<th>( P_{\text{max}} ) (mLH₂)</th>
<th>( R_m ) (mLH₂/gVSS-d)</th>
<th>( \lambda ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.1</td>
<td>0</td>
<td>23 ± 1</td>
<td>0.62 ± 0.04</td>
<td>22.7</td>
<td>69.8</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>25 ± 3</td>
<td>0.69 ± 0.09</td>
<td>25.4</td>
<td>45.2</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>20 ± 2</td>
<td>0.55 ± 0.05</td>
<td>20</td>
<td>37.4</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>12 ± 1</td>
<td>0.31 ± 0.03</td>
<td>11.6</td>
<td>11.8</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3 ± 0</td>
<td>0.09 ± 0.01</td>
<td>3.3</td>
<td>3.9</td>
<td>31.1</td>
</tr>
<tr>
<td>1</td>
<td>2.3</td>
<td>0</td>
<td>60 ± 2</td>
<td>0.83 ± 0.05</td>
<td>60.3</td>
<td>82.6</td>
<td>13.2</td>
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<tr>
<td></td>
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<td>60 ± 4</td>
<td>0.84 ± 0.03</td>
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<td>63.0</td>
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<tr>
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<td>53 ± 5</td>
<td>0.74 ± 0.06</td>
<td>53.8</td>
<td>65.9</td>
<td>15.5</td>
</tr>
<tr>
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<td></td>
<td>2</td>
<td>51 ± 4</td>
<td>0.71 ± 0.05</td>
<td>51</td>
<td>71.8</td>
<td>20.8</td>
</tr>
<tr>
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<td></td>
<td>4</td>
<td>35 ± 5</td>
<td>0.49 ± 0.07</td>
<td>32.1</td>
<td>40.3</td>
<td>32.6</td>
</tr>
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<td>2</td>
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<td>0</td>
<td>138 ± 5</td>
<td>0.95 ± 0.03</td>
<td>138.8</td>
<td>63.0</td>
<td>10.3</td>
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<tr>
<td></td>
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<td>0.5</td>
<td>135 ± 9</td>
<td>0.93 ± 0.06</td>
<td>134.7</td>
<td>61.0</td>
<td>13.1</td>
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<td>1</td>
<td>148 ± 4</td>
<td>1.02 ± 0.03</td>
<td>147.5</td>
<td>134.8</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>134 ± 2</td>
<td>0.92 ± 0.01</td>
<td>134.4</td>
<td>93.4</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>95 ± 1</td>
<td>0.65 ± 0.19</td>
<td>92.9</td>
<td>73.8</td>
<td>36.8</td>
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<tr>
<td>4</td>
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<td>0</td>
<td>288 ± 7</td>
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<td>278.7</td>
<td>69.8</td>
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<tr>
<td></td>
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<td>0.5</td>
<td>252 ± 13</td>
<td>0.87 ± 0.08</td>
<td>247.3</td>
<td>110.2</td>
<td>10.2</td>
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<tr>
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<td>1</td>
<td>343 ± 5</td>
<td>1.18 ± 0.02</td>
<td>345.9</td>
<td>55.1</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>261 ± 10</td>
<td>0.90 ± 0.03</td>
<td>255.8</td>
<td>62.0</td>
<td>16.2</td>
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3.3.3 Biohydrogen Production in Continuous-flow Systems

The hydrogen production rates and yields in both continuous-flow systems were not inhibited at 0.25 and 0.5 g/L furfural, as shown in Table 4-5. In R1, the hydrogen yields and production rates increased from 2.3 molH$_2$/mol glucose and 67 LH$_2$/d in phase 1 to 2.7 molH$_2$/mol glucose and 79 LH$_2$/d at 0.25 g/L furfural in phase 2 and 2.4 molH$_2$/mol glucose and 71 LH$_2$/d at 0.5 g/L furfural in phase 3. The hydrogen yields in R2 increased from 1.6 molH$_2$/mol xylose and 55 LH$_2$/d in phase 1 to 1.7 molH$_2$/mol xylose and 59 LH$_2$/d at 0.25 g/L furfural in phase 2, but slightly decreased to 1.5 molH$_2$/mol xylose and 54 LH$_2$/d at 0.5 g/L furfural in phase 3. Hydrogen yields and production rates significantly decreased with increasing furfural concentrations above 0.5 g/L in both R1 and R2. The hydrogen yields in R1 decreased to 1.8 molH$_2$/mol glucose and 53 LH$_2$/d, 1.6 molH$_2$/mol glucose and 48 LH$_2$/d, and 0.9 molH$_2$/mol glucose and 26 LH$_2$/d at 1, 2, and 4 g/L furfural, respectively. Similarly, the hydrogen yields and production rates in R2 were 1.4 molH$_2$/mol xylose and 48 LH$_2$/d, 1.2 molH$_2$/mol xylose and 41 LH$_2$/d, and 0.6 molH$_2$/mol xylose and 20 LH$_2$/d at 1, 2, and 4 g/L furfural. In phase 7 of R1, the hydrogen yield with furfural-free glucose was 1.6 molH$_2$/mol glucose which was 72% of the yield of phase 1 and the maximum hydrogen production rate recovered by 95%.
Table 3-5. Summary of end-products in the effluent from continuous-flow systems: R1 and R2.

