MICROBIAL ELECTROLYSIS CELLS: HYDROGEN PRODUCTION FROM
GLYCEROL AND ALTERNATIVE CATHODE MATERIALS

A Dissertation in
Chemical Engineering

by
Priscilla A. Selembo

© 2010 Priscilla A. Selembo

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2010
The thesis of Priscilla A. Selembu was reviewed and approved* by the following:

Joseph M. Perez  
Senior Research Scientist of Chemical Engineering  
Dissertation Co-advisor  
Co-Chair of Committee

Bruce E. Logan  
Kappe Professor of Environmental Engineering  
Dissertation Co-advisor  
Co-Chair of Committee

Wallis A. Lloyd  
Adjunct Professor of Chemical Engineering

Douglas D. Archibald  
Research Associate in Agricultural Analytical Chemistry

Andrew L. Zydney  
Walter L. Robb Chair and Professor of Chemical Engineering  
Head of the Department of Chemical Engineering

*Signatures are on file in the Graduate School
ABSTRACT

Microbial electrolysis cells (MECs) are promising systems for producing sustainable energy while treating organic waste. MECs contain exoelectrogenic bacteria that produce hydrogen from organic matter via electrohydrogenesis. Glycerol is a low cost commodity, major byproduct of biodiesel production and a potential source of organic matter for MECs. Glycerol was used for hydrogen production via anaerobic fermentation or electrohydrogenesis. MECs have to be affordable to be commercialized. Low cost transition metals were evaluated as alternatives to platinum catalysts for use in MEC cathodes.

Hydrogen was produced at 0.28 mol-H$_2$/mol-glycerol from glycerol fermentation while 1.06 mol-H$_2$/mol-glucose (0.53 mol-H$_2$/mol-3C) was obtained from glucose fermentation. The main product of glycerol fermentation was 1,3-propanediol which adversely affects H$_2$ gas yields. Higher hydrogen yields were achieved using MECs from glycerol (3.9 mol-H$_2$/mol-glycerol) with efficiencies similar to those achieved with glucose (7.2 mol-H$_2$/mol-glucose or 3.6 mol-H$_2$/mol-3C, E$_{ap}$=0.9V). 1,3-propanediol was produced in MEC but subsequently consumed, achieving better substrate utilization than fermentation. Hydrogen production from the glycerol byproduct from biodiesel was higher via electrohydrogenesis (1.8 mol-H$_2$/mol-glycerol) than fermentation (0.31 mol-H$_2$/mol-glycerol), but lower than pure glycerol due to the presence of methanol and soaps.

Stainless steel and nickel alloys were compared to platinum sheet metal for use as cathodes in MECs. SS A286 was superior to platinum in cathodic and energy recovery,
and hydrogen production rate (1.5 m$^3$/m$^3$d SSA286, 0.68 m$^3$/m$^3$d Pt, $E_{ap}$=0.9V).

Performance was further increased by nickel oxide electrodeposition. Smaller particles reduce total mass of the material and material costs. Commercially available nickel and stainless steel powders were applied to cathodes and compared to typical Pt cathodes (0.002 µm). Cathodes made with Ni (0.5-1 µm) had similar Coulombic efficiencies, cathodic, hydrogen and energy recoveries in MECs compared to Pt-cathodes but slightly lower hydrogen production rates (1.2-1.3 m$^3$/m$^3$/d Ni; 1.6 m$^3$/m$^3$/d Pt, $E_{ap}$=0.6V).

Avoiding exposure of the Ni catalyst to air minimized Ni dissolution. Analysis of the anodic biofilms showed that $G$.sulfurreducens and $P$.propionicus were the most abundant bacteria. Non-precious metals can therefore achieve higher hydrogen production rates than those obtained with platinum and can be used in MEC cathodes allowing large scale production.
TABLE OF CONTENTS

LIST OF FIGURES ..................................................................................................... ix

LIST OF TABLES ....................................................................................................... xii

ACKNOWLEDGEMENTS ......................................................................................... xiii

Chapter 1  Introduction ............................................................................................ 1

1.1. The need for sustainable energies ................................................................. 1
1.2. Hydrogen as a sustainable energy carrier ...................................................... 2
1.3. Hydrogen production via microbial electrolysis cells (MEC) ....................... 3
1.4. Research summary ....................................................................................... 4
   1.4.1. Hydrogen production from biodiesel byproduct ..................................... 4
   1.4.2. Alternative MEC cathode materials .................................................... 6
1.5. References ...................................................................................................... 8

Chapter 2  Background and Literature Survey ............................................................ 10

2.1. Microbial Electrolysis Cells .......................................................................... 10
   2.1.1. Development/background .................................................................... 10
   2.1.2. Exoelectrogenic Microorganisms ......................................................... 14
   2.1.3. Reactor operation and design ............................................................... 15
       2.1.3.1. Cathode ..................................................................................... 16
       2.1.3.2. Anode ....................................................................................... 17
       2.1.3.3. Membrane ............................................................................... 17
       2.1.3.4. Electrolyte effects .................................................................... 19
   2.1.4. MEC scalability and outlook ............................................................... 20
2.2. Glycerol Manufacture and Use ..................................................................... 21
   2.2.1. Glycerol production as a byproduct from biodiesel fuel production ... 22
   2.2.2. Glycerol conversion to value-added products ...................................... 23
       2.2.2.1. Hydrogen from glycerol via fermentation ................................. 24
       2.2.2.2. Hydrogen from glycerol via MEC ............................................ 28
       2.2.2.3. Hydrogen from glycerol via thermochemical methods ............. 30
   2.2.3. Future outlook ..................................................................................... 31
2.3. Catalyst Design and Evaluation ................................................................... 31
   2.3.1. Rate of reaction ................................................................................... 31
   2.3.2. Butler-Volmer model ........................................................................ 33
   2.3.3. Volcano plot for HER ....................................................................... 34
   2.3.4. Potential-pH (Pourbaix) Diagram ......................................................... 35
   2.3.5. Electrochemical testing ....................................................................... 39
       2.3.5.1. Cyclic voltammetry scan ........................................................... 39
       2.3.5.2. Linear voltammetry scan ............................................................. 41
Chapter 5 The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells ............................................. 111

Abstract .............................................................................................................. 111
5.1. Introduction.................................................................................................... 113
5.2. Materials and methods ................................................................................ 116
   5.2.1. MEC reactor construction ..................................................................... 116
   5.2.2. Cathodes .............................................................................................. 117
   5.2.3. Nickel Oxide Electrodeposition .......................................................... 117
   5.2.4. Analysis ............................................................................................... 118
   5.2.5. Calculations ......................................................................................... 119
5.3. Results ............................................................................................................ 122
   5.3.1. Flat cathodes ........................................................................................ 122
   5.3.2. Particles on carbon cloth cathodes compared to metal sheet cathodes .................................................................................. 129
   5.3.3. Nickel Oxide cathodes ......................................................................... 129
5.4. Discussion ...................................................................................................... 135
   5.4.1. Cathode Efficiency .............................................................................. 135
   5.4.2. Cathode Costs ...................................................................................... 137
5.5. Conclusions .................................................................................................... 138
5.6. Acknowledgements ........................................................................................ 138
5.7. References ...................................................................................................... 139

Chapter 6 Hydrogen production with nickel powder cathode catalysts in microbial electrolysis cells ......................................................... 141

Abstract ............................................................................................................... 141
6.1. Introduction.................................................................................................... 143
6.2. Materials and methods ................................................................................ 145
   6.2.1. Cathodes .............................................................................................. 145
   6.2.2. Electrochemical evaluation of catalysts .............................................. 146
   6.2.3. MEC reactor construction .................................................................... 146
   6.2.4. Analysis after MEC cycles .................................................................. 147
   6.2.5. Calculations ......................................................................................... 148
   6.2.6. Community Analysis ........................................................................... 149
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.3</td>
<td>Results</td>
<td>150</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Cathode selection by LSV</td>
<td>150</td>
</tr>
<tr>
<td>6.3.2</td>
<td>MEC performance</td>
<td>154</td>
</tr>
<tr>
<td>6.3.2.1</td>
<td>Volumetric gas production and composition</td>
<td>154</td>
</tr>
<tr>
<td>6.3.2.2</td>
<td>Current production</td>
<td>157</td>
</tr>
<tr>
<td>6.3.2.3</td>
<td>Coulombic efficiency, recoveries and hydrogen production rates at 0.6 V</td>
<td>157</td>
</tr>
<tr>
<td>6.3.2.4</td>
<td>MEC performance with Ni210 cathodes as a function of applied voltage</td>
<td>158</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Cathode catalytic activity and surface analysis of used cathodes</td>
<td>160</td>
</tr>
<tr>
<td>6.3.4</td>
<td>Nickel stability</td>
<td>162</td>
</tr>
<tr>
<td>6.3.5</td>
<td>Microbial community</td>
<td>165</td>
</tr>
<tr>
<td>6.4</td>
<td>Discussion</td>
<td>166</td>
</tr>
<tr>
<td>6.4.1</td>
<td>Cathode Performance</td>
<td>166</td>
</tr>
<tr>
<td>6.4.2</td>
<td>Microbial community</td>
<td>169</td>
</tr>
<tr>
<td>6.4.3</td>
<td>Cathode Costs</td>
<td>170</td>
</tr>
<tr>
<td>6.5</td>
<td>Conclusions</td>
<td>170</td>
</tr>
<tr>
<td>6.6</td>
<td>Acknowledgements</td>
<td>171</td>
</tr>
<tr>
<td>6.7</td>
<td>References</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>Chapter 7 Conclusions and Future Research</td>
<td>175</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 2.1. Schematic of a single chamber MEC. ...................................................... 11

Figure 2.2. Biodiesel production mechanism............................................................. 22

Figure 2.3. Most important metabolic pathways during anaerobic fermentation of glucose and glycerol fermentation. The reactions indicated by the arrows labeled a-f are alternative pathways depending on the microorganism............... 25

Figure 2.4. Generic potential energy diagram showing the effect of a catalyst in a chemical reaction $X + Y \rightarrow Z$................................................................. 32

Figure 2.5. Volcano plot for log $i_0$ values for the HER as a function of M-H bond energy ........................................................................................................... 35

Figure 2.6. Pourbaix diagram for nickel ................................................................. 37

Figure 2.7. Pourbaix diagram for iron with wet corrosion products....................... 38

Figure 2.8. Pourbaix diagram for iron considering Fe, Fe$_3$O$_4$ and Fe$_2$O$_3$ as the dry corrosion products ................................................................. 38

Figure 2.9. Examples of how CV can be used to distinguish efficiency of different cathodes. (a) A lower voltage ($V_e$) is needed by material “A” to initiate current generation, thus this material performs better than “B”. (b) material “C” has a steeper slope and generates more current at a given voltage, thus the performance of material “C” is better than “D”. ............... 40

Figure 2.10. CV with different cathode catalysts for hydrogen evolution, where Pt was the most effective catalyst with lower values of $V_e$ (V) and higher values of $V_h$ (mA/cm$^2$ V)......................................................................................... 40

Figure 2.11. EIS: Nyquist plots limited to the determination of ohmic resistance of MEC systems with (a) cation exchange membrane and (b) anion exchange membrane ............................................................................................................. 42

Figure 3.1. Gas production from glucose, pure glycerol (P-glycerol), or glycerol byproduct from biodiesel production (B-glycerol): (A) total gas production; (B) hydrogen yield ........................................................................................................ 60

Figure 3.2. Liquid and gas composition for glucose and P-glycerol fermentation: (A) product quantity; (B) Coulombic balance ............................................................... 62

Figure 3.3. Gas production using different heat-treated inocula. (A reactor size of 300 mL was used here, compared to 500 mL in other studies). ......................... 64
Figure 3.4. Gas production from P-glycerol using different pretreatment methods on wheat soil................................................................. 65

Figure 3.5. Effect of initial substrate concentration on gas production. .......... 66

Figure 3.6. Effect of initial cell concentration using different buffers on hydrogen production using glycerol or glucose........................................ 67

Figure 3.7. Modified Gompertz model fit for glucose or glycerol fermentation.... 68

Figure 3.8. Hydrogen production based on actual measurements compared to that predicted from liquid product formation. ........................................ 73

Figure 4.1. Gas production of glucose or glycerol in MEC, 50 mM PBS, pH 7.0, (A) applied voltage 0.9 V, (B) applied voltage 0.5 V. Standard deviation bars missing for some cycles due to respirator malfunction. ......................... 87

Figure 4.2. Gas composition of glucose and glycerol during MEC fourth cycle, (A) applied voltage 0.9 V, (B) applied voltage 0.5 V................................. 88

Figure 4.3. Hydrogen yields of glucose, glycerol or glycerol from biodiesel at applied 0.5 V and 0.9 V................................................................. 91

Figure 4.4. Hydrogen production rate ($Q$), hydrogen composition ($H_2$) and energy efficiency ($\eta_e$) for B-glycerol as a function of applied voltage. ..................... 92

Figure 4.5. (A) Product formation and (B) charge balance of 1 g/L P-glycerol at applied 0.5 V......................................................................................... 94

Figure 4.6. (A) Product formation and (B) charge balance of 1 g/L glucose at applied 0.5 V......................................................................................... 96

Figure 4.7. Gas composition and cycle times for different initial P-glycerol concentrations at applied 0.5 V. ................................................................. 97

Figure 4.8. (A) Product formation and (B) charge balance of 3 g/L glycerol at applied 0.5 V......................................................................................... 99

Figure 5.1. Gas production of MEC’s with different stainless steels and nickel cathodes, compared to a platinum disk, at an applied voltage of 0.9 V. .......... 123

Figure 5.2. Gas production of MEC’s with different stainless steels and nickel cathodes, compared to a platinum disk, at an applied voltage of 0.6 V. .......... 125

Figure 5.3. Current densities for MEC’s with a platinum disk, Ni 625 and SS A286 cathodes at applied voltages of 0.6 V and 0.9 V................................. 126
Figure 5.4. Tafel plots for MECs for (A) stainless steel 286 alloy and (B) platinum metal cathodes. .......................................................... 128

Figure 5.5. Gas production of MECs with and without electrodeposited nickel oxide layers on SS A286 and Ni 625 at an applied voltage of 0.6V. ......................... 130

Figure 5.6. Total gas and current production versus time with (A) Ni 625 + NiOx and (B) SS A286 + NiOx cathodes. ......................................................... 132

Figure 5.7. SEM images of SS A286 + NiOx (A and C) before and (B and D) after 8-day use as cathode in MEC. A and B are at 2,000× magnification and C and D are at 20,000× magnification ......................................................... 134

Figure 6.1. Tafel plots for select experimental cathodes in 2mM phosphate buffer, scan rate 2 mV/s, third scan ................................................................. 152

Figure 6.2. (A) Total gas production and (B) maximum current for MECs with Ni210, Ni210+CB, eNiOx or Pt catalyst cathodes, as a function of cycle number at an applied voltage of 0.6 V. Gas production for cycles 1-6 were not recorded for Pt and eNiOx due to equipment malfunction .................................. 156

Figure 6.3. MEC performance for Ni210 catalyst cathodes at different applied voltages. (A) Hydrogen production rate (B) Cathodic recovery and Coulombic efficiency (C) Energy recovery based on electrical input and overall energy recovery. ................................................................. 160

Figure 6.4. Examples of Tafel plots for cathodes before and after use in 12 MEC cycles. Test conditions: 2mM phosphate buffer, scan rate 2 mV/s, third scan ..... 161

Figure 6.5. SEM images of Ni210 catalyst cathodes (A and B) and eNiOx catalyst cathodes (C and D), before (A and C) and after (B and D) 12 cycles in MEC. Images are at 20,000× magnification ......................................................... 162

Figure 6.6. Nickel content via ICP-AES analysis in MEC solution after (A) aerobic feeding and (B) anaerobic feeding ................................................................. 164

Figure 6.7. Microbial community and phylogenetic distances to closest match based on 16S rRNA sequences recovered from the MEC anode with Ni210 catalyst cathode ................................................................. 165
LIST OF TABLES

**Table 3.1.** Best fit parameters using modified Gompertz model ........................................... 68

**Table 3.2.** Yields of hydrogen and 1,3-propanediol from pure glycerol and glycerol from biodiesel production. (NR, not reported, P-glycerol, pure glycerol, B-glycerol, glycerol byproduct from biodiesel production). ...................... 71

**Table 4.1.** Half cell reactions and number of moles of electrons per mol of substrate ................................................................. 84

**Table 4.2.** MEC results for glucose or glycerol ........................................................ 89

**Table 5.1.** Stainless Steel and Nickel Alloys Composition (% by weight) ............... 117

**Table 5.2.** Summary of MEC results for different metal cathodes (stainless steel, nickel and platinum) at an applied voltage of 0.9V .................................................. 124

**Table 5.3.** Tafel plots’s slopes and Y-intercepts for MEC’s with different metal cathodes. ............................................................................................................... 127

**Table 5.4.** Summary of MEC results for metal cathodes with electrodeposited nickel oxide layer, compared to platinum, at an applied voltage of 0.6V. .......... 131

**Table 5.5.** Tafel plots’s slope and Y-intercepts for MECs with and without nickel oxide electrodeposited on Ni 625 and SS 286 alloys. .................................................. 133

**Table 5.6.** Metal composition by SEM-EDS before and after 8 days of use in MEC as cathode. ................................................................................................................ 134

**Table 6.1.** Overpotentials vs SHE at current density of -3.2 log A/cm² for cathodes during third LSV scan at 2 mV/s. Surface area calculated using equation (1), NA – not applicable, ND- not determined. ........................................ 151

**Table 6.2.** Summary of MEC results for Ni210, Ni210+CB, eNiOx and Pt catalyst cathodes at an applied voltage of 0.6V, eighth cycle of operation........... 155
ACKNOWLEDGEMENTS

My tenure at Penn State has been an enjoyable and rewarding experience. I am moving forward from this chapter of my life, feeling enriched not only by what I have learned technically but also by what I have learned from the wonderful people that have been working with me for the last four years. Now that it is time to move on, I find myself treasuring every moment in the Sackett laboratory.

I would like to foremost thank my advisors Dr. Bruce Logan and Dr. Joe Perez. Dr. Logan has taught me everything from microbial fuel cells to improving my technical writing while keeping a good sense of humor. Dr. Perez has been part of my life since my first tenure at Penn State and not only gave me sound technical advice but also made me feel like I was part of his family. I feel lucky to have found such great mentors and role models.

I would also like to sincerely thank my “honorary advisor” Dr. Lloyd. Dr. Lloyd has shared many of his creative ideas and inventions with me which made some of my projects possible. Thank you also to Dr. Archibald for his recommendations, use of the infrared and for agreeing to be part of my committee even though the project was continually changing.

I would like to thank all my lab mates, especially Yi Zuo and Shaoan Cheng for showing me the ropes when I joined the group, Doug Call, Rachel Wagner, Valerie Watson and Farzaneh Rezaei for being my original group of friends and coworkers, and Matt Merrill, Pat Kiely and Maha Mehanna for all their invaluable advice and guidance. Thank you to Dave Jones, Ellen Bingham and Peg Van Ornum for providing the much
needed behind the scenes support. I’d also like to thank Jonathan Chin for helping me with all those “this is the last batch” HPLC samples.

This research was supported by the Global Research Partnership from King Abdullah University of Science and Technology, the General Electric First-Year Faculty for the Future Fellowship, the Arthur and Elizabeth Rose Memorial Fellowship and Air Products and Chemicals. Thank you to Nittany BioDiesel for use of equipment and providing glycerol samples from their biodiesel production.

Finally and most importantly, I would like to thank my dear husband George, who provides me with infinite motivation, love and support to make this milestone a reality. I am grateful to my parents and grandparents for showing me the importance of education and supplying the means to achieve this goal. To George’s family (who is also my family) and our au pair Eveling Lopez for caring for the kids, anytime, anyplace. And to my wonderful children Taylor, Talia, Alexander and Connor, who grant me motivation to keep working and the much needed breaks!
This work is dedicated to the memory of my grandparents mamá Alicia y papá César. Thank you for giving me unconditional love and for the knowledge that I can achieve whatever I set my mind to achieve.


Chapter 1

Introduction

1.1. The need for sustainable energies

“Alternative energy is a future idea whose time is past. Renewable energy is a future idea whose time has come” - Bill Penden (Atlas World Press Review, April 1977). Since these words were said more than three decades ago, the world needs energy that is not only renewable (naturally replenished) but also sustainable (energy that can be provided without compromising the needs of future generations and causes no long-term damage to the environment).

The world’s current economy depends on fossil fuels: 86% of the energy used comes from fossil fuels [1]. A fossil fuel economy presents several problems. Fossil fuels are non-renewable resources which will be depleted. For example, known petroleum reserves are projected to be depleted in less than 50 years at the present rate of consumption [2]. The excavation, transportation and use of fossil fuels increase the emission of poisonous and greenhouse gases, which lead to changes in global climate, ecology, biodiversity and health. Relying on foreign oil creates an economic dependence on exporting countries. We need to increase our use of sustainable energies and minimize fossil fuel consumption.
1.2. Hydrogen as a sustainable energy carrier

Electricity, biofuels and hydrogen are the major energy carriers that have the potential to contribute to our increasing energy need [2]. The world energy consumption reached 472 quadrillion BTUs in 2006 and it is projected to increase an additional 44% by 2030 [1]. A combination of energy carriers, technologies and energy sources may be needed to supply this increasing energy demand as each technology has advantages and limitations. For example, while biofuels are a renewable energy source, supplying biomass for biofuels typically competes with food, water and land needs, plus it increases water and air pollution due to use of fertilizers and pesticides [3, 4]. Also, the production of biofuels creates an overabundance of byproducts (such as glycerol from biodiesel) [5] or waste products that need to be handled appropriately.

In recent years hydrogen has gained considerable interest as a potential energy carrier expected to replace fossil fuels. Burning hydrogen does not contribute to greenhouse gas emissions, acid rain or ozone depletion because its oxidation product is water. Hydrogen is highly efficient: it has the highest energy content per unit weight among the gaseous fuels (energy content 120 MJ/kg for H₂; 44 MJ/kg for gasoline). It can be produced from a variety of energy sources including organic wastes, so it can be cost-effective, clean, sustainable and renewable. Unfortunately, 96% of the hydrogen produced today comes from fossil fuels via thermo-chemical conversion (pyrolysis), gasification and reforming. Another drawback is that the costs for production, delivery and storage of hydrogen are currently high [2].
Renewable hydrogen production technologies include water electrolysis and biological methods such as biophotolysis and fermentation. Water electrolysis requires a large electrical input (50 kilowatt hours of electricity per kilogram of hydrogen) with an energy efficiency of 56-73% [6]. Hydrogen production by dark fermentation is less energy intensive, however, thermodynamic limitations of the fermentation process result in only partial conversion of the substrate, production of byproducts and low hydrogen yields [7]. Biophotolysis and photofermentation conversion rates are orders of magnitude lower than dark fermentation [8].