(a) R1

<table>
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<tr>
<th></th>
<th>H₂ yield (mol/mol)</th>
<th>HPR (L/d)</th>
<th>VSS (mg/L)</th>
<th>SCOD (mg/L)</th>
<th>Acetate (mg/L)</th>
<th>Propionate (mg/L)</th>
<th>Butyrate (mg/L)</th>
<th>Formate (mg/L)</th>
<th>Lactate (mg/L)</th>
<th>Ethanol (mg/L)</th>
<th>Glucose (mg/L)</th>
<th>Furfural (mg/L)</th>
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<tbody>
<tr>
<td>Phase 1</td>
<td>2.3±0.1</td>
<td>67±4</td>
<td>915±80</td>
<td>7936±581</td>
<td>2415±206</td>
<td>529±260</td>
<td>2112±184</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>12±11</td>
</tr>
<tr>
<td>Phase 2</td>
<td>2.7±0.1</td>
<td>79±4</td>
<td>1009±63</td>
<td>8651±456</td>
<td>2620±215</td>
<td>411±93</td>
<td>2143±154</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Phase 3</td>
<td>2.4±0.1</td>
<td>71±3</td>
<td>923±70</td>
<td>8825±753</td>
<td>1888±100</td>
<td>290±110</td>
<td>2518±458</td>
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<td>0.31±0.21</td>
<td>118±32</td>
<td>126±114</td>
<td>ND</td>
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<tr>
<td>Phase 4</td>
<td>1.8±0.2</td>
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<td>966±107</td>
<td>9631±505</td>
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<td>606±723</td>
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<td>201±149</td>
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<td>986±97</td>
<td>11453±676</td>
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<td>468±341</td>
<td>2346±152</td>
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<td>447±66</td>
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<td>Phase 6</td>
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<td>1069±109</td>
<td>13749±217</td>
<td>2361±494</td>
<td>176±89</td>
<td>2266±166</td>
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<td>774±97</td>
<td>396±41</td>
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<td>Phase 7</td>
<td>1.6±0.1</td>
<td>48±3</td>
<td>831±62</td>
<td>8073±230</td>
<td>1117±90</td>
<td>655±57</td>
<td>2474±330</td>
<td>ND</td>
<td>ND</td>
<td>464±116</td>
<td>16±11</td>
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(b) R2

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<th></th>
<th>H₂ yield (mol/mol)</th>
<th>HPR (L/d)</th>
<th>VSS (mg/L)</th>
<th>SCOD (mg/L)</th>
<th>Acetate (mg/L)</th>
<th>Propionate (mg/L)</th>
<th>Butyrate (mg/L)</th>
<th>Formate (mg/L)</th>
<th>Lactate (mg/L)</th>
<th>Ethanol (mg/L)</th>
<th>Xylose (mg/L)</th>
<th>Furfural (mg/L)</th>
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<tr>
<td>Phase 1</td>
<td>1.6±0.1</td>
<td>55±6</td>
<td>952±105</td>
<td>7988±258</td>
<td>1871±231</td>
<td>165±78</td>
<td>2428±336</td>
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<td>ND</td>
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<td>ND</td>
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</tr>
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<td>Phase 2</td>
<td>1.7±0.2</td>
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<td>839±57</td>
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<td>1674±268</td>
<td>187±76</td>
<td>2555±343</td>
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<td>65±73</td>
<td>ND</td>
<td>35±39</td>
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<td>Phase 3</td>
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<td>55±5</td>
<td>811±89</td>
<td>8564±644</td>
<td>1601±190</td>
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<td>2622±360</td>
<td>14±23</td>
<td>14±22</td>
<td>30±32</td>
<td>122±172</td>
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<td>Phase 4</td>
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<td>48±6</td>
<td>901±112</td>
<td>9576±781</td>
<td>1583±207</td>
<td>573±179</td>
<td>2614±393</td>
<td>40±106</td>
<td>129±132</td>
<td>330±111</td>
<td>48±9</td>
<td>ND</td>
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<tr>
<td>Phase 5</td>
<td>1.2±0.1</td>
<td>41±4</td>
<td>931±88</td>
<td>11157±664</td>
<td>2000±305</td>
<td>691±114</td>
<td>2146±355</td>
<td>155±77</td>
<td>417±136</td>
<td>380±60</td>
<td>59±29</td>
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<tr>
<td>Phase 6</td>
<td>0.6±0.1</td>
<td>20±2</td>
<td>1124±85</td>
<td>14060±317</td>
<td>2643±260</td>
<td>870±124</td>
<td>1792±114</td>
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<td>661±82</td>
<td>650±97</td>
<td>404±28</td>
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3.3.4 Effects of Furfural on Current Density and Hydrogen Production in MECs