1.3. Hydrogen production via microbial electrolysis cells (MEC)

Hydrogen can also be produced in a microbial electrolysis cell (MEC) via electrohydrogenesis from a variety of organic sources, including waste materials and complex, non-fermentable substrates [9-13]. In an MEC, exoelectrogenic bacteria oxidize organic matter and generate CO₂, electrons and protons. The bacteria transfer the electrons to the anode and the protons are released to the solution. Electrons and protons are catalyzed at the cathode to form hydrogen gas when the endothermic barrier of hydrogen formation (0.414 V) is overcome. This can be achieved by adding a small voltage to that produced by the bacteria (~0.3 V). Typically voltages of ~0.3 V or larger are needed to overcome electrode overpotentials, which is less than the voltages required for water electrolysis (typically 1.8 – 2.0 V) [14].

The ability for exoelectrogens to utilize a variety of organic sources in MECs not only makes electrohydrogenesis sustainable, but also economically and environmentally
beneficial to society. For example, the potential energy available in raw wastewater exceeds the electricity requirements of its treatment process by a factor of 9.3, and it accounts for 1.5% of the total electricity usage in the USA [14, 15]. Hydrogen can be produced while treating waste in MECs instead of using energy to treat this waste. Also, because MECs are stand alone systems, an economic independence can exist even for remote rural areas that allows these societies to fulfill their energy needs and simultaneously treat their waste.

1.4. Research summary

1.4.1. Hydrogen production from biodiesel byproduct

Glycerol is a major byproduct of biodiesel production: one liter of glycerol is produced for every ten liters of biodiesel. If biodiesel plants reach capacity (10.2 billion liters biodiesel /yr in 2009) [16], glycerol production will exceed glycerol demand (0.2 billion liters/yr glycerol) [5] by a factor of five. The increased biodiesel production has already resulted in glycerol plant closures and substantially lower glycerol prices. New uses for glycerol are needed to help stabilize biodiesel and glycerol product pricing and to avoid accumulation of a material that can have a negative environmental impact.

The use of glycerol for hydrogen gas production was examined using two different approaches: anaerobic fermentation; and electrohydrogenesis using MECs. The main advantage of anaerobic fermentation for hydrogen production over other methods is that fermentation is less energy intensive and therefore less expensive [17]. It is possible
to obtain acceptable hydrogen yields using mixed cultures for glucose fermentation, but low yields were reported in the only published study with glycerol and untreated mixed cultures [18]. Heat and other pre-treatment methods with inocula from four different sources (tomato soil, wheat soil, compost, and sludge) were evaluated to increase hydrogen production from glycerol fermentation.

Pure glycerol and the unpurified glycerol byproduct from biodiesel fuel production (B-glycerol) were used as substrates in fermentation and electrohydrogenesis studies. B-glycerol contains methanol, excess catalyst (sodium hydroxide), and other impurities that can inhibit hydrogen production and cell growth. Glucose was used as a positive control, as it is a fermentable substrate which shares similar stoichiometry and metabolic pathways as glycerol during bacterial degradation. The main difference between glucose and glycerol fermentation is the formation of 1,3-propanediol (PD) during glycerol fermentation [18]. One mole of PD requires one mole of H₂, and thus its production adversely affects H₂ gas yields.

Electrohydrogenesis can achieve higher conversion rates than fermentation as there is complete oxidation of the substrate. The greatest hydrogen yield theoretically possible from bio-organisms is 3 mol-H₂/mol-glycerol based on fermentation to acetate, despite a stoichiometric potential of 7 mol-H₂/mol-glycerol. Glycerol had not yet been used in a mediatorless or membraneless MEC, while B-glycerol was used in a two-chamber MEC with a mediator resulting in low conversion of glycerol to hydrogen (0.77 mol-H₂/mol-glycerol) [19]. A single chamber mediatorless MEC was used in this study to evaluate electrohydrogenesis of glycerol, aiming to reduce PD production and achieve yields closer to the maximum theoretical yield.
The results of this work on glycerol fermentation was summarized in a paper by Selembo P.A., Perez J.M., Lloyd W.A. and Logan B.E. titled “Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures” and published in Biotechnology and Bioengineering [20]. My advisors Dr. Perez and Dr. Logan provided guidance and support for the research on this paper and the rest of the dissertation. Dr. Lloyd provided advice for the experiments on all the glycerol work. Dr. Logan also helped with thorough editing and responding to reviewer comments for this paper and subsequent papers. This paper is included as Chapter 3 of this dissertation.

The work on electrohydrogenesis with glycerol was summarized in a paper by Selembo P.A., Perez J.M., Lloyd W.A. and Logan B.E. titled “High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells” and published in the International Journal of Hydrogen Energy [21]. This paper is included as Chapter 4.

1.4.2. Alternative MEC cathode materials

Non-precious metal alternatives need to be used to improve system sustainability and reduce costs of MECs, as platinum is commonly used as the cathode catalyst. Nickel, nickel alloys and stainless steels are widely used as cathode materials for alkaline water electrolysis due to their relatively good catalytic activity, high corrosion stability, and low cost [22]. Their performance has been well studied for the hydrogen evolution reaction (HER) but only under alkaline conditions, while MECs typically operate at neutral pHs.
Nickel and stainless steel alloys were compared to platinum sheet metal in MECs. A comparison of these materials using flat surface metals provides better information on the intrinsic catalytic activity of these metals by removing surface area variability. Smaller particles were evaluated as catalysts on cathodes to reduce total mass of the material thereby reducing material costs. By demonstrating that transition metals can be used as viable alternatives to Pt cathodes for MECs, we are one step closer to full-scale application of this promising technology.

The work on flat surface metals for use as cathodes was summarized in a paper by Selembo P.A., Merrill M.D. and Logan B.E. titled “The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells” published in the Journal of Power Sources [23]. My colleague Matt Merrill provided assistance with electrochemical and MEC experiments, provided general advice during the research and helped with editing of the manuscript. This paper is included as Chapter 5.

The work with powder metals was summarized in a paper by Selembo P.A., Merrill M.D. and Logan B.E. titled “Hydrogen production with nickel powder cathode catalysts in microbial electrolysis cells” accepted for publication in the International Journal of Hydrogen Energy [24]. My colleague Matt Merrill provided advice on the electrochemical tests and helped with editing of the manuscript. This paper is included as Chapter 6.
1.5. References


2.1. Microbial Electrolysis Cells

2.1.1. Development/background

Microbial electrolysis cells (MECs) are novel systems that can convert a wide variety of organic materials into hydrogen [1]. These systems have the potential to provide clean, renewable and sustainable energy for our future as hydrogen can be produced from waste materials and wastewater [2-5]. An added advantage is the simultaneous treatment of this waste material or wastewater, which would otherwise consume additional electricity.

In MECs bacteria oxidize organic matter and release protons to the solution and electrons to the anode anode (Figure 2.1). The electrons flow to the cathode where they combine with protons and release hydrogen gas if enough energy is provided to overcome the endothermic energy barrier of hydrogen formation [6, 7]. MECs are similar to microbial fuel cells (MFCs) as bacteria in MFCs also oxidize organic matter and release carbon dioxide, electrons and protons. In MFCs power is produced instead of hydrogen when the protons combine with oxygen or another electron acceptor at the cathode without the need for additional energy.
Higher hydrogen production yields can be obtained with MECs than fermentation reactors. Fermentation yields are limited by the formation of side products while in MECs the substrate is fully oxidized. For example, the maximum hydrogen yield from glucose fermentation is 4 mol-H$_2$/mol-glucose if acetate is formed (Equation 2.1) or 2 mol-H$_2$/mol glucose if butyrate is formed (Equation 2.2) while the maximum theoretical yield for complete oxidation of glucose is 12 mol-H$_2$/mol-glucose (Equation 2.3) [8, 9].

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad (2.1)$$

$$\Delta G^{\circ} = -206.3 \text{ kJ/mol}$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2 \quad (2.2)$$

$$\Delta G^{\circ} = -256.8 \text{ kJ/mol}$$

$$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 6H^+ + 12H_2 \quad (2.3)$$

$$\Delta G^{\circ} = +3.2 \text{ kJ/mol}$$

**Figure 2.1.** Schematic of a single chamber MEC.
Glucose and its fermentation byproducts cannot be directly converted to hydrogen without an external energy input as these reactions are endothermic and are not spontaneous under standard conditions (25 °C, pH 7, 1 atm) (equations 2.3, 2.4 and 2.5). This external energy input is provided to MECs thus theoretically achieving the higher conversion rates for fully oxidized substrates.

\[
\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + \text{H}^+ + 4\text{H}_2 \quad (2.4)
\]
\[\Delta \text{G}^\circ' = +104.6 \text{ kJ/mol}\]

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 10\text{H}_2\text{O} \rightarrow 4\text{HCO}_3^- + 3\text{H}^+ + 10\text{H}_2 \quad (2.5)
\]
\[\Delta \text{G}^\circ' = +257.3 \text{ kJ/mol}\]

The minimum cell potential that needs to be added for a system to produce hydrogen at the cathode with acetate as the substrate is 0.114 V (equation 2.11) [8]. This is calculated using the Nernst equation (equations 2.8-2.10) based on hydrogen formation at the cathode (equation 2.6) and acetate oxidation at the anode (equation 2.7).

The hydrogen formation reaction at the cathode is:

\[
2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2 \quad (2.6)
\]

The acetate oxidation reaction at the anode is:

\[
2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \quad (2.7)
\]

The cell potentials \(E_{\text{an}}\) or \(E_{\text{cat}}\) are calculated based on the Nernst equation

\[
E_{\text{an}} = E_{\text{an}}^\circ - \frac{RT}{nF} \ln \Pi \quad (2.8)
\]

where \(E_{\text{an}}^\circ\) is the standard cell electromotive force at standard conditions (25 °C, 1 bar, 1 mol/L), \(n\) is the number of electrons per reaction mol, \(F\) is Faraday’s constant (96,485.3 C/mol) and \(\Pi\) is the reaction quotient (ratio of the activities of the products divided by the reactants raised to their stoichiometric coefficients).
The cell potential at the cathode $E_{\text{cat}}$ is:

$$
E_{\text{cat}} = E^\circ - \frac{RT}{nF} \ln \frac{\text{H}_2}{[\text{H}^+]^2}
$$

$$
= 0 - \frac{8.31 \text{J/molK} \times 298.15 \text{K}}{2 \times 9.65 \times 10^4 \text{C/mol}} \ln \frac{1}{[10^{-7}]^2} = -0.414 \text{V}
$$

(2.9)

The cell potential at the anode $E_{\text{an}}$ is:

$$
E_{\text{an}} = E^{\circ}_{\text{an}} - \frac{RT}{nF} \ln \frac{[\text{CH}_3\text{COO}^-]}{[\text{HCO}_3^-]^2[\text{H}^+]^9}
$$

$$
= 0.187 - \frac{8.31 \text{J/molK} \times 298.15 \text{K}}{8 \times 9.65 \times 10^4 \text{C/mol}} \ln \frac{0.0169}{[0.005]^2[10^{-7} \text{ M}]^9} = -0.300 \text{V}
$$

(2.10)

for a solution with $\text{HCO}_3^-$ = 5mM, $\text{CH}_3\text{COO}^-$ = 16.9 mM and pH 7 [8]

The total cell potential is

$$
E_{\text{cell}} = E_{\text{cat}} - E_{\text{an}} = (-0.414 \text{V}) - (-0.3 \text{V}) = -0.114 \text{V}
$$

(2.11)

In reality, a higher potential than the equilibrium potential, called the overpotential, is required to drive the hydrogen evolution reaction (HER). The overpotential can be due to activation loss (to initiate the reactions on the electrons and extracellular electron transfer to the anode), bacteria metabolism loss (bacteria acquiring energy for growth), mass transfer loss (limited flux of the reactants to the electrodes) and ohmic loss (proton diffusion resistance and charge transfer resistance). Typically voltages of ~0.3 V or larger are needed to overcome electrode overpotentials. This electrical input is less than the voltages required for water electrolysis (typically 1.8 - 2.0 V) [8].
2.1.2. Exoelectrogenic Microorganisms

Exoelectrogens are microorganisms that can directly transfer electrons outside the cell to the electron acceptor (anode) without the need for exogenous mediators. There is an expansive diversity of exoelectrogenic microorganisms including *Alpha-proteobacteria* (*Rhodopseudomonas, Acidiphilium, Ochrobactrum*), *Beta-proteobacteria* (*Rhodoferax*), *Gamma-proteobacteria* (*Shewanella, Pseudomonas, Escherichia and Klebsiella*), *Delta-proteobacteria* (*Aeromonas, Geobacter, Geopsychrobacter, Desulfuromonas, Desulfovibrio, Desulfobulbus*), *Firmicutes* (*Clostridium, Thermincola*), *Acidobacteria* (*Geothrix*) and *Fungi* (*Pichia*) [10].

There are three known electron transfer mechanisms: direct contact through outer membrane cytochromes, nanowires (conductive pili) and mediators (electron shuttles) produced by the bacteria [11]. Extracellular electron transfer mechanisms are not mutually exclusive within a single species. For example, *Shewanella oneidensis* can transfer electrons using three different methods: 1) outer membrane cytochromes for direct electron transfer, 2) electrically conductive nanowires, and 3) production of riboflavins that can function as electron shuttles. *Geobacter sulfurreducens* also has outer membrane cytochromes and can produce nanowires that were able to conduct electrons through a 50 µm thick anodic biofilm [12] but do not produce flavins or other mediators (i.e. pyocyanin, melanin, or quinones).

The MEC microbial community is less diverse than the MFC microbial community [13]. While MFCs have oxygen diffusion into the anode chamber from air cathodes, MECs can be operated under completely anaerobic conditions. This promotes
the growth of anaerobic bacteria such as the exoelectrogen \textit{Geobacter},
nonexoelectrogenic fermentative bacteria, and methanogenic microorganisms.

\textit{Pelobacter propionicus} and \textit{Geobacter} spp. were found to be dominant bacteria in
MECs, previously operated as MFCs, and fed a phosphate buffer solution with acetate as the
substrate [13, 14]. \textit{Shewanella} and \textit{Pseudomonas} were the dominant bacteria in a 2
chamber MEC with acetate as the substrate, carbonate buffer and sewage sludge as the
inoculum [15]. \textit{Pelobacter} was the dominant bacteria in the suspension and anodic
fractions of an ethanol-fed MEC inoculated with sludge [16].

\textit{Pelobacter} is a fermentative bacterium but not an exoelectrogen [17]. Its presence
in the MEC is due to a three-way syntrophy among this fermenter, exoelectrogens and H$_2$
scavengers. Exoelectrogens \textit{Geobacter}, \textit{Rhodopseudomonas} and \textit{Pseudomonas} were
present in very low levels (0.5% or less) in an ethanol-fed MEC. The H$_2$ scavengers
present in MECs were H$_2$-oxidizing methanogens (\textit{methanobacteriales}) but no homo-
acetogenic bacteria when methanogenesis was allowed to occur, and homo-acetogenic
bacteria (\textit{Acetobacterium}) but no H$_2$-oxidizing methanogens when a methanogenic
inhibitor (N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES)) was added [14, 16].

\subsection{2.1.3. Reactor operation and design}

MEC designs and materials have made significant improvement since their
conception in 2005 but need to continue improving for successful use in large scale
applications.
2.1.3.1. Cathode

Hydrogen production occurs at the cathode. A catalyst is used to reduce the overpotential needed to drive the hydrogen evolution reaction (HER). Platinum is typically used as the catalyst in MEC research as it is the best known catalyst for HER [18]. The high cost of platinum, the negative environmental impacts during its mining, and its susceptibility to poisoning has led to the search for alternatives. HER can be catalyzed by bacteria in the absence of a metal catalyst (biocathode) achieving \( Q = 0.63 \) m\(^3\)/m\(^3\) d at \( E_{ap} = 0.7 \) V with ferrocyanide at the anode [19] or \( Q = 0.035 \) m\(^3\)/m\(^3\) d at \( E_{ap} = 0.5 \) V with both a biocathode and bioanode [20]. Since the publication of the work with nickel and stainless steel flat metal sheets (Chapter 5), a few other researchers have also looked at transition metals other than platinum as cathode materials in MECs. With stainless steel (SS) type 304 brush cathodes, MECs produced hydrogen \( (Q = 1.7 \) m\(^3\)/m\(^3\) d, applied voltage of \( E_{ap} = 0.6 \) V) at rates similar to those with platinum-based cathodes \( (Q = 0.5-2.0 \) m\(^3\)/m\(^3\) d) due to the catalytic activity of the SS and the increased surface area of the brush [21]. Electrodeposited nickel alloys (NiMo and NiW) were used in MEC cathodes that produced hydrogen at a rate of \( Q = 2.0 \) m\(^3\)/m\(^3\) d \( (E_{ap} = 0.6 \) V) [22]. Tungsten carbide was examined as a catalyst for hydrogen production under neutral pH conditions in electrochemical tests [23] but not in an MEC. An MEC with a cathode lacking a metal catalyst produced hydrogen at the higher applied voltage of \( E_{ap} = 1 \) V at the low rate of \( Q = 0.57 \) m\(^3\)/m\(^3\) d [14].
2.1.3.2. Anode

MEC anodes have to be highly electrically conductive, non-corrosive, have a high surface area, high porosity, and be non-fouling, inexpensive and easy to manufacture. Advances in MFC anode materials also apply to MEC anodes. Anode materials used in MECs include carbon cloth, carbon paper, graphite felt, granules and brushes [6]. High surface areas have been achieved with graphite fiber brushes [24] so reactor performance is usually not limited by the anodic reactions. High temperature ammonia gas treatment (700 °C, 1 hr, 5% ammonia gas) results in faster start up and increased current densities in MFCs due to the more favorable adhesion of microorganisms to the positively charged anode [25]. Heat treatment (450 °C, 30 min) is a large scale alternative for ammonia treatment, achieving maximum power densities of 720 mW/m² in an MFC with a heat treated anode, compared to 801 mW/m² with an ammonia treated anode [26]. Carbon mesh was identified in the same study as a cheaper alternative for carbon cloth anodes when reducing electrode spacing for increased power in MFCs.

2.1.3.3. Membrane

MECs can be designed either by placing the anode and the cathode in two different chambers (2-chamber MEC) or by having both electrodes in one chamber (single chamber). Membranes separate the anode and the cathode in 2-chamber MECs. They are also used in some single chamber MECs integrated with the cathode as a membrane electrode assembly (MEA). The membrane is used to improve the purity of
the hydrogen and to prevent microbial consumption of the hydrogen. The presence of membranes does not prevent hydrogen diffusion back to the anode chamber.

The most common membranes are cation exchange membranes (CEMs) which allow free protons (H⁺) to pass. However, they can also transport other cationic species present in wastewater or media such as N⁺, K⁺, NH₄⁺, Ca⁺ and Mg²⁺. The protons consumed at the cathode are not replenished by protons generated at the anode which leads to a pH increase at the cathode and decrease at the anode. This pH gradient can lead to performance losses of 0.06 V per unit change in pH, as predicted by the Nernst equation (equation 2.8). Anion exchange membranes (AEM) transport anions such as phosphate and bicarbonate ions from the cathode to the anode, instead of cations from the anode to the cathode as in the CEM membrane. Higher hydrogen production and lower pH differentials have been achieved with AEM membranes (2.1 m³/m³ d) compared to CEM membranes (0.4 m³/m³ d) [27], but this was counteracted by higher cathode overpotentials [4, 28]. Other membranes tested were bipolar and charge mosaic membranes, but these did not perform as well as AEM or CEM membranes [4].

MECs can be operated without membranes, thus simplifying architecture and reducing capital costs. Removing the membrane reduces ohmic resistance from the membrane and reduces the bulk pH gradient in the liquid, but it does not prevent localized pH gradients at the electrodes or hydrogen consumption by methanogens growing on the electrodes or the solution [6]. Hydrogen production rates from acetate of 6.3 m³/m³ d (Eₐp = 1 V) was achieved in a membraneless continuous MEC with a J-cloth separating the anode and the cathode [29] and 3.12 m³/m³ d (Eₐp = 0.8V) in a batch MEC with no separation between the anode and cathode [30]. The average methane level was
0.9% when exposing the reactors to air in between batches [30] demonstrating that high hydrogen production and purity are possible in MECs without a membrane.

**2.1.3.4. Electrolyte effects**

The electrolyte in an MEC affects reactions and transport properties in the cathode, anode and bulk solution; therefore it is a very important variable in MEC design. The anode is covered with a biofilm of exoelectrogenic bacteria which grow best under neutral pH conditions. The lowest anode polarization resistance was obtained at pH 7 for an air cathode MFC as neutral pH is beneficial to bacterial activity [31]. The transport of H\(^+\) out of the biofilm can be a main limitation [32, 33]. If the concentration gradient of H\(^+\) in the biofilm is too large, the pH drops and causes bacterial inhibition causing a decrease in current generation. The use of buffers helps to stabilize pH and facilitate transport of the H\(^+\) protons from the biofilm to the cathode.

The HER overpotential in an MEC can be reduced by optimizing the type of buffer and its pKa at the expected operational pH. Buffers affect the cathodic HER in MECs by a weak acid catalytic effect [34]. Weak acids lower the cathode potential by facilitating HER catalysis depending on the weak acid’s activity. Concurrently, the conjugate bases facilitate the conduction of ionic current between electrodes and increases conductivity. An increase in buffer concentrations increases conductivity, reduces solution resistance and increases current density [24, 33]. The weak acid HER catalytic effect is dominant at lower pHs (pH 5-6) and the conductivity effect is dominant at higher pHs (pH 8-9) at the current densities where MECs typically operate (-4.5 to -2.5
log A/cm²) [34]. The greatest increase in weak acid activity is at 0.5-1.5 pH units below its pKa, therefore buffers with pKas slightly higher than 7 such as phosphate, tris(hydroxymethyl)aminomethane (Tris) and 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) are recommended for typical MECs [35].

The use of buffer has to be evaluated for the specific operating conditions. The buffer can be retained in the cathode chamber in MECs with membranes but it can result in pH gradients across the membrane. The addition of buffer to membraneless MECs may not be cost effective. Typical domestic wastewaters have an alkalinity of 50–200 mg/L, equivalent to 1-4 mM phosphate buffer. Proton transfer out of the biofilm will limit performance at these buffer strengths [4]. Therefore MEC designs have to maximize proton transfer such as flow through the anode and reduced electrode spacing [36]. Bicarbonate buffer is a cheaper alternative to phosphate buffer. MFCs with bicarbonate buffer produced more power than those with phosphate buffer at the same concentration due to the higher mass transfer coefficient of bicarbonate in water (1.34×10⁻⁵ cm²/s vs. 1.0×10⁻⁵ cm²/s at 30 °C) [33]. The buffering effect may not be as important in a continuous MEC compared to a batch MEC because the constant flux of higher pH electrolyte may facilitate transport of the protons from the biofilm.