The profile of current densities of the MEC fed with acetate and spiked with furfural is illustrated in Figure 3-3. Adding 0.5 g/L furfural in the 2nd cycle showed no lag phase, whereas spiking 2 g/L furfural in the 3rd cycle affected a long lag phase of about 10 days. Spiking another 2 g/L of furfural at the beginning of the 4th cycle, the lag phase was about 4 days. Cycles 5 and 6 showed no pronounced lag phases with the immediate peaking of the current after feeding 2.0 g/L of acetate alone. In the 1st cycle, the current density peaked at about 3.5 A/m² when only acetate was fed, as compared to about 4.0 A/m² when 2 g/L furfural was added in the 3rd and 4th cycles, whereas, in the 2nd cycle at 0.5 g/L furfural along with 2 g/L acetate, the current peaked at about 2.6 A/m². The observed lower current density at the low concentration of furfural of 0.5 g/L in 2nd cycle reflected the short-term inhibition as a result of furfural introduction. This inhibition appears to be reversible as in the last two cycles without furfural, similar current densities to the 1st cycle were observed. The reproducibility of the current density peaks when the same feed condition was repeated (in 5th and 6th cycles), proves that no change has occurred in the performance of the ARB as evidenced by the constant current densities. The performance of the acetate-fed MEC is summarized in Table 3-6. Electrons generated from furfural degradation in the acetate-fed MEC were not used for hydrogen production since the amount of hydrogen and current slightly increased, but the hydrogen yields and coulombic efficiencies decreased despite the increase in SCOD removal upon furfural addition. Moreover, the hydrogen production rates were stable except for the 3rd cycle which also indicates that furfural did not inhibit hydrogen production by ARB. The average current density at 0.5 and 2 g/L furfural decreased to 1.8 and 1.2 A/m², but recovered to 2.3 A/m² in the 4th cycle.
Figure 3-3. Current densities during six consecutive acetate fed-batches over time recorded from MEC (SA: sodium acetate).
Table 3-6. Calculated parameters for the consecutive batch cycles using the acetate-fed MEC.

| Cycle | Substrate          | Duration | ΔSCOD  | Current density | n_{CE} | n_{th} | n_{H2} | CE | r_{cat} | R_{H2} | HPR | Y_{H2} | η_{E+S} |
|-------|--------------------|----------|--------|-----------------|--------|--------|--------|----|--------|--------|-----|--------|---------|-----|
|       |                    |          |        |                 | Ave.   | Max.   |        |     |        |        |     |        |         |     |
|       |                    | days     | mg     | A/m²            | mmolH₂ | mmolH₂ | mmolH₂ | %  | %      | %      |     | m³H₂/m³d | molH₂/mol substrate | %  |
| 1     | SA (2 g/L)         | 13.0     | 520    | 2.6             | 3.6    | 26.9   | 32.5   | 83 | 85     | 70.5   | 0.24 | 2.8     | 56      |     |
| 2     | SA (2 g/L) + FF (0.5 g/L) | 15.1     | 814    | 1.8             | 2.6    | 22.0   | 50.8   | 43 | 110    | 47.4   | 0.22 | 1.9     | 46      |     |
| 3&4   | SA (2 g/L) + FF (2 g/L) | 20.0±5.4 | 1630±2 | 1.8±0.8         | 4.0    | 25.8±4.9 | 101.9±0.1 | 26.1±0.6 | 25±5 | 103±17 | 25.7±0.6 | 0.19±0.05 | 1.0±0.0 | 38±2   |     |
| 5&6   | SA (2 g/L)         | 13.2±0.9 | 519±10 | 2.3±0.1         | 3.5    | 24.6±3.4 | 32.4±0.6 | 21.1±0.4 | 76±12 | 87±10 | 65.3±2.6 | 0.22±0.01 | 2.6±0.1 | 54±1   |     |

SA: sodium acetate; FF: furfural; Glu: glucose; n_{CE}: the theoretical H₂ from current; n_{th}: the maximum theoretical H₂ calculated from SCOD removal; n_{H2}: the amount of H₂ produced; CE: the coulombic efficiency; r_{cat}: the cathodic H₂ recovery; R_{H2}: the overall H₂ recovery; HPR: the H₂ production rate; Y_{H2}: H₂ yield; η_{E+S}: the energy recovered based on the energy input and the energy from substrate.
In contrast, different results were observed in the glucose-fed MEC. Figure 3-4 presents that the current densities of the glucose-fed MEC were significantly affected by the presence of furfural at 0.7 and 1.4 g/L. The addition of furfural to the glucose-fed MEC evidently decreased current densities and prolonged cycles from 3.8±0.1 A/m² and 6.2 days in the control to 2.8±0.3 A/m² and 12.7 days at 0.7 g/L furfural and 2.3±0.1 A/m² and 9.6 days at 1.4 g/L furfural. There was no lag phase in all cycles. The maximum current densities were 6.0, 4.5, and 2.9 A/m² from glucose-, glucose and furfural- and furfural- fed MEC, respectively. Table 4-7 shows the performance of the glucose-fed MEC in each operating condition. The average current density decreased from 3.8±0.1 A/m² without furfural to 2.8±0.3 and 2.3±0.1 at 0.7 g/L furfural with 1 g/L glucose and 1.4 g/L furfural alone. Additionally, hydrogen production decreased from 8.4 molH₂/mol glucose_initial at 2 g/L glucose to 6.0 molH₂/mol glucose_initial at 0.7 g/L furfural with 1 g/L glucose and 4.8 molH₂/mol glucose_initial at 1.4 g/L furfural only. Decreasing hydrogen yields and production rates were also observed by adding furfural in the glucose-fed MEC. After feeding glucose alone without furfural, current density, hydrogen yield and production rate did not recover which indicates that the furfural inhibition of FBB and ARB in the glucose-fed MEC is irreversible.
Figure 3-4. The trends of current density with different composition of substrate: glucose fed cycles (1-3), glucose and furfural fed cycles (4-6), furfural fed cycles (7-9) and glucose fed cycles (10-12). The influent TCOD of all cycles was 2417±180 mg/L.
Table 3-7. Calculated parameters for the consecutive batch cycles using the glucose-fed MEC.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Substrate</th>
<th>Duration</th>
<th>ΔSCOD</th>
<th>Current density</th>
<th>n_{CE}</th>
<th>n_h</th>
<th>n_{H2}</th>
<th>CE</th>
<th>r_{cat}</th>
<th>R_{H2}</th>
<th>HPR</th>
<th>Y_{H2}</th>
<th>η_{E+S}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ave.</td>
<td>Max.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>mg</td>
<td>A/m^2</td>
<td>mmolH_2</td>
<td>mmolH_2</td>
<td>mmolH_2</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>m^3H_2/m^3/d</td>
<td>molH_2/mol substrate</td>
</tr>
<tr>
<td>1-3</td>
<td>Glu (2 g/L)</td>
<td>6.2±0.2</td>
<td>514±12</td>
<td>3.8±0.1</td>
<td>6.0</td>
<td>19.2±1.1</td>
<td>32.1±0.8</td>
<td>24.7±0.4</td>
<td>560±5</td>
<td>129±9</td>
<td>76.9±0.8</td>
<td>0.67±0.03</td>
<td>8.44±0.01</td>
</tr>
<tr>
<td>4-6</td>
<td>Glu (1 g/L) + FF (0.7 g/L)</td>
<td>12.7±4.1</td>
<td>577±23</td>
<td>2.8±0.3</td>
<td>4.5</td>
<td>18.1±3.0</td>
<td>36.1±1.5</td>
<td>20.0±0.3</td>
<td>50±4</td>
<td>73±15</td>
<td>55.4±2.3</td>
<td>0.28±0.08</td>
<td>5.98±0.16</td>
</tr>
<tr>
<td>7-9</td>
<td>FF (1.4 g/L)</td>
<td>9.6±2.1</td>
<td>513±57</td>
<td>2.3±0.1</td>
<td>2.9</td>
<td>19.1±4.9</td>
<td>32.4±4.3</td>
<td>14.5±1.7</td>
<td>58±8</td>
<td>78±13</td>
<td>44.9±0.8</td>
<td>0.25±0.03</td>
<td>4.75±0.18</td>
</tr>
<tr>
<td>10-12</td>
<td>Glu (2 g/L)</td>
<td>8.0±0.4</td>
<td>481±23</td>
<td>2.5±0.1</td>
<td>3.0</td>
<td>16.3±1.0</td>
<td>30.1±1.4</td>
<td>15.9±0.8</td>
<td>54±4</td>
<td>98±3</td>
<td>53.0±4.8</td>
<td>0.34±0.01</td>
<td>5.76±0.31</td>
</tr>
</tbody>
</table>

SA: sodium acetate; FF: furfural; Glu: glucose; n_{CE}: the theoretical H_2 from current; n_h: the maximum theoretical H_2 calculated from SCOD removal; n_{H2}: the amount of H_2 produced; CE: the coulombic efficiency; r_{cat}: the cathodic H_2 recovery; R_{H2}: the overall H_2 recovery; HPR: the H_2 production rate; Y_{H2}: H_2 yield; η_{E+S}: the energy recovered based on the energy input and the energy from substrate.
3.3.5 Statistical Analysis

Based on the average of hydrogen yields from hydrogen batches with and without furfural FFA, FA, and ETF or current densities in the acetate-fed and glucose-fed MECs, the t-test at a 95% confidence level was done. The statistical tests indicated that the observed differences in batch hydrogen yields, as well as between current densities in the acetate- and glucose-fed MECs, were statistically significant.

The t-tests confirmed that the increasing hydrogen yield by ETF was significant, but the inhibition of ETF on hydrogen yield was also significant. In addition, that the increase of hydrogen yield from FA was significant while that from FFA was not.