2.1.4. MEC scalability and outlook

Electrohydrogenesis is a promising technology for sustainable energy production as it can produce hydrogen from wastewater and other organic matter such as cellulose and fermentation products, but the cost and ease to manufacture MECs need to improve.
The largest MECs reported so far had 3.3 L chambers and achieved hydrogen production rates up to 0.3 m$^3$/m$^3$ d ($E_{ap} = 1$ V) [28], compared to the maximum hydrogen production rate of 6.3 m$^3$/m$^3$ d with a lab-scale MEC reactor (50 mL chamber, 5 mL/d) ($E_{ap} = 1$ V) [29]. A much larger reactor designed to process 1,000 L/d of winery wastewater to make hydrogen for direct use in a hydrogen fuel cell is currently under evaluation [37]. Advances in the related field of MFCs contribute to advances in MECs. The largest MFC reported has a total working volume of 20 L (1,368 mL/day) and achieved a power density of 0.14 kW/m$^3$ [38] compared to the maximum achieved of 1 kW/m$^3$ in lab scale (6 mL chamber, 864 mL/d) [39]. Additional research on reactor designs, materials, operating conditions and our understanding of bacteria should further increase hydrogen yields and reduce costs to reach commercialization of MECs.

### 2.2. Glycerol Manufacture and Use

Glycerol ($C_3H_5(OH)_3$) is a colorless, odorless, viscous, nontoxic liquid. Glycerol has over 1,500 known end uses, including many applications in cosmetics, toiletries, personal care, drugs, tobacco, and food products. Glycerol forms the backbone of triglycerides and it is formed as a byproduct during soap, oleochemicals, and biodiesel production. It is subsequently concentrated and purified for commercial sale. Synthetic glycerol is petroleum-based. Its manufacture is no longer economically viable due to the surplus glycerol from biodiesel production which has caused prices to fall. Dow chemical is the only manufacturer in the U.S. that produces synthetic glycerol. Dow mainly
supplies the pharmaceutical market, which requires a purity of 99.7%+ and this purity cannot be reached economically from purifying the transesterification byproduct.

2.2.1. Glycerol production as a byproduct from biodiesel fuel production

Biodiesel is made via a transesterification process where triglycerides react with methanol in the presence of a catalyst to form mono-alkyl esters (biodiesel) and glycerol (Figure 2.2). The glycerol phase is denser than the biodiesel phase and can be gravity separated. The excess alcohol is removed and the mixture is neutralized. The final byproduct stream contains glycerol, unused catalyst, soaps, residual methanol, heavy metals and water [40].

\[
\begin{align*}
\text{Triglyceride} & \quad \text{Methanol} \quad \text{Fatty Acid Methyl Esters} \quad \text{Glycerol} \\
\text{CH}_2\text{-}O\text{-}C\text{-}R_1 & \quad 3\text{CH}_3\text{OH} \quad \text{catalyst} \quad \text{CH}_3\text{-}O\text{-}C\text{-}R_2 \\
\text{CH}_2\text{-}O\text{-}C\text{-}R_3 & \quad \text{CH}_3\text{-}O\text{-}C\text{-}R_2 \\
\end{align*}
\]

\text{Figure 2.2. Biodiesel production mechanism}

The glycerol byproduct can be sold as crude glycerol to refiners or used as animal feed or boiler fuel. When used as a fuel, high temperatures must be reached (>572°F) to prevent toxic acrolein fumes. The revenue for all these options is very small as the market
value for this byproduct is $0.035–0.05/lb minus transportation costs [41]. It is expected that this revenue will further decrease as biodiesel production continues to increase.

The glycerol byproduct can also be refined to pure glycerol. The traditional method to remove the salts from the biodiesel byproduct is to distill the glycerol in a flash separation process. This process is effective but requires significant capital, maintenance and utility costs. As biodiesel production increases, refined glycerol prices will continue to decrease and make the purification process uneconomical. Moreover, the current glycerol demand (216 million liters/yr) [41] is 5 times lower than the crude glycerol capacity (1,020 million liters of glycerol/yr, based on biodiesel plants reaching their maximum capacity of 10.2 billion liters/yr) [42]. This glycerol is therefore becoming a waste product unless alternative uses can be found.

2.2.2. Glycerol conversion to value-added products

Glycerol can be converted through catalytic and biological conversion processes into various value-added products. The conversion of glycerol can be broken into two types: the oxidation or reduction of glycerol into other compounds or the reaction of glycerol with other molecules to form new species. The oxidation or reduction of glycerol can produce compounds such as 1,3-propanediol, acrylic acid, propanol, allyl alcohol, acrolein, propylene glycol and propionic acid which have significant price differentials and large market capacity [41, 43]. Glycerol can also be broken down to produce hydrogen [44]. Reaction of glycerol with other compounds also holds great potential. Some examples are glycerol carbonate, glycidol, epichlorohydrin and quinoline [41, 45].
2.2.3. Glycerol conversion to hydrogen

Hydrogen is an attractive option as a clean, efficient and renewable energy carrier which could replace fossil fuels. Hydrogen can be produced from glycerol via biological and thermochemical methods.

2.2.3.1. Hydrogen from glycerol via fermentation

Hydrogen generation from glycerol via biological methods has only been attempted via dark fermentation. There are two main pathways for glycerol fermentation (Figure 2.3). One pathway is the oxidation of glycerol into glyceraldehyde 3-P and pyruvate. This pathway leads to formation of formate, lactate, succinate, propionate, ethanol, acetate and butyrate. The second pathway is the reduction of glycerol to 1,3-propanediol. The products formed and their ratios depend on the microbial community present [46].
Figure 2.3. Most important metabolic pathways during anaerobic fermentation of glucose and glycerol fermentation. The reactions indicated by the arrows labeled a-f are alternative pathways depending on the microorganism [Temudo MF, Poldermans R, Kleerebezem R, van Loosdrecht MCM. Glycerol Fermentation by (open) mixed cultures: a chemostat study. Biotechnol and Bioeng 2008; 100:1088-1098].

Glucose and glycerol share the same metabolic pathway from glyceraldehyde-3-phosphate to the final products. One difference is that the conversion of glycerol into pyruvate generates 2 mol of NADH (reduced nicotinamide adenine dinucleotide) while only 1 mol of NADH is involved in the case of glucose (per 3 carbons). If only acetate is produced during glycerol fermentation, 2 H₂ could be produced from 2 NADH and one additional H₂ from pyruvate to acetyl-CoA or formate, for a total of 3 H₂ per glycerol (1
H\textsubscript{2} per carbon). For glucose fermentation, only 2 H\textsubscript{2} are produced from 2 NADH with 2 additional H\textsubscript{2} from pyruvate to acetyl-CoA or formate, for a total of 4 H\textsubscript{2} per glucose (0.67 H\textsubscript{2} per carbon). Thus the theoretical maximum hydrogen yield for glycerol (3 mol H\textsubscript{2}/mol glycerol) is higher than that of glucose (4 mol H\textsubscript{2}/mol glucose or 2 mol H\textsubscript{2}/3C mol) with the production of acetate. Another difference between glucose and glycerol fermentation is that formation of 1,3-propanediol does not occur during glucose fermentation. This pathway regenerates NAD from NADH but requires H\textsubscript{2}.

Most of the glycerol fermentation studies have been performed with pure, refined glycerol (P-glycerol) and pure cultures, mainly \textit{Enterobacter}, \textit{Escherichia coli}, \textit{Clostridium} and \textit{Klebsiella}, with yields in the range of 0-1.05 mol-H\textsubscript{2}/mol glycerol [47-54]. Fermentation of glycerol from the biodiesel process (B-glycerol) with pure cultures yielded 0.69-1.12 mol-H\textsubscript{2}/mol glycerol [47, 55]. The main coproducts were 1,3-propanediol, ethanol, acetate or butyrate depending on the organism and test conditions.

The use of mixed cultures allows for lower processing costs due to working under non-sterile conditions. Mixed culture inocula can be pretreated to select for a particular bacterial community to enhance hydrogen production and decrease hydrogen consumption by other microorganisms such as methanogens. Most pretreatments are based on the bacterial stress response (BSR) of spore forming hydrogen producing bacteria. Spore-forming bacteria produce spores that can tolerate extreme conditions when the environment becomes stressed (i.e. high temperatures, desiccation, nutrient limitation and presence of adverse chemicals) [56]. Heat-treatment (\geq 100 \degree C, 15 min-2 hrs) has been used to effectively pretreat soil, digested sludge, activated sludge, cow
dung, compost and river sediments to convert glucose and other substrates to hydrogen [57-59].

Acid and basic pretreatment methods are based on the inhibition of methanogenic activity at pHs below 6.3 or above 7.8. Alkaline pretreated sludge (pH 12, 30 min) increased yields to 16.6 ml from 9.1 ml H₂ from sewage sludge [60]. Acid pretreatment (pH 2, 2-4 hr) of cattle manure sludge gave the highest hydrogen yield from glucose, compared to wet heat treatment (boiled for 20 min), addition of a methanogen inhibitor, freeze/thaw (-10 °C, 24 hr), dry heat treatment (105 °C, 2 hr) and no treatment [56]. Methanogens are strict anaerobes, thus their activity can be inhibited by aeration. An aeration pretreatment (aeration with dissolved oxygen <0.5 mg/L and glucose) gave a higher yield than heat-shock (121 °C, 20 min), acid (pH 3, 24 h) and alkaline (pH 11, 24 hr) pretreatments from sludge inoculum using glucose as the substrate [61]. However, it is not clear if there was residual substrate in the inoculum after the aeration pretreatment or if the addition of substrate during the pretreatment stage helped to acclimatize the culture. Exposure of the inoculum (sludge) to electrical current (3.0-4.5 V) has also been used as another pretreatment method to screen for hydrogen producing bacteria [59].

The dominant bacteria are different depending on the inoculum, substrate, test conditions and pretreatment. The microbial community was mainly *Clostridium spp* after heat-shock, acid and alkaline treatments [61, 62]. *Eubacterium multiforme* and *Penibacillus polymixa*, which grow better at higher pHs, were more prominent in another alkaline treatment study [60]. *Ethanoligenes harbinens* were dominant after aeration treatment [61].
Pretreatments such as heat treatment are not always effective. Homoacetogens and sulfate-reducing bacteria can also cause hydrogen consumption. Some homoacetogens (i.e. *Clostridium aceticum* and *Clostridium autotrophicum*) and sulfur reducing bacteria are spore formers which can survive pretreatments based on BSR [56]. Also, bacterial responses are different for different substrates. Heat treatment of cow dung culture was effective in improving hydrogen production from hexose, but not from cellulose or xylose [63].

The fermentation of P-glycerol with untreated mixed cultures yielded a low 0.05 mol-H$_2$/mol glycerol in a chemostat reactor [46]. There were no studies which included pretreatment of mixed cultures for glycerol fermentation published before submitting our study for publication (Chapter 3). Since then, fermentation with a pretreated (boiled and frozen) digested sludge achieved 0.41 mol-H$_2$/mol glycerol with P-glycerol and 0.71 mol-H$_2$/mol glycerol with B-glycerol by varying inoculum and substrate concentration, but there was no explanation provided for the pretreatment choice [64].

### 2.2.3.2. Hydrogen from glycerol via MEC

Hydrogen production from glycerol at pH 7 theoretically needs very little energy to proceed ($E_{cell}^{\circ}$ = - 0.011V) based on equations 2.12 - 2.18. However, if glycerol degradation proceeded via fermentation to acetate and other byproducts, more energy would be needed for hydrogen formation ($E_{cell}^{\circ}$ = - 0.114V with acetate).

The anodic half-reaction for glycerol oxidation [65] is:

\[
3 \text{CO}_2 + 14 \text{H}^+ + 14 \text{e}^- = \text{C}_3\text{H}_8\text{O}_3 + 3 \text{H}_2\text{O} \quad \Delta G^{\circ} = 544 \text{ kJ} \quad (2.12)
\]
The resulting Gibbs free energy ($\Delta G^\circ$) is calculated from the standard Gibbs free energy ($\Delta G^\circ'$).

$$\Delta G^\circ = \Delta G^\circ' - RT \ln \frac{1}{[H^+]}$$  \hspace{1cm} (2.13)

$$\Delta G^\circ = 544,000 \text{J} - 8.31 \text{J/molK} \times 298.15 \text{K} \ln \frac{1}{[10^{-7}]} = -14,783 \text{J/mol}$$  \hspace{1cm} (2.14)

The standard potential $E^\circ$ for the anodic reaction (25°C, 1 mol/L, 1 atm) is:

$$E^\circ_{an} = \frac{-\Delta G^\circ}{nF} = \frac{(-14,783 \text{J/mol})}{14 \times 96,485 \text{C/mol}} = 0.011 \text{V}$$  \hspace{1cm} (2.15)

The anodic cell potential $E_{an}$ is calculated from $E^\circ$ using the Nernst equation (equation 2.8).

$$E_{an} = 0.011 \text{V} - \frac{8.31 \text{J/molK} \times 298.15 \text{K}}{14 \times 96,485 \text{C/mol}} \ln \frac{1}{[10^{-7}]} = -0.403 \text{V}$$  \hspace{1cm} (2.17)

The cell electromotive force ($E_{cell}$) is calculated from the difference between the cathode potential and the anode potential (equations 2.9 and 2.17).

$$E_{cell} = E_{cat} - E_{an} = (-0.414 \text{V}) - (-0.403 \text{V}) = -0.011 \text{V}$$  \hspace{1cm} (2.18)

B-glycerol was used in a two-chamber MEC with a mediator, but the maximum yield was only 0.77 mol-H$_2$/mol-glycerol [55]. By the time of publication (Chapter 4), glycerol had not yet been used in a mediatorless or membraneless MEC. A continuous membraneless MEC later achieved a hydrogen production rate of 0.6 m$^3$/m$^3$ d and a yield of 5.4 mol-H$_2$/mol glycerol at applied 1V [66]. This was a lower hydrogen production rate but higher yield than our study (2.0 m$^3$/m$^3$ d, 3.9 mol-H$_2$/mol glycerol) done at a lower applied voltage of 0.9 V. B-glycerol was not tested in that study.
2.2.3.3. Hydrogen from glycerol via thermochemical methods

The thermochemical methods studied for hydrogen production from glycerol include steam reforming, gasification, autothermal reforming, aqueous-phase reforming (APR) and supercritical water reforming [44]. The pyrolitic decomposition of glycerol can almost reach its theoretical maximum of 7 mol-H$_2$/mol glycerol but this technology requires high process temperatures, leading to high energy losses. The few thermochemical studies that used B-glycerol indicated that the presence of impurities caused catalyst deactivation (mainly Pt based catalysts) and impeded performance of the catalyst [44].

Recently, the electrochemical reforming of glycerol into hydrogen was achieved in a proton exchange membrane (PEM) electrolysis cell with a maximum hydrogen production of 10 m$^3$/m$^3$ d at a cell voltage of 0.7 V, but poisoning of the anodic catalyst resulted in performance loss [67]. The PEM electrolysis cell is very similar to an MEC where the organic compound is oxidized at the anode to carbon dioxide, protons and electrons. The protons, just like in an MEC, combine with electrons at the cathode and release hydrogen gas. An external power source provides the potential to drive this reaction. Unlike an MEC, microbes do not contribute to the external potential to overcome the energy requirement, a catalyst needs to catalyze the oxidation reaction at the anode and the reactor is maintained at higher temperatures (50-100 °C).
2.2.4. Future outlook

U.S. consumption of diesel fuel is 52.4 billion gallons per year [68]. If biodiesel is mandated as an alternative fuel, 0.05 billion gallons of glycerol will be produced for every 1% of diesel fuel replaced. For example, if biodiesel makes up 2% of petroleum diesel, over 0.1 billion gallons of glycerol will be produced as a byproduct, thus exceeding the stable demand of 0.06 billion gallons of glycerol [41]. In Europe, the EU directives predict that in 2020 the consumption of biodiesel will reach 20% of all used fuels [64]. New uses for glycerol are needed to help stabilize both biodiesel and glycerol product pricing, of which hydrogen production via electrohydrogenesis is a promising alternative.

2.3. Catalyst Design and Evaluation

A catalyst is a substance that increases the rate of a chemical reaction without itself suffering any permanent chemical change. The main purpose of a catalyst is to drive the desired reaction at a high rate (i.e. high current density) and a low overpotential. An ideal catalyst is efficient (high rate for the desired reaction), selective (low rate for side reactions) and stable.

2.3.1. Rate of reaction

The rate of reaction \( k \) is calculated using the Arrhenius equation [69, 70]:

\[
k = A e^{-E_a / RT}
\]  

(2.19)
where $A$ is the frequency factor, $E_a$ (J) is the activation energy and $T$ (K) is the temperature. The frequency factor is a function of the frequency of molecular collisions and the probability of the molecules colliding in a certain orientation. The units of the frequency factor are identical to those of the rate constant and will vary depending on the order of the reaction (i.e. if the reaction is first order they have units s$^{-1}$).

The activation energy $E_a$ is the minimum amount of energy needed for a reaction to proceed. A catalyst can lower the activation energy and increase the rate of reaction by providing an alternative reaction mechanism (Figure 2.4). The final product and overall thermodynamics typically do not change.

![Generic potential energy diagram showing the effect of a catalyst in a chemical reaction X + Y → Z.](image)

**Figure 2.4.** Generic potential energy diagram showing the effect of a catalyst in a chemical reaction $X + Y \rightarrow Z$. 
2.3.2. Butler-Volmer model

The Butler-Volmer model describes how the electrical current on an electrode depends on the electrode potential \([69, 70]\). The equation is valid when the electrode reaction is controlled by electrical charge transfer at the electrode, but not by the mass transfer. The Butler-Volmer equation considers both forward and reverse rates of an equilibrium reaction (equation 2.20)

\[
J = J_o \left[ e^{\frac{\alpha_c n F (E - E_o)}{RT}} - e^{\frac{\alpha_a n F (E - E_o)}{RT}} \right]
\]  

(2.20)

where \(J\) is the electrode current (A), \(J_o\) is the exchange current density (A/m\(^2\)), \(E\) is the electrode potential (V), \(E_o\) is the midpoint potential (V), \((E - E_o)\) is the overpotential of the working electrode, \(n\) is the number of electrons for the reaction (2 for HER), \(\alpha_c\) is the charge transfer coefficient for the cathodic current, \(\alpha_a\) is the charge transfer coefficient for the anodic current.

The Butler-Volmer model can be simplified for the HER reaction (reduction) (equation 2.21) assuming the reverse current is negligible. The efficiency of a HER catalyst can therefore be improved by raising the exchange current density \((J_o)\) or the charge transfer coefficient for the cathodic reaction \((\alpha_c)\).

\[
\log J = \log J_o + \frac{\alpha_c n F}{2.303RT} (E - E_o)
\]  

(2.21)
2.3.3. Volcano plot for HER

Volcano plots represent trends of kinetic behavior of heterogeneous processes as a function of a particular surface metal property which exhibit a maximum (volcano shaped). Volcano plots are based on the Sabatier principle which states that the interactions between the catalyst and the substrate should be neither too strong nor too weak. If the interaction is too weak, the substrate will fail to bind to the catalyst and the reaction will not take place. If the interaction is too strong, the catalyst gets blocked by substrate or product fails to dissociate.

The volcano plot for the HER is obtained by plotting the catalytic activity (log of the exchange current density) as a function of the hydrogen-metal bond strength for a material (Figure 2.5). The maximum corresponds to the free Gibbs energy of adsorption of H ($\Delta G_H$) equal to zero [18]. The highest reaction rate for HER can be obtained with platinum as this metal is at the top of volcano curve. Metals to the left of platinum (Au, Ni, Co, Fe, Cu, Ag, etc.) have slower reaction rates because the rate of adsorption is slow and rate-limiting. Metals to the right of platinum (Rh, Ir, Re, W, Mo, Ti, Nb, Ta) have slower reaction rates as desorption is the rate limiting step.
Figure 2.5. Volcano plot for log io values for the HER as a function of M-H bond energy
[Conway BE, Jerkiewicz G. Nature of electrosorbed H and its relation to metal
dependence of catalysis in cathodic H2 evolution. Solid state ionics 2002; 150: 93-103].

2.3.4. Potential-pH (Pourbaix) Diagram

The stability of a metal in a given environment depends on a multitude of factors
that may vary with pH and potential of that environment. Pourbaix diagrams or potential-
pH (E-pH) are thermodynamic charts based on the Nernst equation (equation 2.8) which
indicate the state with the lowest free energy of a system as a function of pH and
electrochemical potential. Each line of a Pourbaix diagram represents conditions of
thermodynamic equilibrium for a particular reaction. Corrosion rates cannot be predicted
from Pourbaix diagrams.
Pourbaix diagrams can be constructed for many metallic elements or alloys and for many environments. Nickel and iron Pourbaix diagrams [71, 72, 73] are illustrated in this section as these metals were used during this research. Typically, the thermodynamic lines for water are depicted in Pourbaix diagrams as dashed lines. Above the top dashed line (starts around 1.4 V at pH -2) oxygen is evolved according to equation 2.22.

\[
\text{H}_2\text{O} \rightarrow \frac{1}{2} \text{O}_2 + 2 \text{H}^+ + 2e^- \quad E_0=1.228\text{V} \quad (2.22)
\]

Below the bottom dashed line (starts around 0.1 V at pH -2) hydrogen is evolved according to equation 2.23.

\[
2\text{H}^+ + 2e^- \rightarrow \text{H}_2 \quad E_0=0.000\text{V} \quad (2.23)
\]

Water is stable between these two lines.

Nickel is stable at voltages < -0.4 V and at neutral to acidic conditions (Figure 2.6). Ni is not stable and can exist in either Ni\(^{2+}\) or Ni(OH)\(_2\) states at voltages -0.5 to 0.5 V and pH 7. In alkaline conditions and voltages -1 V or higher, the stable substances are Ni(OH)\(_2\), HNiO\(_5\)\(^-\), Ni\(_3\)O\(_4\), Ni\(_2\)O\(_3\) and NiO\(_2\) depending on the voltage and the pH. Typical MEC conditions are neutral pH and cathode potentials of -0.4 to -1.0 V, therefore nickel should be stable as an MEC cathode according to Pourbaix diagrams.
Iron is the basis for steel. It can exist in two oxidation states (+2 or +3). The Pourbaix diagram for iron in the presence of water or humid environments at 25 °C takes into account all possible reactions associated with iron in aqueous conditions (Figure 2.7). The Pourbaix diagram for iron in dry environments only includes drier forms of corrosion products such as Fe$_3$O$_4$ or Fe$_2$O$_3$ (Figure 2.8). Iron is stable at potentials below approximately -0.5 V in neutral or acidic environments. At potentials more positive than -0.6 V and at pH values below 9, iron will corrode to ferrous ion (Fe$^{2+}$). The corrosion of iron in other aqueous conditions produces ferric ions (Fe$^{3+}$), ferric hydroxide (Fe(OH)$_3$), and ferrous hydroxide (Fe(OH)$_2$). The solid corrosion products in a dry environment are ferric oxide (Fe$_2$O$_3$) and magnetite (Fe$_3$O$_4$) (Figure 2.8). At very alkaline conditions, complex HFeO$_2^-$ ions will form at certain potentials in wet or dry environments.
Figure 2.7. Pourbaix diagram for iron with wet corrosion products. [Pourbaix M. Atlas of electrochemical equilibria in aqueous solutions. NACE Int. Houston, TX 1974].

Figure 2.8. Pourbaix diagram for iron considering Fe, Fe$_3$O$_4$ and Fe$_2$O$_3$ as the dry corrosion products [Revie RW, Uhlig HH. Corrosion and corrosion control. John Wiley & sons. Hoboken, NJ 2008].
2.3.5. Electrochemical testing

Electrochemical tests can be used to evaluate the performance of experimental materials (including catalysts) for MEC electrodes. They can also be used to determine the electrochemical activity of microbial strains, redox potentials, potential losses and resistances. A three-electrode setup is used to study the behavior of an individual electrode. This setup consists of a working electrode, a reference electrode and a counter electrode. The overall MEC performance can be assessed using a two electrode setup (no reference electrode). The different electrochemical techniques that can be used for MEC evaluation are described below.

2.3.5.1. Cyclic voltammetry scan

In cyclic voltammetry scans (CV) the potential of the working electrode is varied at a certain scan rate between two potentials using forward and backward sweeps. Typically one reduction peak and one oxidation peak are observed. Multiple peaks may appear due to parallel mechanisms or the presence of different redox species. CV has been used in MEC studies to evaluate the performance of catalysts [23, 74]. The performance of catalysts can be evaluated in terms of the voltage needed at a particular current density, the current density reached at a particular applied voltage and the slope of the voltammogram (Figures 2.9 and 2.10). CV is a quick and simple technique but the results can be affected by several factors such as the electrode surface pretreatment, the rate of electron transfer and the chemical and biological species present (Zhao et al 2009).
Figure 2.9. Examples of how CV can be used to distinguish efficiency of different cathodes. (a) A lower voltage ($V_e$) is needed by material “A” to initiate current generation, thus this material performs better than “B”. (b) material “C” has a steeper slope and generates more current at a given voltage, thus the performance of material “C” is better than “D”. [Cheng S, Logan BE. Evaluation of catalysts and membranes for high yield biohydrogen production via electrohydrogenesis in microbial electrolysis cells (MECs). Water Sci & Technol 2008; 58:853-857].

Figure 2.10. CV with different cathode catalysts for hydrogen evolution, where Pt was the most effective catalyst with lower values of $V_e$ (V) and higher values of $V_h$ (mA/cm²-V) [Cheng S, Logan BE. Evaluation of catalysts and membranes for high yield biohydrogen production via electrohydrogenesis in microbial electrolysis cells (MECs). Water Sci & Technol 2008; 58:853-857].
2.3.5.2. Linear voltammetry scan

During linear voltammetry (LV) the current is measured while the voltage is varied at a constant rate. It is similar to CV but the scan only proceeds in one direction: at the appropriate positive voltage values for oxygen evolution and at the appropriate negative voltage values for hydrogen evolution. Typically, the first LV scan discharges and polarizes the catalyst. Subsequent scans produce a more accurate relationship of current density with respect to potential. LV has been used to assess electrolyte effects on hydrogen evolution kinetics for MECs [34].

2.3.5.3. Polarization curves

Polarization curves are plots of the electrode potential (or MEC cell potential) as a function of current density. The curves can be obtained by several methods including: constant resistance discharge (measure current and voltage at different resistances), potentiodynamic polarization (measure current at a defined voltage scan rate), galvanodynamic polarization (measure voltage at a defined current scan rate), galvanostatic discharge (measure voltage while controlling current) and potentiostatic discharge (measure current while controlling voltage) [75]. The evaluation of polarization curves is the same as described under CV curves (section 2.3.5.1). Polarization curves have been used in MECs to test different buffers [35], to study the performance of the biofilm in a biocathode [19, 20] and to determine the kinetic parameters of an anodic biofilm [76].
2.3.5.4. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is used to study voltage loss phenomena in the solution and at the interfaces. Nyquist plots show the impedance vector at a particular frequency. Bode plots show magnitude and phase angle of the impedance vector versus frequency. EIS can be used to obtain information such as kinetic parameters, determination of reaction mechanisms, electrolyte and electrode conductivities and biofilm behavior. EIS has been used to evaluate the ohmic resistance of different types of ion exchange membranes in MECs (Figure 2.11) [28]. While EIS can provide very useful and complete information, the EIS data collection and analysis is very complex and interpretation of results needs to be done very carefully.

Figure 2.11. EIS: Nyquist plots limited to the determination of ohmic resistance of MEC systems with (a) cation exchange membrane and (b) anion exchange membrane [Rozendal RA, Hamelers HVM, Molenkamp RJ, Buisman CJN. Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membranes. Water Res 2007; 41:1984-1994].
2.3.5.5. Current interrupt

Current interrupt method is used to measure the internal ohmic resistance by interrupting the current flow and observing the voltage transients. The ohmic overpotential is a near instantaneous process, so ohmic losses can be easily separated from other voltage loss phenomena.

2.3.5.6. Differential pulse voltammetry

Differential pulse voltammetry is a voltammetric technique similar to CV and LSV but has an improved sensitivity due to enhanced discrimination of Faradaic currents (electron transfer to and from the electrode).

2.3.5.7. Chronoamperometry and chronopotentiometry

Chronoamperometry is the study of current as a function of time of an electrode operating at a constant potential. Chronopotentiometry is the study of potential as a function of time of an electrode operating at constant current. They can be used to study the effect of applied potentials on electrode materials and biofilms.

2.3.5.8. Rotating disk electrode and rotating ring disk electrode

Rotating disk electrode (RDE) and rotating ring disk electrode (RRDE) are hydrodynamic techniques where mass transfer is carefully controlled. They are used to
study kinetic parameters of electron transfer and detailed probing of electrochemical reaction mechanisms. They are mainly used in the evaluation of catalysts or electrode performances. They may not be suitable for probing electrochemical behavior of biofilms as biofilms are fragile and can be destroyed by the high speed rotation.

2.3.6. Electrochemical Summary

Understanding of the principles of electrochemical reactions combined with electrochemical testing of experimental electrodes is important to continue making MEC advancements. A detailed study of the electrode materials under all conditions during MEC operation is still needed to consider other factors such as microbial effects.
2.4. References


[38] Dekker A, Ter Heijne A, Saakes M, Hamelers HVM, Buisman CJN. Analysis and improvement of a scaled-up and stacked microbial fuel cell. Environ Sci Technol 2009; Available online DOI: 10.1021/es901939r


Chapter 3

Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures

Abstract

The conversion of glycerol into high value products, such as hydrogen gas and 1,3-propanediol (PD), was examined using anaerobic fermentation with heat-treated mixed cultures. Glycerol fermentation produced 0.28 mol-H₂/mol-glycerol (72 ml-H₂/g-COD) and 0.69 mol-PD/mol-glycerol. Glucose fermentation using the same mixed cultures produced more hydrogen gas (1.06 mol-H₂/mol-glucose) but no PD. Changing the source of inoculum affected gas production likely due to prior acclimation of bacteria to this type of substrate. Fermentation of the glycerol produced from biodiesel fuel production (70% glycerol content) produced 0.31 mol-H₂/mol-glycerol (43 ml H₂/g-COD) and 0.59 mol-PD/mol-glycerol. These are the highest yields yet reported for both hydrogen and 1,3-propanediol production from pure glycerol and the glycerol byproduct from biodiesel fuel production by fermentation using mixed cultures. These results

1 Material presented in this chapter was published in the following paper: Selemba PA, Perez JM, Lloyd WA, Logan BE. Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures. Biotechnol Bioeng 2009; 104:1098-1106.
demonstrate that production of biodiesel can be combined with production of hydrogen and 1,3-propanediol for maximum utilization of resources and minimization of waste.
3.1. Introduction

Glycerol is a major byproduct of biodiesel production, with 1 gallon of glycerol produced for every 10 gallons of biodiesel. According to the National Biodiesel Board, there were 176 biodiesel plants in operation in the U.S. with an annual production capacity of 9.8 billion liters/yr (http://www.biodiesel.org). If plants operate to capacity, this would produce 980 million liters of glycerol/yr, compared to the current U.S. demand of 216 million liters/yr [1]. The increased biodiesel production has already resulted in glycerol plant closures and substantially lower glycerol prices. In addition, glycerol could be considered a hazardous waste due to its low flash point (<140 °F). New uses for glycerol are needed to help stabilize biodiesel and glycerol product pricing and supply, and to avoid accumulation of a material that can have a negative environmental impact.

Glycerol can be used to produce hydrogen via anaerobic fermentation. In recent years hydrogen has gained considerable interest as a potential energy carrier that has no emissions in fuel cells other than water. The main advantage of anaerobic fermentation for hydrogen production over other methods, such as electrolytic or thermochemical processes, is that fermentation is less energy intensive and therefore less expensive [2]. On a per-carbon basis, anaerobic glycerol fermentation can theoretically produce hydrogen at yields equal to or higher than those possible with glucose due to similarities in metabolic pathways and thermodynamics. If glycerol is degraded to the same initial intermediate (glyceraldehyde-3-P) as glucose, then from this point the same fermentation pathways could be used to produce hydrogen and other end products [3]. With glycerol,
one mole of NADH (nicotinamide adenine dinucleotide, reduced form) is generated prior to glyceraldehyde-3-P, and thus the hydrogen yield for glycerol could theoretically be higher than that of glucose. Assuming only acetate is produced during glycerol fermentation, 2 H₂ could be produced from 2 NADH and one additional H₂ from pyruvate to acetyl-CoA or formate, for a total of 3 H₂ per glycerol or 1 H₂ on a per carbon basis. For glucose fermentation, only 2 H₂ are produced from 2 NADH with 2 additional H₂ from pyruvate to acetyl-CoA or formate, for a total of 4 H₂ per glucose or 0.67 H₂ on a per carbon basis. In addition, the standard Gibbs free energy for glycerol and glucose oxidation half-reactions have similar values (ΔG°'glucose = 41.35 kJ/e-eq, ΔG°'glycerol = 38.88 kJ/e-eq).

The unique aspect of glycerol versus glucose fermentation is that during glycerol fermentation 1,3-propanediol (PD) is produced as a method of NADH regeneration for respiratory balance. PD is a valuable chemical mainly used as monomer for plastics with special characteristics such as superior biodegradability, such as polytrimethylene terephthalate (PTT). It is also used in resins, engine coolants, mortars and inks. The market for PD is over 100 million pounds per year and growing rapidly [4]. With glucose fermentation, butyrate is often the main end product for NADH regeneration. The production of one mole of PD requires one mole of H₂, and thus its production adversely affects H₂ gas yields. Most prior research on anaerobic glycerol fermentation has been conducted with pure cultures. Maximum yields for hydrogen of 1.05 mol-H₂/mol-glycerol were obtained with Enterobacter aerogenes, with low yields for PD (0.06 mol-PD/mol-glycerol) [5]. Conversely, a maximum yield of 0.69 mol-PD/mol-glycerol was obtained with Clostridium butyricum, but there was no hydrogen production [6].
High yields of hydrogen gas production have been achieved using mixed cultures for glucose fermentation, but so far there has been only one study of hydrogen production using glycerol and yields were low. A mixed culture from a distillery wastewater and a potato processing plant was used to produce 0.05 mol-H2/mol and 0.14 mol-PD/mol [3]. Inocula used for hydrogen production with glucose are usually pre-treated to increase yields through the inhibition of hydrogen-consuming microorganisms such as methanogens while preserving the activity of hydrogen producing bacteria [7]. Heat treatment is the most common pretreatment method and it may be easily and inexpensively achieved in practice [2]. Other pretreatment methods include ultrasonication, acidification, basification, sterilization and freezing/thawing [8-11]. The method used for inoculum pretreatment for glucose fermentation can produce different hydrogen yields and fermentation end products [12]. It is not known how the use of these different pre-treatment methods might affect hydrogen and PD production from glycerol fermentation.

In this study, glycerol fermentation was compared to glucose fermentation for hydrogen production using heat and other pre-treatment methods with inocula from four different sources (tomato soil, wheat soil, compost, and sludge). Two types of glycerol sources were examined: pure glycerol (P-glycerol) and the glycerol byproduct from biodiesel production (B-glycerol). The main limitation in using B-glycerol is that it contains methanol, excess catalyst (sodium hydroxide), and other impurities that can inhibit hydrogen production and cell growth. While B-glycerol can be purified by neutralization, separation of unreacted methanol and splitting of soaps, or by distillation, these processes can require high capital and operating costs. Thus, we examined the
direct use of B-glycerol for hydrogen and PD production without any purification methods.

3.2. Materials and methods

3.2.1. Substrates

P-glycerol (ultrapure) was obtained from MP Biomedicals, LLC (Solon, OH) and glucose (D-glucose, anhydrous) was obtained from J. T. Baker (Phillipsburg, NJ). B-glycerol had a chemical oxygen demand (COD) of 1300 ± 100 mg/L (69.5% glycerol content, analyzed by HPLC) and was the byproduct of transesterification of soybean oil used to make biodiesel fuel (Nittany Biodiesel, State College, PA).

3.2.2. Inocula

Tomato soil was obtained from pots from the Penn State University greenhouse. Wheat soil was taken from soil at a depth of 20 cm from the Pennsylvania State University Farms. Compost (dewatered sludge) and sludge (secondary digester) were obtained from the State College Wastewater Treatment facility. These four inocula were heat treated using slightly different techniques. Soils were placed on an aluminum pan to a depth of 1 cm and heated at 105 °C for 2 hours. The wheat soil was either used without any pre-treatment, or subjected to one of four alternate pretreatment methods: sterilization by autoclaving for 30 minutes; acidification by stirring 0.1 M HCl solution for 30 minutes, followed by a decanting step to remove the excess liquid; alkali
pretreatment by stirring in 4 M NaOH for 30 minutes with a decanting step; or treated by
a freeze/thaw process by storing the soil at -20°C for 24 hours, and thawing for 12 hours
(25 °C). All soil samples were dried overnight at 30 °C (except heat treated samples),
crushed with a mortar and pestle, and sieved through a #45 mesh (354 µm pore size). All
samples were stored in sealed containers at 4°C prior to use.

3.2.3. Experimental Procedure

Experiments were conducted using 500 mL serum glass bottles filled with 250
mL of solution and the inoculum (1 g/L), except for the experiments with different
inocula sources where 300 mL serum glass bottles and 250 mL of solution were used.
Substrates (3 g/L) were dissolved in 70 mM 2-(N-morpholino)ethanesulfonic acid
monohydrate (MES) buffer and nutrient solution (0.31 g/L NH₄Cl, 0.13 g/L KCl, trace
vitamins and minerals) at pH 6.2 [13] except as noted. To examine the effect of the pH
and a different buffer, one experiment was done using a 50 mM phosphate buffer (4.58
g/L Na₂HPO₄ and 2.45 g/L NaH₂PO₄·H₂O; pH = 7.0) nutrient solution [2, 14]. Solutions
in the bottles were sparged with ultra high purity nitrogen for five minutes prior to
sealing the bottles and placing them on a stirrer in a temperature controlled room (30 °C).
In some tests, the solution in the bottle at the end of the fermentation cycle (25 mL) was
used as the only inoculum for a subsequent fermentation test with 225 mL of fresh buffer.
3.2.4. Analysis

Gas production was measured using a respirometer (AER-200, Challenge Technology, AZ). Gas leaving the respirometer was collected in gas bags (250 mL capacity, Cali-5 bond, Calibrated Instruments Inc., NY). The gas composition in the bottle headspace and gas bags was analyzed using two gas chromatographs (models 8610B and 310, SRI Instruments, CA), both equipped with Alltech Molesieve 5A 80/100 stainless steel-tubing columns and thermal conductivity detectors (TCDs). Argon was used as the carrier gas for H₂, O₂, N₂ and CH₄ analysis, and helium was used as the carrier gas for CO₂ analysis.

Liquid product composition was determined by high performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan) using an Aminex HPX-87H column (Bio-Rad Laboratories, CA), a 4 mM H₂SO₄ mobile phase (0.5 mL/min, 45 °C), and ultraviolet (UV, 210 nm) and refractive index detectors. The samples were filtered through a 0.2 µm polyethersulfone membrane prior to analysis. The chemicals measured included: acetate, butyrate, butanol, ethanol, glycerol, glucose, lactate, propionate, succinate, 2,3-butanediol, 1,3-propanediol and 1,2-propanediol. The diols and the sugars were only detected by refractive index, while the organic acids and alcohols were detected by both refractive index and ultraviolet detectors. Organic acids and alcohol concentrations were verified by gas chromatography (GC) (6890N Agilent, CA) by acidifying the samples with 50 µl of 50% formic acid per 1 ml of sample, and using a CA-FFAP 30 m × 0.32 mm column (Chromatography Associates, PA), FID detector, and
a helium carrier gas with a temperature profile of 60 °C to 240 °C over 10 minutes. GC samples were also filtered through a 0.2 µm polyethersulfone membrane prior to analysis.

Cell numbers were measured using the epifluorescence microscopic standard method [15]. Dry cell weights were calculated using 0.2 µm polycarbonate filters before and after filtration and heating at 105 °C overnight.

3.2.5. Calculations

A Coulombic balance was done on the solution constituents following the method of Huang and Logan [16] to assess the fate of electrons during the fermentation process. The total Coulombs at the beginning of the experiment are:

\[ C_{x0} = n_s b_s F \]  

(1)

where \( n_s \) is the number of moles of substrate, \( b_s \) is the moles of electrons per mole of substrate calculated from the half-cell reaction (\( b_{glucose} = 24 \), \( b_{glycerol} = 14 \)), and \( F \) is Faraday’s constant (\( F = 96,484.3 \) C/mol). Once fermentation begins, the total Coulombs are calculated as:

\[ C_I = C_I + C_S + C_B + C_L \]  

(2)

where \( C_I \) are the Coulombs calculated from the measured intermediates, \( C_S \) the Coulombs calculated from substrate remaining in the medium, \( C_B \) are the Coulombs in the produced biomass, and \( C_L \) are the Coulombs lost during the process (i.e. respiration). The Coulombs in biomass were calculated on the basis of COD as:

\[ C_B = \frac{F \rho_{O2} \Delta COD_{cell}}{M_{O2}} \]  

(3)
where \( b_{eO2} = 4 \) is the number of electrons exchanged per mole of oxygen, \( M_{O2} = 32 \) g/mol the molecular weight of oxygen and \( \Delta COD_{cell} \) is the change in the COD estimated from the change in dry cell weight using the conversion formula of 1.39 COD/g-DW [17].

A modified Gompertz equation [18] was used to fit the gas production for each cycle.

\[
H = P \exp \left\{ - \exp \left[ \frac{R_m}{P} (\lambda - t) + 1 \right] \right\}
\]

\( H \) is the cumulative gas production (mL), \( \lambda \) is the lag-phase time (h), \( P \) is the gas upper asymptote (maximum gas production) (mL), \( R_m \) is the production rate (mL/h), and \( t \) is the incubation time (h). The data was fitted using the Solver function in Microsoft Excel while minimizing the sum of the squared errors.

3.3. Results

3.3.1. Glycerol and glucose fermentation

Total gas production with P-glycerol was 133 ± 18 mL, resulting in a hydrogen yield of 0.28 ± 0.01 mol-H\(_2\)/mol for the first batch cycle with a heat-treated wheat soil (Figure 3.1 A). Total gas production with B-glycerol was 71 ± 7 mL, resulting in a hydrogen yield of 0.31 ± 0.01 mol-H\(_2\)/mol (adjusted to 70% glycerol, based on HPLC analysis of B-glycerol). These results are both lower than those with glucose of 296 ± 20 mL, for a hydrogen yield of 1.06 ± 0.15 mol-H\(_2\)/mol at the same mass loading (COD basis) (Figure 3.1 B). On a three-carbon basis, the yield for P-glycerol (0.28 ± 0.01 mol-
H$_2$/mol-C$_3$) was about half that of glucose (0.53 ± 0.07 mol-H$_2$/mol-C$_3$). Hydrogen gas concentrations were higher for B-glycerol (61% H$_2$, with balance CO$_2$) than for either P-glycerol (53% H$_2$) or glucose (54% H$_2$). The gas production lag time for P-glycerol and B-glycerol (~18 hours) was almost three times longer than for glucose (~6.5 hours).

**Figure 3.1.** Gas production from glucose, pure glycerol (P-glycerol), or glycerol byproduct from biodiesel production (B-glycerol): (A) total gas production; (B) hydrogen yield.
Subsequent hydrogen production cycles were performed using an inoculum from the previous experiment (10% serial transfer) to try to reduce the lag time and increase hydrogen yields. Lag time decreased from 7-18 to 2-5 hours for all three substrates. There were slight increases in hydrogen gas concentrations for P-glycerol (53-57%) and B-glycerol (59-61%), likely due to further acclimatization of the bacteria to the substrate. For glucose, however, the concentration of hydrogen gas decreased from 54% in the first cycle to 42% in the third cycle, likely due to acetogenesis (conversion of H₂ and CO₂ to acetate) as acetate increased concurrently.

The major fermentation product of glycerol degradation (both P-glycerol and B-glycerol) was PD, followed by acetate and ethanol (Figure 3.2 A). Lactate, succinate and formate were also present, but at concentrations <1 mM. The major fermentation product with glucose was acetate, followed by ethanol and 2,3-butanediol, with <1 mM concentrations of lactate, succinate and formate. The fermentation end products of acetate and ethanol are typical of those found in batch tests with glucose using heat-treated soils [13]. While butyrate is also a common fermentation product from glucose, it was not produced at detectable concentrations here with the wheat soil inoculum. This finding based on HPLC analysis was verified by GC analysis. 2,3-butanediol was detected in glucose fermentation. This chemical is not frequently reported in glucose fermentation tests, although this may be because it is not detected by GC using mol sieve columns typically used for product analysis in fermentation tests [13].
A Coulombic balance showed that 99% of the Coulombs were accounted by the fermentation products during P-glycerol fermentation, 76% during B-glycerol fermentation and 92% during glucose fermentation (Figure 3.2 B). The Coulombic
balance was calculated for three cycles and duplicate reactors. For P-glycerol, 77% of the Coulombs were recovered in PD and 14% in acetate. For B-glycerol, 65% of the Coulombs were recovered in PD and 10% in acetate. For glucose, 32% were in ethanol, 32% in 2,3-butanediol and 24% in acetate. Biomass accounted for only 0.018% of the Coulombs for glucose fermentation, and 0.008% of the Coulombs for P-glycerol fermentation. The CE for B-glycerol did not take into account mass that could be contributed by residual salts and soaps. These constituents might add to values obtained by dry weight that were assumed to only reflect the weight of the cells. Even without considering bacterial growth, this CE still a much lower value than that found for the pure substrates (P-glycerol and glucose). It is possible that the electrons went to other electron acceptors found in B-glycerol such as sulfate. Sodium sulfate is produced from the neutralization of sodium hydroxide with sulfuric acid during the esterification process in biodiesel production and is removed along with the B-glycerol layer. Another electron sink possibility is in the formation of intracellular storage polymers which allows the cells to store carbon [19].