The t-tests of current densities in the glucose-fed MECs for the effect of furfural on ARB confirmed that the inhibition of furfural was significant at 0.7 and 1.44 g/L furfural. The addition of 2 g/L furfural in the acetate-fed MECs significantly increased the current density.

3.4 Discussion

3.4.1 Electrochemical Treatment of Furfural

The electrochemical degradability of furfural and end-products vary with operational factors including electrode materials, catalyst, applied voltage, and electrolyte\(^{35}\). Nilges and Schroder\(^{35}\) used several electrode materials (e.g. Cu, Ni, Pt, C, etc.) to remove furfural, and observed that furfural was degraded to pinacol (70%), FA (15%), FFA (10%), and methylfuran (5%) using carbon electrodes with an applied voltage of 1.05 V (vs. Ag/AgCl), but it was transformed to 22% methyl furan, 57 % FFA, and 21% FA using Pt. Zeng et al.\(^{24}\) observed that furfural was oxidized to FA by an electrochemical reaction using carbon felt electrode at applied voltage 0.6 V for 7 days and ARB could consume FA for current generation. In this study, 90% of furfural was degraded to FA electrochemically using a graphite plate without a catalyst at the applied voltage of 1.0 V for 3 hours. However, the residual furfural of 0.22 g/L inhibited hydrogen production which meant that further treatment is required. For a practical application of
electrochemical degradation of furfural, further studies need to improve the removal efficiency and optimize the conditions.

3.4.2 Hydrogen potential of furfural degradation products

As shown in Figure 3-1, the addition of FFA, FA, and ETF exhibited a two-stage hydrogen production which is not consistent with the Gompertz model. The two-stage hydrogen production is due to sequential consumption of glucose and FFA or FA. The batch bottles which contained FFA or FA only as a substrate began hydrogen production after 48 hours, and the second stage hydrogen production in the batches of glucose and FFA, FA, and ETF started around 50 hours as shown Figure 3-2. Thus, FBB required about 2 days to adapt to and utilize FFA and FA for hydrogen production.

FFA and FA showed more hydrogen potential than furfural. In Table 3-1, the cumulative hydrogen from glucose increased by 4% and 13% with FFA and FA, respectively. However, the maximum specific hydrogen production rates were slower than the control and hydrogen production periods were prolonged. It is clear that FFA and FA did not inhibit hydrogen production and FA increased hydrogen production and yield, but FFA and FA decreased the maximum hydrogen production rates. Further studies are required to identify the mechanisms of hydrogen production from FAA and FA.

3.4.3 Effects of Furfural on Biohydrogen Production

Boopathy and Daniels\textsuperscript{36} and Haroun et al.\textsuperscript{13} reported that 1 mole of acetate is produced from 1 mole of furfural by sulfate reducing bacteria (e.g. \textit{Desulfovibrio sp.}) with ethanol or hydrogen production. The aforementioned studies suggested the thermodynamic reaction (Eq. 3-3) of furfural degradation to acetate at low concentration of furfural.

\[
\text{C}_5\text{H}_4\text{O}_2 + 6\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 3\text{CO}_2 + 6\text{H}_2, \Delta G = -152 \text{ kJ/mol} \quad 3-3
\]

At a furfural concentration lower than 0.5 g/L as shown in Table 3-3, a part of furfural is degraded to acetate and hydrogen by the Eq. 4-2 at 0.25 g/L furfural in phase 2, but furfural was degraded to lactate and ethanol instead of hydrogen. The concentrations of propionate, ethanol and lactate in the effluents from R1 and R2 increased and glucose and
xylose did not completely consume which indicates that microbial pathway was shifted by furfural.

Table 3-6 summarizes the inhibition results of furfural in literature and this study including concentrations of substrate and furfural, \( \frac{S^o}{X^o} \), furfural-to-substrate (F/S), furfural-to-biomass (F/B) and hydrogen yield. Based on the data of Table 3-6, no mathematical correlation between hydrogen yield, \( H_2 \) production inhibition, furfural concentration, furfural-to-substrate, and furfural-to-biomass ratios could be developed, emphasizing that the short-term inhibitory impact of furfural was primarily dictated by the quality of seed sludge. However, Figure 3-5 shows the linear correlation between \( H_2 \) production inhibition (%) against furfural concentrations (g/L) and F/S (g furfural/g substrate) in a continuous-flow system. As shown in Figures 3-5 (a) and (b), \( H_2 \) production inhibition increased with increasing furfural concentration and F/S. The inhibitory threshold levels of furfural and F/S are 0.56 g/L furfural and 0.058 g furfural/g substrate, respectively. In the continuous-flow systems, hydrogen production was not inhibited at 0.25 and 0.5 g/L furfural since these furfural concentrations were lower than the threshold level. Moreover, the F/S were 0.03 and 0.05 g furfural/g substrate which were lower than the inhibition level. \( H_2 \) production depends both on furfural concentrations and F/S, as evidenced by the correlation as shown in Figure 3-5 (c). The amount of substrate can be determined at a given furfural concentration using the aforementioned correlation. For example, if a sample contains furfural at 2 g/L, it requires a F/S of 0.21 g furfural/g substrate corresponding to 9.7 g/L sugars to avert inhibition in the continuous-flow system.