3.3.2. Effect of start-up conditions

The source of the inoculum substantially affected gas production from P-glycerol fermentation (Figure 3.3). Wheat soil (97 mL/g COD) was the best inoculum on the basis of total gas production, followed by tomato soil (56 mL/g COD), compost (28 mL/g COD) and sludge (4 mL/g COD). In contrast, there were fewer differences among the inocula for glucose fermentation. Wheat, tomato and sludge inocula all produced ~180
mL/g COD gas, with 210 mL/g COD gas for the compost. The inoculum also affected the lag time for fermentation of both substrates. The shortest lag times were observed with compost (glucose 10 hrs; glycerol 11 hrs), followed by tomato (glucose 12 hrs; glycerol 25 hrs), sludge (glucose 15 hrs), and wheat soil (glucose 20 hrs; glycerol 56 hrs).

Glycerol did not show any gas production when using heat-treated sludge as inoculum.

![Figure 3.3.](image)

**Figure 3.3.** Gas production using different heat-treated inocula. (A reactor size of 300 mL was used here, compared to 500 mL in other studies).

The use of alternate pretreatment methods did not increase gas production from P-glycerol fermentation (Figure 3.4). Heat, acid, or base pre-treatment, or a lack of pretreatment, resulted in fermentation cycles that had similar gas production and lag times. Gas production (117 ± 18 mL/g COD) with P-glycerol was 2 to 3 times lower than gas production obtained using glucose (296 ± 20 mL/g COD). Sterilization and freezing of the inoculum resulted in batches that had longer lag times, and experiments with these
inocula produced little or no gas when the solution was used (via serial transfer) for a second batch test.

**Figure 3.4.** Gas production from P-glycerol using different pretreatment methods on wheat soil.

The volume of gas produced per gram of COD decreased with increasing substrate concentrations (**Figure 3.5**). In addition, the difference in gas production using glucose and P-glycerol became larger as the concentrations increased. Gas production per g of COD from glucose fermentation was 2.1 times higher than P-glycerol fermentation at 1 g/L, 2.3 times higher at 3 g/L and 2.6 times higher at 6 g/L.
Final cell densities were 3 times higher for glucose ($1.69 \pm 0.2 \times 10^9$ cells/mL) than for P-glycerol ($5.17 \pm 0.2 \times 10^8$ cells/mL). Increasing the initial cell density in a fermentation cycle (by a factor of three) did not substantially affect gas production (Figure 3.6). No change in gas production resulted from changing the buffer from MES (pH = 6.2) to 50 mM phosphate buffer (pH = 7.0) (Figure 3.6).
Figure 3.6. Effect of initial cell concentration using different buffers on hydrogen production using glycerol or glucose.

3.3.3. Gompertz Modeling

A modified Gompertz model was used to fit data for gas production using P-glucose and glycerol substrates over the first three cycles. (Table 3.1, Figure 3.7). The model fit was very good ($R^2 > 0.978$) for all the cycles. The total gas production parameter ($P$) for glucose (295 mL) was more than double than that for glycerol (142 mL). Total gas production did not significantly change with successive inoculations as shown by constant values of $P$, but the lag time parameter ($\lambda$) decreased significantly from the first cycle to the next two cycles (glucose from 8.5 hrs to 2.8 hrs; glycerol from 19.4 hrs to 2.1 hrs).
Table 3.1. Best fit parameters using modified Gompertz model

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cycle</th>
<th>$P$</th>
<th>$\lambda$</th>
<th>$R_m$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1</td>
<td>295</td>
<td>8.5</td>
<td>51.0</td>
<td>0.996</td>
</tr>
<tr>
<td>Glucose</td>
<td>2</td>
<td>316</td>
<td>2.8</td>
<td>62.3</td>
<td>0.987</td>
</tr>
<tr>
<td>Glucose</td>
<td>3</td>
<td>346</td>
<td>2.0</td>
<td>54.6</td>
<td>0.987</td>
</tr>
<tr>
<td>P-glycerol</td>
<td>1</td>
<td>142</td>
<td>19.4</td>
<td>24.9</td>
<td>0.978</td>
</tr>
<tr>
<td>P-glycerol</td>
<td>2</td>
<td>139</td>
<td>2.1</td>
<td>17.7</td>
<td>0.985</td>
</tr>
<tr>
<td>P-glycerol</td>
<td>3</td>
<td>138</td>
<td>5.2</td>
<td>17.0</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Figure 3.7. Modified Gompertz model fit for glucose or glycerol fermentation.
3.4. Discussion

3.4.1. Fermentation products

P-glycerol fermentation with mixed cultures using a wheat soil inoculum produced 0.28 mol-H₂/mol, a value substantially higher than that previously reported using a mixed culture inoculum (0.05 mol-H₂/mol) [3]. Varying inoculum sources affected hydrogen yields, as found by others using glucose [20] and sucrose [7]. In previous studies using sucrose by Van Ginkel et al. [7], potato soil was determined to be the best inoculum, generating three times more hydrogen gas than soybean soil or compost. Baghchehsaraee et al. [20] found that a heat-treated digested sludge (95°C) produced twice as much hydrogen with glucose than heat-treated activated sludge. Wheat soil was found here to be the best inoculum for glycerol fermentation, compared to tomato soil, compost, or sludge, while compost (digested sludge) was the best inoculum for glucose fermentation. The reason for the better results for glycerol with wheat soil may be better natural acclimatization of bacteria in that soil for degradation of glycerols. For example, wheat has more triglycerides (2.5%) than tomatoes (0.2%). For glucose or sucrose fermentation, sugar content may be more relevant, with potatoes having higher sugar content (1.15%) than soybeans (negligible).

Hydrogen yields using tomato and wheat soil were not substantially different when glucose was used as a substrate. Different inoculum sources contain different microbial communities, and the composition of these communities change when large concentrations of a specific substrate are used in fermentation tests. Heat treated soils typically result in the predominance of various Clostridia [20, 21], which is consistent
with the use of heat treatment to inactivate non-spore formers. Others have found that *Klebsiella* species became predominant in tests using glycerol, and *Clostridium* species with glucose, when both samples were inoculated with a mixture of distillery wastewater and potato starch sludge [22]. Therefore, it is clear that the specific substrate used in the fermentation tests can affect which bacteria become predominant, and thus hydrogen yields and end products.

Hydrogen yields from P-glycerol fermentation (0.28 mol \(\text{H}_2/\text{C}_3\) mol-glycerol) were only half of those produced with glucose (0.53 mol-\(\text{H}_2/\text{C}_3\) mol-glucose). This is not as large a difference in hydrogen production between glucose and P-glycerol as found in another study [3] where glycerol fermentation yields (0.05 mol-\(\text{H}_2/\text{C}_3\) mol glycerol) were only one third of those for glucose (0.17 mol-\(\text{H}_2/\text{C}_3\) mol glucose). The lower yields found by Temudo et al. [3] are likely not only due to the different inoculum, but also to different experimental conditions (continuous flow conditions, 30 °C, pH 8, dilution rate 0.12/h, 10-24 g/L). Yields obtained here with glucose (0.53 mol-\(\text{H}_2/\text{C}_3\) mol-glucose) were similar to those previously obtained by our laboratory (0.46 mol-\(\text{H}_2/\text{C}_3\) mol-glucose) [23] under similar experimental conditions (26 °C, batch, pH 6, 4 g/L COD here, compared to 30 °C, batch, pH 6.2, 3 g/L COD). While the hydrogen yields obtained here are higher than those found by Temudo et al. [3], the overall conversion efficiency is only 9.3% for glycerol (based on 3 mol-\(\text{H}_2/\text{mol-glycerol}\) for production of acetate) and 26% for glucose (based 4 mol-\(\text{H}_2/\text{mol-glucose}\) for production of acetate). Higher hydrogen yields than those obtained here have been achieved, but only by using genetically engineered cultures (0.94-1.05 mol-\(\text{H}_2/\text{mol-glycerol}\)) (Table 3.2). This suggests that further improvements in hydrogen yields are possible, with maximum (stoichiometric) yields for
complete conversion of these substrates of 7 mol-H\textsubscript{2}/mol-glycerol and 12 mol-H\textsubscript{2}/mol-glucose.

**Table 3.2.** Yields of hydrogen and 1,3-propanediol from pure glycerol and glycerol from biodiesel production. (NR, not reported, P-glycerol, pure glycerol, B-glycerol, glycerol byproduct from biodiesel production).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inoculum</th>
<th>$Y\text{_{mol-H\textsubscript{2}/mol}}$</th>
<th>$Y\text{_{mol-PD/mol}}$</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Glycerol</td>
<td><em>Enterobacter aerogenes</em></td>
<td>1.05</td>
<td>0.06</td>
<td>[5]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Enterobacter aerogenes</em></td>
<td>0.62</td>
<td>NR</td>
<td>[27]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Escherichia coli</em></td>
<td>0.94</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Clostridium acetobutylicum</em></td>
<td>0</td>
<td>0.64</td>
<td>[26]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Clostridium butyricum</em></td>
<td>0</td>
<td>0.69</td>
<td>[6]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Clostridium butyricum</em></td>
<td>NR</td>
<td>0.58</td>
<td>[29]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>0.61</td>
<td>0.42</td>
<td>[30]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>Mixed (wastewater)</td>
<td>0.05</td>
<td>0.14</td>
<td>[3]</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>Mixed (wheat soil)</td>
<td>0.28</td>
<td>0.69</td>
<td>This study</td>
</tr>
<tr>
<td>B-glycerol</td>
<td><em>Enterobacter aerogenes</em></td>
<td>1.12</td>
<td>0.2</td>
<td>[5]</td>
</tr>
<tr>
<td>B-glycerol</td>
<td><em>Enterobacter aerogenes</em></td>
<td>0.69</td>
<td>NR</td>
<td>[31]</td>
</tr>
<tr>
<td>B-glycerol</td>
<td><em>Clostridium butyricum</em></td>
<td>NR</td>
<td>0.56</td>
<td>[29]</td>
</tr>
<tr>
<td>B-glycerol</td>
<td>Mixed (wheat soil)</td>
<td>0.31</td>
<td>0.59</td>
<td>This study</td>
</tr>
</tbody>
</table>

Heat treatment has been used to eliminate methane gas production, but even without heat treatment, there was no methane gas produced in tests here. However, heat treatment does not prevent hydrogen losses by acetogenesis as homoacetogenic bacteria may survive the heat treatment and consume hydrogen for acetate production [13]. The effect of heat-treatment on hydrogen production varies with the substrate. While several authors reported improvement in hydrogen production from heat-treatment using several different carbohydrates [2], others have found no improvement with xylose or cellulose [24]. Other possible causes for low hydrogen yields are hydrogen consumption by the mixed culture present [2] or inhibition of the culture due to changes in hydrogen partial
pressure [25]. Other pretreatment methods were also unsuccessful in improving hydrogen production during glycerol fermentation during this study.

The main product of glycerol fermentation using mixed cultures here was PD. The PD yield here (0.69 ± 0.09 mol-PD/mol-glycerol) was the same as the highest reported yield with pure cultures (0.69 mol-PD/mol-glycerol), and it surpassed the maximum yield with mixed cultures (0.14 mol-PD/mol-glycerol) (Table 3.2). High PD production lowers hydrogen production as the formation of PD requires hydrogen gas (1 mol H₂ is consumed per mol PD formed). Therefore, we see in Table 3.2 that the highest hydrogen yields (0.94-1.05 mol-H₂/mol-glycerol) were always obtained under the conditions that resulted in the lowest PD production (0-0.06 mol-PD/mol-glycerol). The formation of fermentation products other than PD can have a positive or neutral effect on hydrogen production (Figure 3.8). Our results are also different from previous results in that substantial hydrogen gas was recovered along with PD. In the studies by Gonzalez-Pajuelo et al [26] and Saint-Amans et al [6], which had the highest PD yields (0.64-0.69 mol-PD/mol-glycerol), there was no hydrogen production. Our results therefore show that it is possible to obtain both hydrogen and PD using mixed cultures. While it may be possible to further increase yields of both hydrogen and PD, production of PD will always adversely affect hydrogen yields.
Figure 3.8. Hydrogen production based on actual measurements compared to that predicted from liquid product formation.

3.4.2. Outlook for B-glycerol fermentation for hydrogen gas production

These studies show for the first time that biodiesel byproduct (B-glycerol) can directly be used in fermentation with mixed cultures for hydrogen and PD production, with overall yields based on glycerol content of 0.31 mol-H₂/mol and 0.59 mol-PD/mol. Despite the presence of potentially inhibitory compounds in the biodiesel byproduct, yields obtained for B-glycerol were similar to those obtained with P-glycerol using mixed cultures here, and with pure cultures by others (Table 3.2).

The high yields of B-glycerol compared to P-glycerol open the possibility for integrating hydrogen and PD production into biodiesel production. Environmentally, this would achieve production of two clean and renewable fuels and production of a high commodity chemical which is currently made from petrochemical sources, while
minimizing waste streams. Economically, the integration of these bioprocesses would achieve the valorization of the waste streams and thus make the enterprise more profitable. Hydrogen gas prices are currently in the range of $3 to $4/kg and PD prices are $172/L or $661/gallon (Sigma Aldrich, St. Louis, MO). These higher value products can be achieved without purification of the biodiesel byproduct, which is valued at $0.021/gallon [32]. As a comparison, pure glycerol prices are $88/L or $338/gallon (Sigma Aldrich, St. Louis, MO). Fermentation does not completely remove organic matter, so additional products processes could be produced from biodiesel wastes through anaerobic digestion (methane) [33] or electrohydrogenesis (hydrogen) [34], further increasing production of valuable products.

3.5. Acknowledgements

The authors thank S. Cheng and D. Jones for assistance with experiments and analysis, and Nittany Biodiesel for providing B-glycerol samples. Special thanks to Dr. Pat Cirino and Jonathan Chin for help with HPLC analysis. This research was supported in part by the Global Research Partnership (GRP) from KAUST University, the General Electric First-Year Faculty for the Future Fellowship and the Arthur and Elizabeth Rose Memorial Fellowship.
3.6. References


Chapter 4

High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells

Abstract

The use of glycerol for hydrogen gas production was examined via electrohydrogenesis using microbial electrolysis cells (MECs). A hydrogen yield of 3.9 mol-H$_2$/mol was obtained using glycerol, which is higher than that possible by fermentation, at relatively high rates of 2.0±0.4 m$^3$/m$^3$d ($E_{ap}$=0.9 V). Under the same conditions, hydrogen was produced from glucose at a yield of 7.2 mol-H$_2$/mol and a rate of 1.9±0.3 m$^3$/m$^3$d. Glycerol was completely removed within 6 hours, with 56% of the electrons in intermediates (primarily 1,3-propanediol), with the balance converted to current, intracellular storage products or biomass. Glucose was removed within 5 hours, but intermediates (mainly propionate) accounted for only 19% of the electrons. Hydrogen was also produced using the glycerol byproduct of biodiesel fuel production at a rate of 0.41±0.1 m$^3$/m$^3$d. These results demonstrate that electrohydrogenesis is an effective

2 Material presented in this chapter was published in the following paper: Selombo PA, Perez JM, Lloyd WA, Logan BE. High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. Int J Hydrogen Energy 2009; 34:5373-5381.
method for producing hydrogen from either pure glycerol or glycerol byproducts of biodiesel fuel production.
4.1. Introduction

Glycerol is a commodity chemical widely used by the pharmaceutical industry. However, it is being overproduced as a result of biodiesel fuel production as 1 liter of glycerol is made for every 10 liters of biodiesel fuel produced. At the current annual production capacity of 9.8 billion liters (www.biodiesel.org), 980 million liters of glycerol/yr are produced compared to a demand of only 216 million liters/yr [1].

One alternative use for glycerol is hydrogen gas production by anaerobic fermentation [2-4]. However, only a maximum of 3 moles of H₂ can be produced per mole of glycerol if acetate is the main soluble fermentation end product. Further conversion to hydrogen without additional energy is not possible due to an overall endothermic reaction. Hydrogen yields obtained from pure glycerol (P-glycerol) fermentation are often substantially lower than this maximum value, mainly due to formation of 1,3-propanediol (PD), reaction which requires hydrogen [5]. Yields obtained during glycerol fermentation were 0.05 - 0.28 mol-H₂/mol using mixed cultures [3, 5], and 0.61- 1.05 mol-H₂/mol using pure cultures [2, 4]. The actual glycerol byproduct from biodiesel (B-glycerol) produced up to 0.31 mol-H₂/mol using mixed cultures and 1.12 mol-H₂/mol using pure cultures [2, 5].

An alternative to glycerol fermentation for hydrogen production is the process of electrohydrogenesis using microbial electrolysis cells (MECs) [6, 7]. In an MEC, exoelectrogenic bacteria oxidize organic matter and release electrons to the anode and protons into solution. A small electrical input (~0.2 V) is added, in addition to that
supplied by the bacteria (-0.3 V anode open circuit potential for acetate) to overcome the
endothermic barrier of hydrogen formation (0.414 V) [8]. With an MEC it is possible to
achieve nearly stoichiometric conversion of a substrate to hydrogen. For example, 3.9
mol-H₂/mol-acetate (6.32 L/L d, applied 1 V) was obtained in a membraneless MEC [9]
and 2.1 mol-H₂/mol-acetate (0.05 L/L d, applied 0.8 V) [10] was obtained in a two-
chamber MEC with a proton exchange membrane compared to the stoichiometric limit of
4 mol-H₂/mol-acetate. The theoretical minimum electrical input needed is 0.12 V for
acetate [8], but in practice a higher voltage is needed to overcome electrode
overpotentials and to increase rates. MECs are especially useful when the substrate
originates from a “waste product” such as wastewater [11].

The glycerol byproduct from biodiesel (B-glycerol) was used in a two-chamber
MEC with a mediator, but the maximum yield was only 0.77 mol-H₂/mol-glycerol [12].
Glycerol has not yet been used in a mediatorless or membraneless MEC. Single chamber
membraneless systems with acetate have shown higher hydrogen production rates than
systems with membranes due to reduced ohmic resistance and pH gradients in the system
[9, 13]. Also, membraneless systems are simpler to manufacture and they have reduced
capital costs.

In this study, P-glycerol and B-glycerol were evaluated in a single chamber
mediatorless MEC. The goal was to obtain higher hydrogen yields than those obtained by
fermentation, and therefore to achieve yields closer to the maximum theoretical yield of 7
mol-H₂/mol-glycerol by oxidation. Glucose was used as a positive control, as it is also a
fermentable substrate and it shares similar stoichiometry and metabolic pathways as
glycerol during bacterial degradation [3]. The intermediate product formation was
examined over time to follow charge balances of these fermentable substrates. MEC performance and methane formation were also evaluated at different substrate concentrations and applied voltages for P-glycerol and B-glycerol.

4.2. Materials and methods

4.2.1. Substrates

P-glycerol (ultrapure) was obtained from MP Biomedicals, LLC (Solon, OH). Glucose (D-glucose, anhydrous) was obtained from J.T. Baker (Phillipsburg, NJ). B-glycerol was donated by Nittany Biodiesel (State College, PA). This B-glycerol, produced from the transesterification of soybean oil with methanol, sodium hydroxide and sodium methylate, had a chemical oxygen demand (COD) of 1160 ± 100 mg/L and a glycerol content of 69.5%.

4.2.2. MEC reactor construction and operation

Single-chamber membraneless MEC reactors consisted of a 4-cm long by 3-cm diameter cylindrical chambers formed from a solid block of Lexan, as developed by Call and Logan [13]. The anodes were ammonia-treated graphite brushes (25 mm diameter × 25 mm length, 0.22 m² surface area) [14, 15]. The brush anodes were first enriched in microbial fuel cells (MFCs) with carbon cloth air cathodes [16] initially inoculated with domestic wastewater (first cycle only) and the substrate (1 g/L) to be used in MEC mode. On a molar basis, this represents a starting substrate quantity of 10.9 mM of P-glycerol,
7.52 mM of B-glycerol and 5.5 mM of glucose. The brush anodes remained in MFC cells until they reached three repeatable cycles and then they were transferred to MEC reactors. The MEC cathodes (surface area 7 cm²) were wet-proofed carbon cloth (B-1/B/30WP; BASF Fuel Cell, Somerset, NJ) with a platinum catalyst (10wt% on Vulcan XC-72; BASF Fuel Cell, Somerset, NJ). The media for both MFC and MEC consisted of a 50 mM phosphate buffer solution (4.58 g/L Na₂HPO₄ and 2.45 g/L NaH₂PO₄·H₂O; pH = 7.0), 0.31 g/L NH₄Cl, 0.13 g/L KCl, and trace vitamins and minerals [17].

Voltage to the MECs was applied by a power source (3645A; Circuit Specialists, Inc, AZ). After each MEC cycle, the reactors were drained, exposed to air for 10 min to inhibit methanogen growth [13], refilled with substrate solution (1 g/L unless noted), and sparged with ultra high purity nitrogen gas for 5 min. The reactors were run in duplicate and maintained in a 30°C constant temperature room.

### 4.2.3. Analysis

Continuous gas production was measured using a respirometer (AER-200; Challenge Technology, Springdale, AR). Gas leaving the respirometer was collected in sampling bags (250 ml capacity, Cali-5 bond; Calibrated Instruments Inc., Hawthorne, NY). Gas composition (MEC headspace and gas bags) was analyzed by gas chromatography [18]. Liquid product composition was determined by High Performance Liquid Chromatography (LC-10AD; Shimadzu, Japan) with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) and 4 mM H₂SO₄ as the mobile phase (0.5 ml/min, 45 °C). Glycerol, glucose, and diols (2,3-butanediol, 1,3-propanediol, 1,2-
propanediol) were detected using a refractive index detector (RID). Organic acids (acetate, butyrate, lactate, propionate, succinate) and alcohols (butanol, ethanol) were detected by both RID and ultraviolet (210 nm) detectors.

### 4.2.4. Calculations

A charge balance was used to determine the fate of electrons in the MEC [19].