F/B is also a one of the significant factors affecting furfural. A low furfural concentration with a high VSS concentration (low F/B) theoretically shows high hydrogen yield. Besides, increasing furfural concentration causes decrease of hydrogen yields in batches regardless of \( \frac{S^o}{X^o} \). In the continuous-flow systems, the average F/B of R1 and R2 varied from 0.13 (Phase 2) to 3.1 (Phase 6) g furfural/gVSS-d and 0.15 (Phase 2) to 3.1 g furfural/gVSS-d (Phase 6), respectively. Quemeneur et al.\(^\text{10}\) also observed that the hydrogen yield from xylose decreased from 1.67 molH\(_2\)/mol substrate\(_{\text{initial}}\) without furfural to 0.52 molH\(_2\)/mol substrate\(_{\text{initial}}\) at 5.7 g furfural/g VSS\(_{\text{initial}}\) at constant \( \frac{S^o}{X^o} \) 28
g xylose/g VSS\textsubscript{initial} and this trend was shown in other studies in Table 4-6. The observed hydrogen yields at various S°/X° from 0.5 to 4 g sugars/g VSS in this study were higher than other studies at similar furfural concentrations since a lower range of S°/X° from 0.5 to 4 g substrate/g VSS was used in this study as compared to 7 and 28 g substrate/g VSS\textsuperscript{10,11}. 
Figure 3-5. Hydrogen production inhibition (%) plotted against (a) furfural concentration (g/L), (b) furfural-to-substrate, (c) furfural concentration and furfural-to-substrate in continuous-flow systems (R1 and R2). The negative H₂ production inhibition means that H₂ production increased.
3.4.4 Effects of Furfural on ARB

In this study, ARB in the acetate-fed MEC were not inhibited by furfural in this study as shown in Figure 3-3. In contrast, Figure 3-4 presents that the performance of the glucose-fed MEC was significantly affected by the presence of furfural at 0.7 and 1.4 g/L. The addition of furfural to the glucose-fed MEC reduced current density and prolonged the operational cycle time. FA was detected in the effluent from all cycles after adding furfural as shown in Figure 4-6. Since there was glucose (1 g/L) as an electron source to produce current in the 4th to 6th cycle and furfural was electrochemically oxidized to FA immediately, glucose and FA were utilized to generate hydrogen. Although there was no glucose in the medium between the 7th and the 9th cycle, hydrogen was still produced. During this period, the average hydrogen production yield was 4.75 molH₂/mol glucose\textsubscript{initial} (4.11 molH₂/mol furfural\textsubscript{initial}), 56% of that of cycles 1 and 3. This hydrogen was produced by the degradation of furfural. The theoretical hydrogen yield from furfural is 10 molH₂/mol furfural computed by the half-reaction (C₅H₄O₂ + 8H₂O ↔ 5CO₂ + 20H⁺ + 20e⁻). Zeng et al. 24 observed hydrogen production from furfural in the MEC. First, furfural is degraded to FA and then FA is also transformed to acetate which is a favorable substrate of ARB for hydrogen production. Furfural completely degraded to FA in MECs, but the ARB in the glucose-fed MEC were inhibited because the F/S in the glucose-fed MEC at 0.7 g/L furfural with 1 g/L glucose was 0.7 g furfural/g substrate which was higher than the threshold F/S (0.058 g furfural/g substrate) an F/S of 0.7 affects 100% inhibition as depicted in Figure 4-5 (b). Another reason for ARB inhibition in the glucose-fed MEC relates to the contact time since it took 5 days to completely remove furfural in the glucose-fed MEC as shown in Figure 4-7, although furfural was degraded to FA within 3 hours by an electrochemical treatment. The electrochemical treatment was under acidic condition at pH of 0.2, but the pH in the glucose-fed MEC was 6.63±0.14.
Figure 3-6. Concentration of volatile fatty acids and 2-furoic acid in MEC effluent.
**Figure 3-7.** The degradation of furfural in the glucose-fed MEC.
It is obvious that in this study furfural inhibited ARB in the glucose-fed MEC affecting low current density, coulombic efficiency, and H₂ yield. Catal et al.²⁵ also observed furfural inhibition in glucose-fed MFC. Low ranges of furfural concentrations from 5 to 19 mg/L inhibited the performance of glucose-fed MFC. The output voltage decrease by 17% at 5 mg/L furfural with 1 g/L glucose and there was no electricity generation at 19 mg/L furfural. Moreover, the ARB in the glucose-fed MFC did not recover after the replacement with furfural-free glucose medium and the lethal furfural concentration of ARB was 0 mg/L. Luo et al.²⁶ operated a similar configuration of the glucose-fed MFC and observed maximum power and current densities at 1 g/L glucose were 298 mW/m² and 0.9 A/m², respectively, which were lower than 361 mW/m² and 1.6 A/m² at 0.6 g/L furfural without glucose, indicating that furfural was not inhibitory. When furfural concentration increased from 0.6 g/L to 1.4 and 1.9 g/L, the maximum power density increased to 411 and 368 mW/m², but coulombic efficiencies decreased to 23% and 21%, respectively. In addition, the operational period was prolonged from 30 hours at 0.6 g/L furfural to 58 hours at 1.9 g/L furfural and the threshold level of inhibition was greater than 1.9 g/L furfural without glucose. In the two aforementioned studies, the authors did not analyze microbial community and the inhibitory mechanism of furfural on ARB was not delineated.

The most common substrate in BES studies is acetate since the hydrogen yield is close to the theoretical hydrogen yield of 4 molH₂/mol acetate³⁷ and the hydrogen rate is the highest at about 50 m³H₂/m³-d from acetate in a dual-chamber MEC at an applied voltage of 1 V.³⁸ Glucose is also a widely used substrate in BES, but the energy conversion efficiency of glucose was 3% while that of acetate was 42%.³⁹ Moreover, Chae et al.⁴⁰ observed that the current density in a glucose-fed MFC was lower than that in the acetate-fed MFC due to electron loss by fermentative bacteria. The anaerobic condition of MECs enhances the growth of anaerobic microorganisms including ARB, non-exoelectrogenic fermentative bacteria and methanogens. The majority of the microbial community in acetate-fed MEC is Geobacter and Shewanella, but different species of ARB (e.g. Rhodoferan ferrireducens, Klebsiella pneumoniae and Aeromona hydrophila) are also found in glucose-fed MECs.⁴¹ Moreover, non-exoelectrogenic fermentative bacteria (e.g. Clostridium sp., Desulfovibrio sp. and Pelobacter) exist in glucose- or other substrate-fed
MECs. Zeng et al. found that dominant phyla in the microbial community of the anode were Proteobacteria (68%; Desulforvibrio sp., Geobacter sp., and Pelobacter sp.), Bacteroidetes (17%; Cloacibacillus sp.) and Firmicutes (12%; Clostridium sp. and Eubacterium sp.) in an MEC using a mixture of phenolic and furanic compounds. However, the enteric bacteria (e.g. Klebsiella) and sulfate-reducing bacteria (e.g. Desulfovibriio sp.) can degrade furfural to acetate which indicates that they are not inhibited by furfural. It is conceivable that specific ARB and FBB were inhibited by furfural in glucose-fed BES.

3.5 Conclusions

This study revealed the inhibitory effects of furfural on FBB and ARB in different systems. The hydrogen yield and the maximum hydrogen production rate decreased by 44% and 36% in batch experiments at 1 g/L furfural, respectively. Furfural is generally inhibitory to FBB but may also be stimulatory under certain conditions. Higher furfural concentrations than the threshold of 1 g/L were inhibitory as evidenced by lower yields and longer lag phases. Furfural was electrochemically degraded to FA and the electrochemical treatment enhanced biohydrogen production: hydrogen yield and maximum hydrogen production rate increased by 34% and 29% compared to untreated furfural in batch tests. The maximum hydrogen production rate at 1 g/L FFA and FA decreased by 33% and 22%, respectively, while the hydrogen yields were not significantly affected. In the continuous-flow systems, the acclimatized sludge was tolerant to furfural up to 0.5 g/L. In the glucose-fed reactor, although furfural concentration of 0.25 and 0.5 g/L stimulated hydrogen yields by 17% and 6%, hydrogen yields decreased by 21%, 29% and 62% at 1, 2 and 4 g/L furfural. The acclimatized sludge recovered 95% of the maximum hydrogen production rate after eliminating furfural from the feed indicating that the inhibition was reversible. Furfural concentration and F/S considerably affect hydrogen production and the threshold furfural level and F/S were 0.56 g/L furfural and 0.058 g furfural/g substrate.
In the acetate-fed MEC, ARB in long-term continuous-flow systems were not affected by furfural (up to 2 g/L). On the other hand, 0.7 g/L furfural inhibited the performance of the glucose-fed MEC irreversibly. To avert inhibition at 0.7 g/L furfural in the glucose-fed MEC, the substrate of 6.8 g/L is required, but it will cause the substrate inhibition to ARB due to a high concentration of substrate\textsuperscript{45}. The glucose-fed MEC has more diverse microbial community including FBB and ARB; while FBB (e.g. \textit{Clostridium sp.}) are intolerant of furfural, ARB (e.g. \textit{Geobacter sp.} and \textit{Klebsiella sp.}) can utilize FA the electrochemical degradation as a fuel to product hydrogen.
3.6 References


(43) Kiely, P. D.; Cusick, R.; Call, D. F.; Selembo, P. A.; Regan, J. M.; Logan, B. E. Anode microbial communities produced by changing from microbial fuel cell to


Chapter 4

4 Conclusions and Recommendations

4.1 Conclusions

This study was mainly aimed at investigating the impact of furfural, an inhibitor commonly present in pretreated lignocellulosic biomass, on fermentative biohydrogen producing bacteria and anode respiring bacteria. The major outcomes of this study are summarized below:

- Furfural was degraded to 2-furoic acid by electrochemical treatment and hydrogen yield and maximum hydrogen production rates from electrochemically treated furfural increased by 34% and 29% compared to the untreated furfural.