The number of Coulombs ($C$) can be calculated as:

$$C = nbF$$  \hspace{1cm} (1)

where $n$ is the number of moles, $b$ the moles of electrons per mole of substrate calculated from the half-cell reaction (Table 4.1), and $F$ is Faraday’s constant ($F = 96,484.3 \text{ C/mol}$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
<th>$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>$C_3H_8O_3 + 3 \text{ H}_2\text{O} \rightarrow 3 \text{ CO}_2 + 14 \text{ H}^+ + 14 \text{ e}^-$</td>
<td>14</td>
</tr>
<tr>
<td>Glucose</td>
<td>$C_6H_{12}O_6 + 6 \text{ H}_2\text{O} \rightarrow 6 \text{ CO}_2 + 24 \text{ H}^+ + 24 \text{ e}^-$</td>
<td>24</td>
</tr>
<tr>
<td>Acetate</td>
<td>$C_2H_4O_2 + 2 \text{ H}_2\text{O} \rightarrow 2 \text{ CO}_2 + 8 \text{ H}^+ + 8 \text{ e}^-$</td>
<td>8</td>
</tr>
<tr>
<td>Lactate</td>
<td>$C_3H_6O_3 + 3 \text{ H}_2\text{O} \rightarrow 3 \text{ CO}_2 + 12 \text{ H}^+ + 12 \text{ e}^-$</td>
<td>12</td>
</tr>
<tr>
<td>Formate</td>
<td>$\text{CH}_2\text{O}_2 \rightarrow \text{CO}_2 + 2 \text{ H}^+ + 2 \text{ e}^-$</td>
<td>2</td>
</tr>
<tr>
<td>1,3-propanediol</td>
<td>$C_3H_8O_2 + 4 \text{ H}_2\text{O} \rightarrow 3 \text{ CO}_2 + 16 \text{ H}^+ + 16 \text{ e}^-$</td>
<td>16</td>
</tr>
<tr>
<td>Propionate</td>
<td>$C_3H_6O_2 + 4 \text{ H}_2\text{O} \rightarrow 3 \text{ CO}_2 + 14 \text{ H}^+ + 14 \text{ e}^-$</td>
<td>14</td>
</tr>
</tbody>
</table>

The total Coulombs at any time ($C_T$) are calculated as:

$$C_T = C_f + C_s + C_c + C_L$$  \hspace{1cm} (2)
where $C_i$ are the Coulombs from the measured intermediates (equation 1, Table 4.1), $C_s$ are the Coulombs calculated from the substrate left in the medium, $C_c$ are the Coulombs recovered from the current produced and $C_l$ are the remaining Coulombs lost to non-measured products such as biomass or extracellular polymers. The total Coulombs recovered from current ($C_c$) are calculated by integrating the current over the desired time ($T$) [20]:

$$C_c = \frac{\sum_{i=1}^{T} V_i t_i}{R}$$

where $V$ is the measured voltage ($V$), $t$ is the time interval (1200 s) and $R$ is the applied resistance (10 $\Omega$).

The performance of the reactors was evaluated using equations given in Logan et al. [6] unless otherwise noted. Coulombic efficiency ($CE$) (%) was calculated as the percentage of total Coulombs recovered as current to original Coulombs in the substrate. The cathodic hydrogen recovery ($r_{H_2,\text{cat}}$) (%) was the percentage of electrons that were recovered as hydrogen gas from the total number of electrons that reached the cathode. The volumetric current density ($I_V$) (A/m$^3$) was the average of the maximum current production over a 4-hr period divided by the liquid volume. The maximum volumetric hydrogen production rate ($Q$) (m$^3$ H$_2$/m$^3$ d) was proportional to the current produced and the gas rate per volume of reactor. The hydrogen gas composition (H$_2$ %) was calculated by using the gas bag approach calculation ($V_{H_2}$) (mL) by Logan et al. [6] and dividing it by the total gas produced (mL). The hydrogen yield ($Y_{H_2}$) (mol-H$_2$/mol-substrate) was the moles of hydrogen produced divided by the moles of substrate consumed. This is the only
value adjusted for glycerol content (69.5%) of B-glycerol. The energy efficiency relative to electrical input (\(\eta_E\)) (%) was the ratio of energy content of hydrogen produced to the electrical energy added. The overall energy recovery based on both electric and substrate inputs (\(\eta_{E+S}\)) (%) takes into account both the electrical input and the heat of combustion of the substrate (glycerol \(\Delta H_f = 1655.4\) kJ/mol, glucose \(\Delta H_f = 2802.7\) kJ/mol) [21].

4.3. Results

4.3.1. Volumetric gas production and composition

The use of P-glycerol as a substrate in electrohydrogenesis resulted in a high volume of gas (1428 ± 85 mL/g-COD) with a consistent composition at an applied voltage of 0.9 V over 5 consecutive batch cycles (Figure 4.1 A). Gas production with B-glycerol (444 ± 103 mL/g-COD) was lower than that with P-glycerol. When glucose was used, gas production (1283 ± 42 mL/g-COD) was similar to that achieved with P-glycerol on the basis of COD. The use of a lower applied voltage (0.5 V) produced lower and more variable amounts of gas over successive cycles for both glucose and glycerol (1060 ± 157 mL/g-COD P-glycerol, 530 ± 144 mL/g-COD B-glycerol, 1040 ± 408 mL/g-COD glucose) (Figure 4.1 B). Similar gas production rates observed here using glucose and glycerol with MECs did not occur during anaerobic fermentation with these two substrates as gas production was two times higher for glucose (296 ± 20 mL/g-COD) than for glycerol (133 ± 18 mL/g-COD) fermentation [5].
Figure 4.1. Gas production of glucose or glycerol in MEC, 50 mM PBS, pH 7.0, (A) applied voltage 0.9 V, (B) applied voltage 0.5 V. Standard deviation bars missing for some cycles due to respirator malfunction.
The gas was consistently composed of more hydrogen than carbon dioxide or methane at 0.9 V than at 0.5 V. Using P-glycerol, the gas was 88% H₂ (994 mL-H₂) at 0.9 V, compared to 80% H₂ (766 mL-H₂) at 0.5 V (Figure 4.2, Table 4.2). This effect of applied voltage on hydrogen gas production was not as substantial with B-glycerol, as the percent of hydrogen gas composition was only slightly higher at 0.9 V (87% H₂) than at 0.5 V (82% H₂). Similarly, the gas was relatively enriched with hydrogen using glucose at 0.9 V (87%) compared to 0.5 V (81%).

![Figure 4.2. Gas composition of glucose and glycerol during MEC fourth cycle, (A) applied voltage 0.9 V, (B) applied voltage 0.5 V.](image-url)
Table 4.2. MEC results for glucose or glycerol

<table>
<thead>
<tr>
<th>Substrate Type</th>
<th>Conc</th>
<th>Applied V</th>
<th>CE %</th>
<th>( r_{H_2, cat} ) %</th>
<th>( I_v ) A/m(^3)</th>
<th>( Q ) m(^3)/m(^3) d</th>
<th>( H_2 ) %</th>
<th>( \eta_E ) %</th>
<th>( \eta_{E+S} ) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1</td>
<td>0.5</td>
<td>127±23</td>
<td>51±4</td>
<td>115±4</td>
<td>0.83±0.18</td>
<td>81±5</td>
<td>159±12</td>
<td>50±10</td>
</tr>
<tr>
<td>Glucose</td>
<td>1</td>
<td>0.9</td>
<td>105±10</td>
<td>88±5</td>
<td>182±31</td>
<td>1.87±0.30</td>
<td>87±2</td>
<td>152±8</td>
<td>62±4</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>1</td>
<td>0.5</td>
<td>99±10</td>
<td>64±15</td>
<td>116±5</td>
<td>0.80±0.08</td>
<td>80±0</td>
<td>198±48</td>
<td>47±7</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>1</td>
<td>0.9</td>
<td>104±7</td>
<td>79±18</td>
<td>221±12</td>
<td>2.01±0.41</td>
<td>88±2</td>
<td>139±31</td>
<td>51±10</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>2</td>
<td>0.5</td>
<td>43±1</td>
<td>18±3</td>
<td>116±2</td>
<td>0.10±0.02</td>
<td>31±1</td>
<td>58±10</td>
<td>7±1</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>3</td>
<td>0.5</td>
<td>49±5</td>
<td>1±1</td>
<td>136±45</td>
<td>0.01±0.01</td>
<td>3±2</td>
<td>4±2</td>
<td>1±0</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.3</td>
<td>37±5</td>
<td>1±0</td>
<td>15±3</td>
<td>0.00±0.00</td>
<td>2±0</td>
<td>3±0</td>
<td>0±0</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.5</td>
<td>84±11</td>
<td>45±15</td>
<td>35±8</td>
<td>0.14±0.06</td>
<td>82±5</td>
<td>136±44</td>
<td>28±8</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.6</td>
<td>65±8</td>
<td>72±19</td>
<td>59±10</td>
<td>0.30±0.01</td>
<td>79±1</td>
<td>182±47</td>
<td>36±6</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.8</td>
<td>103±11</td>
<td>52±15</td>
<td>87±11</td>
<td>0.55±0.28</td>
<td>78±13</td>
<td>99±29</td>
<td>35±12</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.9</td>
<td>91±10</td>
<td>65±14</td>
<td>63±14</td>
<td>0.41±0.13</td>
<td>87±4</td>
<td>107±25</td>
<td>37±7</td>
</tr>
</tbody>
</table>
The use of a higher applied voltage resulted in less methane production. For example, when using P-glycerol the gas contained only 1.2% CH₄ at an applied voltage of 0.9 V, compared to 9.5% CH₄ at 0.5 V in the fourth cycle of operation.

4.3.2. Hydrogen yields

Hydrogen yields for P-glycerol at applied 0.9 V reached 3.9 mol-H₂/mol-glycerol (Figure 4.3). This is 56% of the maximum possible yield by oxidation (7 mol-H₂/mol-glycerol), and well above the yield that could be achieved by fermentation to acetate (3 mol-H₂/mol-glycerol). Hydrogen yields for B-glycerol were 1.8 mol-H₂/mol-glycerol, which is 36% of the theoretical maximum by oxidation, and 85% of that possible by fermentation. Higher hydrogen yields of 7.2 mol-H₂/mol-glucose were obtained using glucose, which is 59% of the theoretical maximum by oxidation (12 mol-H₂/mol-glucose). Hydrogen yields for the pure substrates were reduced at the lower applied voltage (0.5 V), with 3.1 mol-H₂/mol-glycerol (P-glycerol), 2.3 mol-H₂/mol-glycerol (B-glycerol), and 6.4 mol-H₂/mol-glucose.
4.3.3. Hydrogen gas-production rates

The hydrogen production rate for P-glycerol (1 g/L) at 0.9 V ($Q = 2.0 \text{ m}^3/\text{m}^3 \text{ d}$) was double that obtained at 0.5 V ($Q = 0.8 \text{ m}^3/\text{m}^3 \text{ d}$) (Table 4.2). Increasing the voltage improved performance of the MEC with P-glycerol in terms of Coulombic efficiency (CE= 99-104%), cathodic recovery ($r_{H_2,cat}=64-79\%$), current ($I_v=116-221 \text{ A/m}^3$) and total energy efficiency ($\eta_{E+S}=47-51\%$). However, the increased voltage resulted in a decrease in energy efficiency based on electrical input ($\eta_{E} = 198-139\%$).

Performance of the MEC with P-glycerol was better than that with B-glycerol. For example, hydrogen production rate ($Q$) was five times lower for B-glycerol (0.41 m$^3$/m$^3$ d) than P-glycerol (2.0 m$^3$/m$^3$ d) at 0.9 V. Hydrogen production was examined
over a range of applied voltages using B-glycerol. Production rates and other MEC performance parameters generally showed a favorable increase with the applied voltage, although at 0.9 V there was a slight decrease in hydrogen production rate (range $H_2 = 79$ to 87%, $Q = 0.14$ to 0.55 m$^3$/m$^3$ d; $\eta_{E+S} = 28$ to 37%; Table 4.2, Figure 4.4). Energy efficiency based on electrical input ($\eta_E$) was optimum at 0.6 V (182%). At 0.3 V, the applied voltage was too small to produce any significant hydrogen ($Q = 0.0$ m$^3$/m$^3$ d). This sharp drop in performance at a low applied voltage was also observed at 0.2 V in MEC tests by others using acetate [13], where $Q = 0.5$ m$^3$/m$^3$ d at an applied voltage of 0.3 V, but no gas production occurred at 0.2 V.

![Figure 4.4](image_url)  

**Figure 4.4.** Hydrogen production rate ($Q$), hydrogen composition ($H_2$) and energy efficiency ($\eta_e$) for B-glycerol as a function of applied voltage.
4.3.4. Charge balance analysis

P-glycerol was completely consumed 6 hours after the start of the fed-batch cycle, with all intermediates consumed within 36 hours (Figure 4.5 A). The main intermediate was 1,3-propanediol (PD), with lesser amounts of acetate and propionate. While PD and acetate were produced almost immediately, propionate formation had a lag time of 4 hours. The total Coulombs accounted for by the intermediates reached a peak at 8 hours and steadily decreased until the end of the cycle (Figure 4.5 B). After 8 hours, 78% of the Coulombs could be accounted for by intermediates (56%) and by current (22%). Coulomb accountability increased to 100% after 16 hours and remained constant for the duration of the cycle.
Figure 4.5. (A) Product formation and (B) charge balance of 1 g/L P-glycerol at applied 0.5 V.
Glucose was completely removed less than 5 hours after the start of the MEC cycle and all intermediates were consumed within 30 hours (Figure 4.6 A). Glucose electrohydrogenesis resulted in the rapid formation of lactate, formate, and acetate, with propionate observed only after 4 hours. After 8 hours, only 44% of the coulombs were accounted for by current production (25 %) and intermediates (19 %) (Figure 4.6 B) compared to 78% for P-glycerol. By the end of the experiment, the recovery of Coulombs was 109%.
Figure 4.6. (A) Product formation and (B) charge balance of 1 g/L glucose at applied 0.5 V.
4.3.5. Effect of organic loading

The effect of organic loading was examined by increasing the initial P-glycerol concentration (1, 2 and 3 g/L or 10.9 mM, 21.8 mM and 32.7mM, respectively) at the lower applied voltage of 0.5 V (Table 4.2). The performance of the reactors decreased with increased organic loading in terms of all operational parameters (\(CE= 104 \text{ to } 49\%\), \(r_{H_2,cat}= 79 \text{ to } 1\%\), \(\eta_E = 139 \text{ to } 4\%\), \(\eta_{E+S} = 51 \text{ to } 1\%\), \(I_v= 221 \text{ to } 136 \text{ A/m}^3\), \(Q= 2 \text{ to } 0.01 \text{ m}^3/\text{m}^3 \text{ d}\) and \(\text{H}_2 \text{ content= } 88 \text{ to } 3\%\)). Cycle times (36 to 48 hrs) and methane composition (7 to 80%) also increased with increasing glycerol concentration (1 to 3 g/L) (Figure 4.7).

![Graph showing gas composition and cycle times for different initial P-glycerol concentrations at applied 0.5 V.](image)

**Figure 4.7.** Gas composition and cycle times for different initial P-glycerol concentrations at applied 0.5 V.
The impact of the higher organic loading on performance can be better understood through the analysis of intermediates over time (Figure 4.8 A). At an initial glycerol concentration of 3 g/L, P-glycerol was consumed during the same time span as the lower glycerol concentration of 1 g/L (within 6-hrs) and formed the same fermentation products (acetate, propionate and 1,3-propanediol) (Figure 4.5 A). However, 4.6 mM of propionate remained in solution at the end of a cycle, accounting for 20% of the total Coulombs added (Figure 4.8 B).
Figure 4.8. (A) Product formation and (B) charge balance of 3 g/L glycerol at applied 0.5 V.
At the higher P-glycerol concentration of 3 g/L, 49% of the Coulombs were unaccounted for during the cycle (between 20-30 hrs) (Figure 4.8 B), compared to only 22% at 1 g/L (8 hrs). The intermediates accounted for 22% and the current accounted for 29% of the Coulombs recovered. The maximum Coulombs accounted at the end of the cycle were 68% at 3 g/L (48% from current, 20% from propionate), compared to 100% at 1 g/L (Figure 4.5 B).

4.4. Discussion

4.4.1. MEC performance in comparison to other studies

The MECs with P-glycerol produced an overall energy efficiency of $\eta_{E+S} = 51 \pm 10\%$, a hydrogen production rate of $Q = 2.0 \pm 0.4 \text{ m}^3/\text{m}^3 \text{ d}$, with a hydrogen yield of 3.9 mol-$\text{H}_2$/mol-glycerol at applied 0.9 V. The use of a lower applied voltage (0.5 V) reduced performance ($\eta_{E+S} = 47 \pm 7\%$, $Q = 0.8 \pm 0.1 \text{ m}^3/\text{m}^3 \text{ d}$, with a hydrogen yield of 3.2 mol-$\text{H}_2$/mol-glycerol) and there was greater variability between cycles. These results are up to five times higher than those achieved in a previous study with glycerol using a two-chamber MEC and an exogenous mediator (0.77 mol-$\text{H}_2$/mol-glycerol) at a lower applied voltage of 0.2 V [12].

The performance of the MECs with glucose was better than that achieved with glycerol, in terms of energy efficiency, hydrogen production rates ($\eta_{E+S} = 62\%$, $Q = 1.87 \text{ m}^3/\text{m}^3 \text{ d}$), and hydrogen yield (7.2 mol-$\text{H}_2$/mol-glucose) (applied 0.9 V). Results with glucose at an applied voltage of 0.9 V were comparable to those in a previous two-
chamber MEC study at a lower applied voltage of 0.6 V in terms of these same parameters (\(\eta_{E+S} = 64\%\), \(Q = 1.23 \text{ m}^3/\text{m}^3 \text{ d}\), and hydrogen yield of 8.6 mol-H\(_2\)/mol-glucose) [16]. Both of these results with glucose are better than those reported in another two-chamber continuous-flow system which had a hydrogen production rate of \(Q = 0.58 \text{ m}^3/\text{m}^3 \text{ d}\) at an applied voltage of 1.2 V [22].

4.4.2. Product formation in MECs

Both glycerol and glucose are fermentable substrates, and it was observed that intermediates rapidly accumulated in solution at the start of the fed batch cycles. This observation in MEC tests of intermediate accumulation was similar to that found in MFC tests where it was observed that 84% of the starting concentration of xylose was depleted within 10 hrs with intermediates formed and consumed over the rest of the 60 hour cycle [19].

One of the main intermediates formed during MEC operation with glucose was propionate. Propionate was produced after a lag time of about 4 hours, with a peak in concentration 16 hrs after glucose was completely consumed (5 hrs). This lag time and observation of continued intermediate formation after the primary substrate was depleted was not observed in MFC tests with xylose [19] where all the intermediates were formed at their maximum concentrations by the time xylose was depleted. This suggests that the propionate formed did not come directly from glucose or glycerol fermentation, but rather from other intermediates such as lactate [23] or acetate [24]. Propionate could have been produced by the same bacteria that may be involved in current generation, for
example by *Pelotomaculum thermopropionicum* [25]. At an initial concentration of 1 g/L of P-glycerol, propionate reached a maximum concentration of 0.8 mM but was subsequently removed. However, at a higher initial concentration of glycerol (3 g/L P-glycerol), propionate accumulated to higher concentrations (4.6 mM) and it was not removed by the end of the cycle (48 hours). The reason for the lack of propionate removal is not known. Propionate accumulation has been observed in anaerobic digestion, and many theories have been proposed for propionate accumulation, such as hydrogen inhibition [26, 27].

There was evidence of substantial carbon storage, especially with glucose as substrate. Current and intermediates did not fully account for all the Coulombs (based on COD removal) during some portions of the total cycle time and electron recovery was 100% or higher at the end of the cycle. Carbon storage occurs when bacteria take up substrate and store it in the form of polymers (commonly poly-β-hydroxyalkanoates and glycogen). These stored materials can subsequently be used as energy sources when needed [28]. Carbon storage has been observed in MFC tests with glucose, acetate and xylose, where carbon storage was observed up to 56% of the Coulombs available [19, 28]. Carbon storage was not as prominent in MEC tests here using P-glycerol, where a maximum of only 22% of the Coulombs were unaccounted (after 16 hrs). This shows that for P-glycerol consumption rates are comparable to production rates (intermediates, biomass and current) without much less intracellular carbon storage compared to other fermentable substrates such as glucose and xylose.

Formation of fermentation products and carbon storage indicates that intermediates were the main chemicals used for subsequent current generation for a large
portion of the fed batch cycle as opposed to the starting material. The minimum potentials needed for chemical oxidation of fermentation products is larger than that needed for the original substrate. For example, less energy is theoretically needed to form hydrogen at the cathode ($E_{ca} = -0.414$ V) with glucose ($E_{an} = -0.428$ V, $E_{emf} = 0.014$ V) or glycerol ($E_{an} = -0.403$ V, $E_{emf} = -0.011$ V) than is needed for acetate ($E_{an} = -0.3$ V, $E_{emf} = -0.114$ V) [8]. According to these values, glucose oxidation could result in spontaneous current generation in an MEC, but once the intermediates are formed, a spontaneous reaction leading to hydrogen production at the cathode is no longer possible [8].

### 4.4.3. Electron recycling and methane production

There was evidence for electron recycling during MEC operation, especially with glucose at an applied voltage of 0.5 V as Coulombic recovery exceeded 100%. Electron recycling occurs when the hydrogen produced at the cathode is used at the anode. Many bacteria in mixed anaerobic cultures can use hydrogen as an electron donor to reduce volatile fatty acids [29]. In addition, exoelectrogens such as *Shewanella oneidensis* MR-1 [30] and *Geobacter sulfurreducens* [31] can use hydrogen as an electron donor.

Methane formation was substantially reduced at 0.9 V compared to 0.5 V for P-glycerol and glucose. In previous studies, higher voltages corresponded to shorter cycle times and reduced methane formation [5, 18]. In this study, the increased voltage did not reduce the cycle time (36 hours for both voltages), but methane production still decreased by using a higher applied voltage for P-glycerol and glucose. For B-glycerol, there was no significant change in methane production with applied voltage. Methane formation
was most likely linked to propionate formation, as propionate is an important intermediate in the anaerobic degradation of organic matter to methane [32]. Methane was likely formed during the conversion of intermediates to propionate, as methane can be the most predominant byproduct in the gas phase when volatile organic acids are converted to other volatile organic acids or alcohols [29].

4.4.4. Outlook for hydrogen production from B-glycerol

Results in this study demonstrate that the B-glycerol byproduct from biodiesel fuel production can be converted, without purification, into hydrogen at higher production rates and yields than fermentation. The cost of hydrogen produced via electrohydrogenesis due to the electrical energy input with B-glycerol is $1.06/kg H₂ at applied 0.5 V and $1.18/kg H₂ at applied 0.9 V, based on energy demands of 2.32 kWh/m³ H₂ (0.5 V) and 2.60 kWh/m³ H₂ (0.9 V) and current wholesale electricity prices of $41/MWh (www.eia.doe.gov). These costs are 2.5 times less than that needed for hydrogen produced by water electrolysis ($2.55/kg H₂ based on energy requirements of 5.6 kWh/m³ H₂) [13]. Hydrogen produced by an MEC based on electrical energy would therefore cost one third of that currently charged for pure hydrogen gas ($3-4/kg H₂; Sigma Aldrich, St. Louis, MO).

The main issue remaining with using B-glycerol as a substrate in an MEC is the adverse effect of components other than the glycerol on treatability. B-glycerol is a mixture of glycerol, sodium sulfate, soaps, methanol, and water [33]. Proteins may also be present in B-glycerol depending on the feedstock and the production method [33],
although they would be expected to contribute to current production. Single proteins, such as bovine serum albumin, as well a complex mixtures of proteins (peptone and a meat packing wastewater) have been used as substrates in MFCs [34].

The lower MEC performance using B-glycerol compared to P-glycerol is likely mainly due to methanol. Methanol can be toxic to microorganisms [35], and in tests with methanol in an MFC, which is a system very similar to an MEC, there was no sustained power generation [36]. In fermentation tests with Enterobacter aerogenes, it was also found that there was less hydrogen production with B-glycerol (0.7 mol H₂/mol glycerol) than P-glycerol (0.89 mol H₂/mol glycerol) [2]. Methanol can be removed by evaporation. A detailed economic analysis is needed to determine if the increased cost of methanol removal would be justified by the increased hydrogen yield.

The effect of triglycerides on hydrogen production in an MEC is not clear. Triglycerides have not been used in MFC or MEC systems, but it is likely that they could be used as a substrate for power generation or just degraded via fermentation. Pseudomonas aeruginosa, which has been shown to produce current in an MFC using self-produced mediators [37], can ferment triglycerides at higher rates than glycerol [38]. Triglycerides can also be converted into soaps, and this could negatively influence bacterial growth. Anionic and cationic surfactants, for example, can restrain or retard the growth of P. aeruginosa [39]. Soaps have been found to have a negative effect on docosahexaenoic acid production by algae via fermentation from B-glycerol [40]. Soap removal can be difficult, but a common method for removal is by precipitation through pH adjustments (acidic conditions) [40].
It is unlikely that sodium sulfate in B-glycerol was a factor in reduced hydrogen production. Sodium sulfate is produced from the neutralization of sodium hydroxide with sulfuric acid during the esterification process in biodiesel production. Sodium is required by some but not all organisms, depending on their natural habitat [41]. Sulfate is a source of sulfur for most organisms and is used by sulfate-reducing bacteria, which include *Geobacter metallireducens* and *Geobacter sulfurreducens*, well-known exoelectrogens [37]. Salts at high concentrations may inhibit cell growth. Reduced hydrogen production by fermentation was observed with *Enterobacter aerogenes* when 1% sodium chloride was added to 10 g/L B-glycerol (>200 mM NaCl plus salts in B-glycerol) but not when it was added to P-glycerol (171 mM NaCl) [2].

4.5. Conclusions

High hydrogen yields were obtained from glycerol and glucose in single chamber membraneless MEC reactors. A higher applied voltage (0.9 V) than that typically used for acetate (0.5 V) was needed for consistent operation and methane reduction. The fermentable substrates were consumed rapidly and fermentation products were formed depending on the substrate. 1,3-propanediol was the main intermediate during glycerol electrohydrogenesis, while acetate and propionate were the main products during glucose electrohydrogenesis. All intermediates were consumed by the end of an MEC cycle at 1 g/L, but not at 3 g/L where 4.6 mM propionate remained at the end of the cycle. Electron recycling and cell carbon storage occurred during glucose electrohydrogenesis, and to a smaller extent during glycerol electrohydrogenesis. B-glycerol produced higher hydrogen
yields in MEC than anaerobic fermentation, but less than those with P-glycerol, probably
due to the presence of methanol and soaps in the mixture and complexity of the substrate.

4.6. Acknowledgements

The authors thank S. Cheng, D. Call, E. Lalaurette, D. Jones, J. Chin and P. Cirino for assistance with experiments and analysis and to Nittany Biodiesel for providing glycerol samples from their biodiesel production. This research was supported in part by Award KUS-I1-003-13 by King Abdullah University of Science and Technology (KAUST), the General Electric First-Year Faculty for the Future Fellowship, and the Arthur and Elizabeth Rose Memorial Fellowship.
4.7. References


Chapter 5

The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells

Abstract

Microbial electrolysis cells (MECs) are used to produce hydrogen gas from the current generated by bacteria, but low cost alternatives are needed to typical cathode materials (carbon cloth, platinum and Nafion™). Stainless steel A286 was superior to platinum sheet metal in terms of cathodic hydrogen recovery (61% vs. 47%), overall energy recovery (46% vs. 35%), and maximum volumetric hydrogen production rate (1.5 m³/m³/d vs. 0.68 m³/m³/d) at an applied voltage of 0.9 V. Nickel 625 was better than other nickel alloys, but it did not perform as well as SS A625. The relative ranking of these materials in MEC tests was in agreement with cyclic voltammetry studies. Performance of the stainless steel and nickel cathodes was further increased, even at a lower applied voltage (0.6 V), by electrodepositing a nickel oxide layer onto the sheet metal (cathodic hydrogen recovery: 52%, overall energy recovery: 48%, maximum volumetric hydrogen production rate: 0.76 m³/m³/d). However, performance of the nickel

---

3 Material presented in this chapter was published in the following paper: Selemo PA, Merrill MD, Logan BE. The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells. J Power Sources 2009; 190:271-278.
oxide cathodes decreased over time due to a reduction in mechanical stability of the oxides (based on SEM-EDS analysis). These results demonstrate that non-precious metal cathodes can be used in MECs to achieve hydrogen gas production rates better than those obtained with platinum.
5.1. Introduction

Electrohydrogenesis is a promising process to produce hydrogen gas from organic matter in devices known as microbial electrolysis cells (MECs) [1]. In an MEC, exoelectrogenic bacteria oxidize organic matter, which generate CO₂, electrons and protons. The bacteria transfer the electrons to the anode and the protons are released to the solution. By adding a small voltage to that produced by the bacteria (~0.2 V) to overcome the endothermic barrier of hydrogen formation (0.414 V), electrons and protons are catalyzed at the cathode to form hydrogen gas. Typically voltages of ~0.3 V or larger are needed to overcome electrode overpotentials. This electrical input is less than the voltages required for water electrolysis (typically 1.8 – 2.0 V) [1]. MECs are especially useful when the organic matter used in the process originates from wastewater or non-food biomass sources such as corn stover.

While MEC tests have so far been limited to the laboratory, these reactors will need to be scaled-up to sizes suitable for real-world applications [2]. One of the challenges of scaling up MECs is the cost of the cathode and the cathode catalyst. Most MECs use platinum applied to carbon cloth [3-5] or carbon paper [6, 7] using a binder (i.e. Nafion™), but both the carbon cloth and the binder are expensive. Therefore, it is necessary to identify other cathode materials for this MEC technology to be economical.

There have been very few investigations into alternative cathode materials for MEC’s. Comparisons of the performance of cathode materials among studies can be difficult as the system conditions and architecture also affect reactor performance.
Titanium mesh with platinum catalyst [8, 9] has been used in two-chamber and one-chamber (with membrane) configurations, but hydrogen production rates were low (up to 0.3 L/L/d at 1.0 V applied voltage). A combination of palladium and platinum catalyst on carbon paper [10] was used in a continuous flow reactor, but this system produced high concentrations of methane. In another study, it was observed that hydrogen evolution could be catalyzed using only bacteria on a plain electrode (graphite felt), referred to as a biocathode [11]. The best results have been achieved using a single chamber MEC with acetate as a substrate and platinum on carbon cloth cathodes. At an applied voltage of 0.8 V, for example, hydrogen was produced at a maximum volumetric production rate of $Q = 3.12 \text{ m}^3/\text{m}^3/\text{d}$, with a cathodic hydrogen recovery $r_{H_2,\text{cat}} = 96\%$ and an overall energy efficiency of $\eta_{E+S} = 75\%$ [3]. In contrast, the two-chamber MEC with a biocathode [11], operated at an applied voltage of 0.7 V under continuous flow conditions, produced a gas flowrate of $Q = 0.63 \text{ m}^3/\text{m}^3/\text{d}$ and a cathodic hydrogen recovery of $r_{H_2,\text{cat}} = 49\%$.

Various metals have been examined for use in water electrolysers to catalyze the hydrogen evolution reaction (HER) [12]. First row transition metals are desirable as they are stable, abundant in nature, economical, and have low toxicity to living organisms. The most promising materials identified so far are nickel and stainless steel alloys based on cost, availability, low overpotentials, and stability in solutions which are usually highly alkaline. A comparison of stainless steel alloys (304, 316 and 430) in an alkaline electrohydrolyzer showed that SS 316 was the best of these three materials for HER [13]. In another study, SS 310 was compared to Raney nickel alloys in a commercial electrolyser [14], and it was concluded that the use of SS 310 would theoretically make the system 16 times cheaper at similar hydrogen production rates. Nickel alloys SAF
2205, INCONEL 625 and MONEL 400 were evaluated for hydrogen evolution using cyclic voltammetry [15]. SAF 2205 showed more favorable overpotentials for HER in 1 M NaOH than carbon steel SAE1020. Nickel oxide catalysts also have shown great promise as catalysts for hydrogen evolution [16] by water electrolysis under alkaline conditions. The main limitation in extending the previous results with these metals to MECs is that they have only been examined using highly alkaline conditions. MECs operate at near-neutral pH, and thus the efficacy for hydrogen evolution of nickel and stainless steel materials under these conditions have not been evaluated with respect to more conventional catalysts such as platinum.

In this study, different nickel and stainless steel alloys were compared under neutral pH conditions to platinum using sheet metal cathodes in MECs. Most MECs use particles of the catalyst bound to highly porous carbon cloth. Under these conditions, catalyst particle size, binding efficiency, and other factors can influence current densities. A baseline comparison of these different metals was therefore made here using flat surfaces to remove variability of the materials based on surface area and binders. The performance of two of these materials was also examined with a nickel oxide deposited onto the sheet metal surface. For comparison with typical MEC cathodes, we also conducted MEC tests using platinum bound to a carbon cloth cathode.
5.2. Materials and methods

5.2.1. MEC reactor construction

Single-chamber MEC reactors consisted of 4 cm long by 3 cm diameter cylindrical chambers formed from a solid block of Lexan, as described by Call and Logan [3]. The anodes were ammonia treated graphite brushes (25 mm diameter × 25 mm length, 0.22 m² surface area) [17, 18]. Reactors were inoculated with the anode solution from another acetate-fed MFC reactor that had been running for over one year using acetate (1 g/L) and a phosphate buffer nutrient medium [18]. The inoculum was omitted once a reactor produced > 0.3 mA/cm. The medium consisted of a 50 mM phosphate buffer solution (4.58 g/L Na₂HPO₄ and 2.45 g/L NaH₂PO₄·H₂O; pH = 7.0), 0.31 g/L NH₄Cl, 0.13 g/L KCl, and trace vitamins and minerals [19]. MECs were operated in fed-batch mode. A power source (3645A; Circuit Specialists, Inc, Arizona) was used to apply either 0.6 V or 0.9 V to the reactors. After each cycle, the reactors were drained, refilled with substrate solution, and sparged with ultra high purity nitrogen gas for five minutes. The reactors were maintained in a 30°C constant temperature room. Once reactors reached similar current (~0.57 mA/cm²) and gas production volumes (~30 ml) for three consecutive cycles using carbon cloth cathodes, the cathodes were replaced with sheet metal cathodes. All reactors were run in duplicate, and tests with new cathodes were run for at least three consecutive cycles.
5.2.2. Cathodes

Stainless steel alloys 304, 316, 420 and A286 and nickel alloys 201, 400, 625 and HX were made by cutting sheet metal (McMaster-Carr, Illinois) into 3.8 cm diameter disks. Metal compositions are listed in Table 5.1. A platinum metal disk (99.9% purity) used for comparison to these other metal materials was pre-cut by the manufacturer (Hauser & Miller, Missouri). Metal cathodes were cleaned with ethanol before placing them in the reactors. Carbon cloth cathodes (projected surface area of 7 cm$^2$) were made using a platinum catalyst (0.5 mg/cm$^2$) and a Nafion binder, with the catalyst layer facing the water side [20].

**Table 5.1. Stainless Steel and Nickel Alloys Composition (% by weight).**

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Fe</th>
<th>C</th>
<th>Mn</th>
<th>P</th>
<th>S</th>
<th>Mo</th>
<th>Si</th>
<th>Cr</th>
<th>Ni</th>
<th>Cu</th>
<th>Other</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 304</td>
<td>0.08</td>
<td>2</td>
<td>0.45</td>
<td>0.03</td>
<td>0</td>
<td>1</td>
<td>18-20</td>
<td>8-10.5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 316</td>
<td>0.08</td>
<td>2</td>
<td>0.05</td>
<td>0.03</td>
<td>2-3</td>
<td>1</td>
<td>16-18</td>
<td>10-14</td>
<td>2-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 420</td>
<td>0.15</td>
<td>1</td>
<td>0.04</td>
<td>0.03</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS A286</td>
<td>0.08</td>
<td>2</td>
<td>0.025</td>
<td>0.025</td>
<td>1-1.5</td>
<td>1</td>
<td>13.5-16</td>
<td>24-27</td>
<td>1.9-2.35 Ti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni 201</td>
<td>0.4</td>
<td>0.02</td>
<td>0.35</td>
<td>99</td>
<td>0.25</td>
<td>.35 Si</td>
<td>.01 S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni 400</td>
<td>1.6</td>
<td>1.1</td>
<td>65.1</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni 625</td>
<td>2.5</td>
<td>9</td>
<td>21.5</td>
<td>61</td>
<td>3.6 Nb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni HX</td>
<td>18</td>
<td>0.1</td>
<td>9</td>
<td>22</td>
<td>47</td>
<td>0.6 W</td>
<td>1.5 Co</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2.3. Nickel Oxide Electrodeposition

The nickel oxide catalyst was created through cathodic electrodeposition onto a sheet metal support [16] using a 12.9 cm$^2$ nickel foam anode. Electrodeposition was achieved by applying 20 V at ~2 A for 30 s (1696 power source, B&K Precision, California) in a solution containing 12 mM NiSO$_4$ and 20 mM (NH$_4$)$_2$SO$_4$ at a pH = 2.0,
adjusted by adding H₂SO₄. Cyclic voltammetry (CV) scans were performed on the electrodeposited metal to ensure consistent electrodeposition. Tests were conducted in a Lexan cell using a 50 mM phosphate buffer, a Ag/AgCl reference electrode, and a platinum counter electrode (3 cm × 5 mm) with a scan range of 0.2 to -1.2 V and a scan rate of 3 mV/s. Consistent electrodeposition was confirmed as all nickel oxide cathodes had similar hydrogen evolution potentials between -0.65 to -0.70 V. The electrodes were subsequently cut to size (3.8 cm diameter disks) and rinsed with deionized water before placing them in the reactors.

5.2.4. Analysis

Gas production was measured using a respirometer (AER-200, Challenge Technology, Arizona). Gas flowing out of the respirometer was collected in sampling gas bags (250 ml capacity, Cali-5 bond, Calibrated Instruments Inc., New York). The composition of the MEC headspace and the gas bags were analyzed using two gas chromatographs (models 8610B and 310, SRI Instruments, California) equipped with Alltech Molesieve 5A 80/100 stainless steel-tubing columns and thermal conductivity detectors (TCDs). Argon was used as the carrier gas for H₂, O₂, N₂ and CH₄ analysis, and helium was used as the carrier gas for CO₂ analysis. Voltage across an external resistor (Rₑₓ = 10 Ω) was measured using a multimeter (2700, Keithley Instruments, Inc., Ohio) to calculate current.

Electrochemical experiments were conducted with a potentiostat (PC4/750TM, Echem Analyst, v. 5.5, Gamry Instruments, Pennsylvania). CV scans were done over
three cycles, from 0 to 1 V, at a scan rate of 1 mV/s on the MEC cells after use. CV scans have been previously performed on whole cell MFCs [22, 23] and on separate MEC components [24] for evaluation of biofilms and electron transfer performance. Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDS) analysis was done at 20 kV (Quanta 200, FEI, Oregon).

5.2.5. Calculations

Hydrogen recovery, energy recovery, volumetric density and hydrogen production rates were used to evaluate reactor performance [2]. The theoretical number of hydrogen moles produced ($n_{H2,COD}$), based on COD removal is:

$$n_{H2,COD} = \frac{b_{eO2}v_L\Delta COD}{2M_{O2}}$$  \hspace{1cm} (1)

where $b_{eO2} = 4$ is the number of electrons exchanged per mole of oxygen, $v_L = 32$ ml the volume of liquid in the reactor, $M_{O2} = 32$ g/mol the molecular weight of oxygen, 2 the number of moles of electrons per mole of hydrogen gas, and $\Delta COD$ the change in substrate concentration (g/L).

The theoretical number of hydrogen moles that can be recovered based on the measured current ($n_{H2,cat}$) is:

$$n_{H2,cat} = \frac{\int_{t=0}^{t} I dt}{2F}$$  \hspace{1cm} (2)

where $I=V/R_{ex}$ is the current (A) calculated from the voltage across the resistor (10 Ω) and $dt$ is the time interval (1,200 s) for data collection.
The overall hydrogen recovery \( r_{H_2,\text{COD}} \) is the ratio of hydrogen recovered compared to the maximum theoretical hydrogen produced based on substrate utilization:

\[
r_{H_2,\text{COD}} = \frac{n_{H_2}}{n_{H_2,\text{COD}}}
\]  

(3)

where \( n_{H_2} \) is the actual number of hydrogen moles produced. The cathodic hydrogen recovery \( r_{H_2,\text{cat}} \) is the fraction of electrons that are recovered as hydrogen gas from the total number of electrons that reach the cathode, or

\[
r_{H_2,\text{cat}} = \frac{n_{H_2}}{n_{H_2,\text{cat}}}
\]  

(4)

The Coulombic efficiency \( (C_E) \) is the ratio of electrons recovered as current relative to the total electrons available from substrate consumption, calculated as:

\[
C_E = \frac{n_{H_2,\text{cat}}}{n_{H_2,\text{COD}}} = \frac{r_{H_2,\text{COD}}}{r_{H_2,\text{cat}}}
\]  

(5)

The energy efficiency relative to electrical input \( (\eta_E) \) is the ratio of energy content of hydrogen produced to the input electrical energy:

\[
\eta_E = \frac{W_{H_2}}{W_E} = \frac{n_{H_2,\text{cat}}}{\sum_1^n (IE_{ap} \Delta t - I^2 R_{eq} \Delta t)}
\]  

(6)

where \( W_{H_2} \) (kJ) is the energy produced by hydrogen, \( W_E \) (kJ) the amount of energy added to the circuit by the power source minus the losses across the resistor, \( \Delta H_{H_2} = 285.83 \) kJ/mol the energy content of hydrogen based on the heat of combustion and \( E_{ap} \) (V) the voltage applied by the power source. The number of moles of substrate consumed during a batch cycle based on COD removal \( (n_s) \) is:
\[ n_s = \frac{\Delta CODv_L}{M_s} \]  

(7)

where \( M_s = 82 \text{g/mol} \) is the substrate’s molecular weight. When using sodium acetate, the molecular weight needs to be multiplied by a conversion factor of 0.78 g COD/g sodium acetate. The energy efficiency relative to the substrate (\( \eta_S \)) is:

\[ \eta_S = \frac{W_{H_2}}{W_S} = \frac{n_{H_2} \Delta H_{H_2}}{\Delta H_S n_s} \]  

(8)

where \( \Delta H_S = 870.28 \text{kJ/mol} \) is the heat of combustion of the substrate. The overall energy recovery based on both electric and substrate inputs (\( \eta_{E+S} \)) is:

\[ \eta_{E+S} = \frac{W_{H_2}}{W_E + W_S} \]  

(9)

The hydrogen production rate (\( Q \)) (\( \text{m}^3/\text{m}^3/\text{d} \ H_2 \)) was evaluated in terms of current produced per volume of reactor and the gas rate per volume as:

\[ Q = 3.68 \times 10^{-5} I_v T r_{H_2,cat} \]  

(10)

where 3.68 \( \times 10^{-5} \) is a constant that includes Faraday’s constant, a pressure of 1 atm and unit conversions, \( I_v \) \( (\text{A/m}^3) \) is the volumetric current density averaged over a 4 hour period of maximum current production and divided by the liquid volume, and \( T \) (K) is the temperature.

The Butler-Volmer reaction for hydrogen evolution was used to determine the catalytic performance of the metals, where the reverse current was considered negligible [16, 21]. CV scans for the complete MEC’s were converted to Tafel plots by plotting log \( I \) as a function of voltage. The transformed Butler-Volmer equation was used to obtain slopes and y-intercepts via linear regression of the Tafel plots using:
\[ \log J = \log J_0 + \frac{\alpha_c n_e F}{2.303RT} (E - E_0) \]  

(11)

where \( J \) (A/cm\(^2\)) is the current density, \( J_0 \) (A/cm\(^2\)) is the exchange current density, \( \alpha_c \) is the cathodic transfer coefficient, \( n_e \) is the number of electrons per reaction, \( E \) (V) is the working potential and \( E_0 \) (V) is the equilibrium potential. The equilibrium potential \( E_0 \) is equal to the hydrogen potential \( E_{H2} \):

\[
E_{H2} = 0 + 0.0602 \log \left[ \frac{1/2 H_2}{H^+} \right] = 0 - 0.0602 pH + 0.0301 \log(p_{H_2})
\]  

(12)

The equilibrium potential \( E_0 = E_{H2} = -0.4458 \) V for the experimental conditions presented: \( T = 30^\circ \) C, \( pH = 7 \) and a partial pressure for hydrogen \( p_{H2} = 0.15 \) atm. The hydrogen partial pressure value was the average hydrogen gas composition of all MEC reactors over complete cycles.

5.3. Results

5.3.1. Flat cathodes

SS alloys A286 (21.2 ± 2.2 ml) and 304 (19.1 ± 1.1 ml) produced twice as much hydrogen as Ni 201 (9.5 ± 1.6 ml) or SS 316 (9.5 ± 2.6 ml) at an applied voltage of 0.9 V (Figure 5.1). Platinum sheet metal produced slightly less hydrogen gas (18.9 ± 5.4 ml) than SS A286 and SS 304. While gas production was consistent over multiple cycles with the SS and Ni materials, gas production with platinum sheet metal decreased with continued use. The total gas production during the first cycle using platinum was 34.5 ±
2.6 ml, but only 19.2 ± 1.3 ml by the third cycle. This change in gas production resulted in a higher variability of the gas produced with platinum than with the other metals.

The best performing alloys based on MEC recoveries and efficiencies were SS A286, SS 304 and Ni 625 (Table 5.2) ($E_{ap} = 0.9$ V). Of these three materials, SS A286 consistently had the best performance for all parameters used to evaluate the MECs ($r_{H2,cat}$, $r_{H2,COD}$, $\eta_E$, $\eta_{E+S}$, $I_v$, $Q$, and H$_2$ content). The hydrogen production rate was significantly higher for SS A286 ($Q = 1.5$ m$^3$/m$^3$/d) than for any of the other metals, including platinum ($Q = 0.68$ m$^3$/m$^3$/d). The platinum sheet metal displayed only average performance compared to the other metals, being surpassed by both SS 304 and SS A286 in terms of hydrogen recoveries and energy efficiencies at an applied voltage of 0.9 V.