- Furfuryl alcohol and 2-furoic acid did not affect the hydrogen yield in batches, but hydrogen production rates decreased by 33% and 22%, respectively.

- In continuous-flow systems, the inhibitory threshold level of furfural and furfural-to-substrate were 0.56 g furfural/L and 0.056 g furfural/g substrate, respectively.

- In the acetate-fed MEC, furfural up to 2 g/L did not affect ARB in a long-term continuous-flow system.

- ARB in the glucose-fed MEC were inhibited by 0.7 g/L furfural and did not recover after removing furfural from the feed.

- Fermentative biohydrogen bacteria are intolerant of furfural, but ARB are able to survive in the presence of furfural and utilize 2-furoic acid as a substrate for hydrogen production.
4.2 Recommendations

Based on the results of this study, the following recommendations are suggested for further studies:

- The impact of furfural at low concentrations ranging from 0 to 1 g/L on ARB

- The analysis of microbial community of ARB in acetate- and glucose-fed MECs is required to prove the inhibitory effect of furfural on fermentative bacteria instead of ARB in the glucose-fed MEC.

- The effects of furfural on ARB in different configuration of MEC, e.g. a single-chamber MEC

- The impact of other inhibitors (e.g. HMF and phenolic compounds) on fermentative biohydrogen bacteria in continuous-flow systems and ARB in acetate- and glucose-fed MECs
Appendix A

A1. Pretreatment Methods for MEC

A.1.1 Carbon Fiber

A.1.1.1 1 N HNO₃ for 1 day

- Properties of nitric acid (HNO₃) in the lab
  - Molecular weight: 63.01 g/mole
  - Density: 1.413 g/mL at 20°C
  - 70% (w/w): 70 g HNO₃ in 100 g solution

- Preparation of 1 L of 1 N HNO₃ solution, 1 N HNO₃ = 1 M HNO₃

\[
\frac{1 \text{ mol } HNO_3}{L} = \frac{1.413 \text{ g}}{1 \text{ mL}} \times \frac{1 \text{ mol}}{63.01 \text{ g}} \times V_N \times 0.7
\]

\( V_N \) is the volume of nitric acid in 1 L solution, 63.7 mL HNO₃.

Thus, 63.7 mL of HNO₃ with 936.3 mL of water for 1 L of 1 N HNO₃ solution.

A.1.1.2 1 N acetone for 1 day

- Properties of acetone (CH₃COCH₃) in the lab
  - Molecular weight: 58.08 g/mole
  - Density: 0.792 g/mL at 20°C
  - 99.9% = 100%

- Preparation of 1 L of 1 N acetone solution, 1 N acetone = 1 M acetone

\[
\frac{1 \text{ mol acetone}}{L} = \frac{0.792 \text{ g}}{1 \text{ mL}} \times \frac{1 \text{ mol}}{58.08 \text{ g}} \times V_{ac}
\]

\( V_{ac} \) is the volume of acetone in 1 L solution, 73.3 mL acetone.
Thus, 73.3 mL of acetone with 926.7 mL of water for 1 L of 1 N acetone solution.

A.1.1.3 1 N ethanol for 1 day

Properties of ethanol (CH₃CH₂OH) in the lab
- Molecular weight: 46.07 g/mole
- Density: 0.789 g/mL at 20°C
- 99%

· Preparation of 1 L of 1 N ethanol solution, 1 N ethanol = 1 M ethanol

\[
\frac{1 \text{ mol ethanol}}{L} = \frac{0.789 \text{ g}}{1 \text{ mL}} \times \frac{1 \text{ mol}}{46.07 \text{ g}} \times V_{et}
\]

\(V_{et}\) is the volume of ethanol in 1 L solution, 73.3 mL ethanol.

Thus, 73.3 mL of ethanol with 926.7 mL of water for 1 L of 1 N ethanol solution.

A.1.2 Pretreatment of Membrane

Soak membrane into 5% NaCl solution (5 g NaCl with 100 mL of water) at 40°C for 1 day.
Appendix B

B.1 Continuous-flow system

Figure B.1 A continuous-flow reactor for biohydrogen production: a) a schematic diagram and b) a continuous-flow reactor in the laboratory.
B.2 Microbial Electrolysis Cell

(a) (b) (c)

(d) (e)

(f)

Figure B.2. Microbial electrolysis cell: a) an anode, b) a cathode, c) anion exchange membrane, d) a power supply, e) a data logger, and f) MEC in the laboratory.
Appendix C

C1. Copyright for Figure 2-3.
Curriculum Vitae

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**Oral Presentations:**


**Poster Presentations:**