Figure 5.1. Gas production of MEC’s with different stainless steels and nickel cathodes, compared to a platinum disk, at an applied voltage of 0.9 V.
Table 5.2. Summary of MEC results for different metal cathodes (stainless steel, nickel and platinum) at an applied voltage of 0.9V.

<table>
<thead>
<tr>
<th>Metal</th>
<th>$r_{H_2, cat}$ (%)</th>
<th>$r_{H_2, COD}$ (%)</th>
<th>$\eta_F$ (%)</th>
<th>$\eta_{E+S}$ (%)</th>
<th>$I_v$ (A m$^{-2}$)</th>
<th>$Q$ (m$^3$ m$^{-3}$ d$^{-1}$)</th>
<th>$H_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 304</td>
<td>53±1</td>
<td>49±0</td>
<td>90±2</td>
<td>38±1</td>
<td>100±4</td>
<td>0.59±0.01</td>
<td>77±1</td>
</tr>
<tr>
<td>SS 316</td>
<td>27±6</td>
<td>25±6</td>
<td>47±10</td>
<td>19±4</td>
<td>116±1</td>
<td>0.35±0.08</td>
<td>55±10</td>
</tr>
<tr>
<td>SS 420</td>
<td>43±2</td>
<td>38±1</td>
<td>73±3</td>
<td>30±1</td>
<td>122±10</td>
<td>0.58±0.07</td>
<td>67±2</td>
</tr>
<tr>
<td>SS A286</td>
<td>61±3</td>
<td>62±6</td>
<td>107±5</td>
<td>46±3</td>
<td>222±4</td>
<td>1.50±0.04</td>
<td>80±2</td>
</tr>
<tr>
<td>Ni 201</td>
<td>27±4</td>
<td>26±3</td>
<td>46±7</td>
<td>20±3</td>
<td>127±8</td>
<td>0.38±0.04</td>
<td>57±3</td>
</tr>
<tr>
<td>Ni 400</td>
<td>31±5</td>
<td>31±8</td>
<td>53±9</td>
<td>23±5</td>
<td>116±9</td>
<td>0.41±0.10</td>
<td>62±8</td>
</tr>
<tr>
<td>Ni 625</td>
<td>43±9</td>
<td>41±13</td>
<td>75±16</td>
<td>31±8</td>
<td>160±22</td>
<td>0.79±0.27</td>
<td>67±9</td>
</tr>
<tr>
<td>Ni HX</td>
<td>40±8</td>
<td>38±7</td>
<td>68±14</td>
<td>29±5</td>
<td>124±14</td>
<td>0.55±0.11</td>
<td>69±4</td>
</tr>
<tr>
<td>Pt</td>
<td>47±2</td>
<td>46±4</td>
<td>81±3</td>
<td>35±2</td>
<td>129±7</td>
<td>0.68±0.06</td>
<td>74±2</td>
</tr>
</tbody>
</table>

Overall gas production was reduced for all the metals at a lower applied voltage of 0.6 V (average = 6.8 ± 3.9 ml H$_2$) compared to 0.9 V (21.3 ± 3.8 ml H$_2$) as expected from previous studies [3] (Figure 5.2). Hydrogen concentrations at 0.6 V were reduced to 17.2 ± 13.2 % H$_2$ (vs. 67.5 ± 8.6 % H$_2$ at 0.9 V), and methane concentrations increased (69.0 ± 13.3 % at 0.6 V vs. 23.9 ± 8.3 % at 0.9 V). Ni 625 performed better than the other metals in terms of total hydrogen gas production at this lower applied voltage (6.61 ml H$_2$), but the product gas was mainly methane (47.3 % CH$_4$, 40.8 % H$_2$, 11.9 % CO$_2$). Platinum sheet metal produced only 11.2 ml H$_2$, with a gas composition of 49.8 % CH$_4$, 35.0 % H$_2$ and 15.1 % CO$_2$. Maximum current densities at 0.9 V were higher for both SS A286 (1.01 ± 0.18 mA/cm$^2$) and Ni 625 (0.73 ± 0.099 mA/cm$^2$) than for the platinum sheet metal (0.59 ± 0.03 mA/cm$^2$) (Figure 5.3). At 0.6 V, the difference between current densities of these metals was almost non-existent (0.25 ± 0.014 mA/cm$^2$ to 0.39 ± 0.014
Therefore, a higher applied voltage was needed to properly differentiate these metal surfaces.

![Graph showing gas production of MEC's with different stainless steels and nickel cathodes, compared to a platinum disk, at an applied voltage of 0.6 V.](image)

**Figure 5.2.** Gas production of MEC’s with different stainless steels and nickel cathodes, compared to a platinum disk, at an applied voltage of 0.6 V.
Figure 5.3. Current densities for MEC’s with a platinum disk, Ni 625 and SS A286 cathodes at applied voltages of 0.6 V and 0.9 V.

The performance of the metal alloys for use as cathodes in MECs was evaluated on the basis of the slopes and y-intercepts from Tafel plots (Table 5.3). The Tafel plots for SS A286 and platinum are shown as typical examples in Figure 5.4, with two linear regions: one at high current densities (dashed line) and one at low current densities (solid line). The larger Tafel slopes and y-intercepts indicate better catalytic performance. The Tafel slope is a function of the transfer coefficient $\alpha_c$ and the number of electrons $n_e$ transferred during the reaction. The y-intercept is controlled by the exchange current density $J_0$. The best cathodes based on Tafel slopes and y-intercepts were SS 286, Ni 625, Ni HX and platinum sheet metal, with slopes ranging from 3.68 to 4.31 decade A/cm$^2$/V and y-intercepts of 5.25 to 5.45 A/cm$^2$ at low current densities. V-intersect is the voltage
at which the linear regressions intersect. Ideally, it should be close to zero so that the
MEC operates at a high current density. SS 286 has the lowest V-intersect (0.45 V) of all
the metals tested. The ranking of the metal alloys based on electrochemical results thus
confirms the same relative performance of the materials observed in MEC tests.

Table 5.3. Tafel plots’s slopes and Y-intercepts for MEC’s with different metal cathodes.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Low Current Density</th>
<th></th>
<th>High Current Density</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (decade A cm²V⁻¹)</td>
<td>Y-intercept (A cm⁻²)</td>
<td>Slope (decade A cm²V⁻¹)</td>
<td>Y-intercept (A cm⁻²)</td>
<td>V-intersect (V)</td>
</tr>
<tr>
<td>Ni 625</td>
<td>-3.68</td>
<td>-5.37</td>
<td>-0.98</td>
<td>-3.94</td>
<td>-0.54</td>
</tr>
<tr>
<td>Ni HX</td>
<td>-3.70</td>
<td>-5.25</td>
<td>-0.91</td>
<td>-3.87</td>
<td>-0.51</td>
</tr>
<tr>
<td>Ni 201</td>
<td>-2.38</td>
<td>-4.73</td>
<td>-0.75</td>
<td>-3.74</td>
<td>-0.61</td>
</tr>
<tr>
<td>Ni 400</td>
<td>-2.30</td>
<td>-4.84</td>
<td>-0.76</td>
<td>-3.82</td>
<td>-0.67</td>
</tr>
<tr>
<td>SS 286</td>
<td>-4.44</td>
<td>-5.34</td>
<td>-0.88</td>
<td>-3.76</td>
<td>-0.45</td>
</tr>
<tr>
<td>SS 304</td>
<td>-2.18</td>
<td>-4.53</td>
<td>-0.64</td>
<td>-3.66</td>
<td>-0.56</td>
</tr>
<tr>
<td>SS 420</td>
<td>-2.94</td>
<td>-4.85</td>
<td>-0.88</td>
<td>-3.82</td>
<td>-0.49</td>
</tr>
<tr>
<td>SS 316</td>
<td>-2.39</td>
<td>-4.61</td>
<td>-0.94</td>
<td>-3.84</td>
<td>-0.53</td>
</tr>
<tr>
<td>Pt</td>
<td>-4.31</td>
<td>-5.45</td>
<td>-0.82</td>
<td>-3.75</td>
<td>-0.48</td>
</tr>
</tbody>
</table>
Figure 5.4. Tafel plots for MECs for (A) stainless steel 286 alloy and (B) platinum metal cathodes.
5.3.2. Particles on carbon cloth cathodes compared to metal sheet cathodes

The performance of the platinum sheet metal was compared to the higher surface area platinum particle catalyst bound on carbon cloth usually used in MEC studies. Current densities produced by the platinum sheet metal cathode at an applied voltage of 0.9 V (0.59 ± 0.03 mA/cm²) were similar to the current densities achieved by the platinum particle bound on carbon cloth at an applied voltage of 0.6 V (0.56 ± 0.03 mA/cm²). As expected, the MECs performed better with platinum particle catalyst than with the platinum metal sheet as similar current densities were achieved at lower applied voltages.

5.3.3. Nickel Oxide cathodes

The performance of the best MEC materials (SS A286 and Ni 625) was further improved by electrodepositing a nickel oxide layer on the surface of the sheet metal. Gas production increased from 9.4 to 25 ml for SS A286 and from 16.2 to 25 ml for Ni 625 (Figure 5.5) at an applied voltage of 0.6 V. Methane gas production was reduced from 6.8 to 4.1 ml for SS A286 and from 7.7 to 4.2 ml for Ni 625. Hydrogen production and recoveries were 4 to 40 times higher than the original values without the metal oxide (Table 5.4). Both nickel oxide modified metals reached similar hydrogen production and recovery values, suggesting the sheet metal was less of a factor than the metal oxide surface for performance. For example, energy recovery based on electrical input ($\eta_E$) increased from 3.1% (SS A286) and 31% (Ni 625) to 137 % for both SS A286 and Ni 625 plus nickel oxide. Volumetric hydrogen production rates ($Q$) also improved from
0.01 (SS A286) and 0.1 (Ni 625) to 0.76 m³/m³/d H₂ for both nickel oxide modified metals. In comparison, platinum sheet metal performance at applied 0.6 V was similar to the performance of metals without the nickel oxide layer (Table 5.4): low recoveries ($\eta_E=31\%$, $\eta_{E+S}=4\%$), low gas production ($Q=0.08$ m³/m³/d H₂) and low hydrogen content (H₂=36%).

**Figure 5.5.** Gas production of MECs with and without electrodeposited nickel oxide layers on SS A286 and Ni 625 at an applied voltage of 0.6V.
Table 5.4. Summary of MEC results for metal cathodes with electrodeposited nickel oxide layer, compared to platinum, at an applied voltage of 0.6V.

<table>
<thead>
<tr>
<th>Metal</th>
<th>$r_{H_2,\text{cat}}$ (%)</th>
<th>$r_{H_2, \text{COD}}$ (%)</th>
<th>$\eta_{E}$ (%)</th>
<th>$\eta_{E+S}$ (%)</th>
<th>$I_{v}$ (A m$^{-3}$)</th>
<th>$Q$ (m$^{3}$ m$^{-3}$ d$^{-1}$)</th>
<th>$\text{H}_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS A286</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>3.1±0.1</td>
<td>1.1±0.1</td>
<td>71±3</td>
<td>0.01±0.001</td>
<td>6±1</td>
</tr>
<tr>
<td>Ni 625</td>
<td>12±5</td>
<td>11±4</td>
<td>31±13</td>
<td>10±4</td>
<td>86±3</td>
<td>0.1±0.04</td>
<td>35±2</td>
</tr>
<tr>
<td>Pt</td>
<td>12±5</td>
<td>4±1</td>
<td>31±12</td>
<td>4±2</td>
<td>55±3</td>
<td>0.08±0.03</td>
<td>36±1</td>
</tr>
<tr>
<td>SSA286 + NiO$_x$</td>
<td>52±4</td>
<td>56±2</td>
<td>137±12</td>
<td>48±3</td>
<td>130±21</td>
<td>0.76±0.16</td>
<td>76±2</td>
</tr>
<tr>
<td>Ni625 + NiO$_x$</td>
<td>52±9</td>
<td>56±10</td>
<td>137±24</td>
<td>48±9</td>
<td>131±7</td>
<td>0.76±0.15</td>
<td>76±5</td>
</tr>
</tbody>
</table>

Stability of the MEC’s with nickel oxide cathodes was examined by running the reactors for 15 days (Figure 5.6). The initial high gas production and current densities decreased over the first few cycles. Current appeared to stabilize after the first three cycles, while gas production stabilized after seven cycles. The initial decrease in performance was confirmed through changes in the Tafel plot parameters (Table 5.5).

There was a 30% decrease in Tafel slope values between day 5 and day 15 (1.87 to 1.29 decade A/cm$^2$/V for Ni 625 + NiO$_x$; 1.54 to 1.04 decade A/cm$^2$/V for SS 286 + NiO$_x$), and a slight decrease in the y-intercept values (4.1 to 4.06 A/cm$^2$ for Ni 625 + NiO$_x$; 3.9 to 3.82 A/cm$^2$ for SS A286 + NiO$_x$).
Figure 5.6. Total gas and current production versus time with (A) Ni 625 + NiOx and (B) SS A286 + NiOx cathodes.
Table 5.5. Tafel plots’s slope and Y-intercepts for MECs with and without nickel oxide electrodeposited on Ni 625 and SS 286 alloys.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Day #</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni 625 + NiOx</td>
<td>5</td>
<td>-1.87</td>
<td>-4.10</td>
</tr>
<tr>
<td>Ni 625 + NiOx</td>
<td>15</td>
<td>-1.29</td>
<td>-4.06</td>
</tr>
<tr>
<td>SS 286 + NiOx</td>
<td>5</td>
<td>-1.54</td>
<td>-3.90</td>
</tr>
<tr>
<td>SS 286 + NiOx</td>
<td>15</td>
<td>-1.04</td>
<td>-3.82</td>
</tr>
</tbody>
</table>

The surfaces of the metal oxides deposited on SS A286 observed using SEM (Figure 5.7) indicate finer structures before use, compared to dull structures 8 days after use. The metal composition by SEM-EDS (Table 5.6) shows 15 times higher weight percent values for nickel compared to oxygen on the “before” electrodeposited sample, which is equivalent to 4.2 atoms of nickel per atom of oxygen. The SEM-EDS analysis also shows small metal composition differences in the non-modified SS A286 and Ni 625 alloys before and after use, but large differences in the nickel oxide on SS A286 cathode. The most important finding was the decrease in nickel content (from 52.4 to 21.8 wt %), suggesting that some of the oxide layer was removed from the surface. There were increases in chromium (from 7.98 to 11.3 wt %), oxygen (from 3.5 to 8.5 wt %), and iron (from 31.2 to 43.9 wt %). The increase in chromium and iron suggests that the stainless steel metal contributed 40% more to the “after” composition compared to the “before” composition. Carbon, phosphorus, sodium and sulfur also increased, perhaps as a result of exposure to buffer and bacteria.
Figure 5.7. SEM images of SS A286 + NiOx (A and C) before and (B and D) after 8-day use as cathode in MEC. A and B are at 2,000× magnification and C and D are at 20,000× magnification.

Table 5.6. Metal composition by SEM-EDS before and after 8 days of use in MEC as cathode.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Ni 625 Before (wt%)</th>
<th>Ni 625 After (wt%)</th>
<th>SS A286 Before (wt%)</th>
<th>SS A286 After (wt%)</th>
<th>SS A286 + NiOx Before (wt%)</th>
<th>SS A286 + NiOx After (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.56</td>
<td>7.36</td>
<td>3.58</td>
<td>7.70</td>
<td>2.98</td>
<td>5.17</td>
</tr>
<tr>
<td>O</td>
<td>1.76</td>
<td>2.80</td>
<td></td>
<td></td>
<td>3.50</td>
<td>8.54</td>
</tr>
<tr>
<td>Cr</td>
<td>17.22</td>
<td>16.10</td>
<td>14.80</td>
<td>14.10</td>
<td>7.98</td>
<td>11.32</td>
</tr>
<tr>
<td>Fe</td>
<td>6.04</td>
<td>5.90</td>
<td>58.70</td>
<td>53.70</td>
<td>31.20</td>
<td>43.95</td>
</tr>
<tr>
<td>Ni</td>
<td>61.90</td>
<td>60.00</td>
<td>19.50</td>
<td>17.90</td>
<td>52.40</td>
<td>21.80</td>
</tr>
<tr>
<td>Mo</td>
<td>7.49</td>
<td>7.85</td>
<td>1.22</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td></td>
<td></td>
<td>2.24</td>
<td>1.77</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.41</td>
<td></td>
</tr>
</tbody>
</table>
5.4. Discussion

5.4.1. Cathode Efficiency

Platinum has been assumed to be the most efficient catalyst for electrohydrogenesis in MECs. The results obtained here, however, show that the performance of platinum can be surpassed by certain stainless steel and nickel alloys. In all cases, for example, SS A286 showed better performance than platinum and the other alloys evaluated in terms of hydrogen gas production, total gas production, cathodic hydrogen recoveries ($r_{H_2, \text{cat}}$) and energy recoveries ($\eta_E, \eta_{E+S}$). Furthermore, the volumetric hydrogen production rate ($Q$) for SS A286 was 4.3 times higher than the SS 316, and 2.2 times better than platinum sheet metal disk. Tafel slopes and intercepts confirmed the superior performance of SS A286 and the general ranking of the other alloys evaluated in MEC tests.

Based on the composition of the SS A286 compared to the other materials, the nickel/iron composition of this alloy appears to be an important factor in their hydrogen evolution performance. The SS A286 has the most nickel (24-27%) compared to the other stainless steel alloys tested, and Ni 625 (the best performing nickel alloy) had the second highest iron content (2.5%) of the nickel alloys tested.

The decrease of platinum activity with use (Figure 1) can be due to metal poisoning. Poisonous chemicals can reduce chemical activity and the number of active sites for hydrogen production. Several chemicals can poison the catalytic activity of platinum, such those containing sulfur, hexamethyldisiloxane (HDMS), nitrogen, silicon, nitric oxide and carbon monoxide [25, 26]. In an MEC environment, sulfur and nitrogen
are present and could be contributing to platinum poisoning. This poisoning is not observed in the powder platinum catalyst applied with Nafion™ on carbon cloth, perhaps due to the protection provided by the Nafion™.

There was substantial methane production at the lower applied voltage, but the amount of methane produced decreased in tests at the higher applied voltage. The increased methane production rates at the lower voltage can be explained by the longer cycle times (36 to 80 hours) at 0.6 V, compared to shorter cycle times (~24 hrs) at 0.9 V. The metal cathode with the shortest cycle time at applied 0.6 V (Ni 625; 36 hrs) also had the highest gas production rate and hydrogen concentration. Long cycle times facilitate growth of methanogens. These microorganisms convert organic matter to methane, but they grow slowly. Methanogens decrease hydrogen gas levels by competing for organic matter in the media (acetate), and scavenging existing hydrogen gas to form methane [27]. This increased methane generation concentrations at longer cycle times has also been observed using platinum on carbon cloth cathodes in MEC tests [3].

The use of nickel oxides on the cathode is a promising method for increasing MEC performance, but the material must be stabilized. When a nickel oxide layer was applied to the cathode by electrodeposition, current densities, total hydrogen gas production, cathodic recoveries, energy efficiencies, and hydrogen production rates improved by a factor of four. It was also found that the MEC provided good performance, even at the lower applied voltage of 0.6 V. The use of a lower voltage significantly improved the process energy efficiency based on energy input, for example, from $\eta_E = 3.1\%$ (SS A286) and $\eta_E = 31\%$ (Ni 625) to $\eta_E = 137\%$ (nickel oxide on either metal surface). However, performance of the metal oxides showed an initial decrease but
stabilized with repeated cycles, and thus additional work is needed to maintain the performance of these materials over time. Also, future work should address to what extent improvement in initial performance was due to a surface area, and if other metals used as a base for the metal oxide would be a factor in performance.

5.4.2. Cathode Costs

The cost of the metal sheets used here represents a one to two order of magnitude decrease in costs compared to traditional Pt and carbon cloth cathodes. The cost of the sheet metals varied from $73/m² (SS 304) to $370/m² (Ni 625) (www.mcmaster.com). A typical platinum particle catalyst on a carbon cloth cathode costs ca. $2,300/m² based on $850/m² for the carbon cloth (BASF Fuel Cell, Inc., New Jersey), $700/m² for the platinum particle catalyst (BASF Fuel Cell, Inc., New Jersey), and $750/m² for the binder (Nafion™, Sigma-Aldrich, Missouri). To put these costs in perspective, for an MEC with 100 m² of surface area per cubic meter of reactor volume (100 m²/m³), the cost would be $730 for a sheet metal cathode compared to $200,300 for a typical platinum catalyst on carbon cloth. This cost could be further decreased by using this metal in different surface configurations and thicknesses.

There are additional costs to deposit nickel oxide onto the sheet metal. The cost to treat 100 m² of cathode is estimated to be $240 (1 cm³ of solution per 1 cm² of cathode area) for $0.236 per liter of solution (0.012 M NiSO₄ at $0.196/L, 20 mM (NH₄)₂SO₄ at $0.031/L, H₂SO₄ at $0.01/L) (www.sial.com). The electrical requirement needed for the process is negligible (< 1¢/m² at 10.9¢/kWh)
as the required voltage is only applied for 30 seconds. Therefore, the addition of a nickel oxide layer on the cathode is minor compared to the cost of the support material.

5.5. Conclusions

Performance of stainless steel and nickel alloys was similar or better than platinum sheet metal when used as cathodes in MECs. Stainless steel A286 showed the best performance of all the alloys tested at an applied potential of 0.9 V. Lower applied voltages resulted in long cycle times and low hydrogen production rates. A nickel oxide layer electrodeposited on the metal surfaces improved their catalytic performance by at least a factor of four. Mechanical long term stability of the nickel oxide layer needs to be improved by evaluating combinations of different supports and catalysts, and possibly binding agents. The nickel oxide layer and sheet metal support are relatively inexpensive and good candidates for large scale applications.

5.6. Acknowledgements

The authors thank S. Cheng, D. Call, E. Lalaurette and D. Jones for assistance with MEC experiments, and J.M. Perez and W.A. Lloyd for their advice and insight. This research was supported in part by the Global Research Partnership (GRP) from KAUST University, the General Electric First-Year Faculty for the Future Fellowship and the Arthur and Elizabeth Rose Memorial Fellowship, and Air Products and Chemicals, Inc.
5.7. References


Chapter 6

Hydrogen production with nickel powder cathode catalysts in microbial electrolysis cells

Abstract

Although platinum is commonly used as catalyst on the cathode in microbial electrolysis cells (MEC), non-precious metal alternatives are needed to reduce costs. Cathodes were constructed using a nickel powder (0.5-1 µm) and their performance was compared to conventional electrodes containing Pt (0.002 µm) in MECs and electrochemical tests. The MEC performance in terms of Coulombic efficiency, cathodic, hydrogen and energy recoveries were similar using Ni or Pt cathodes, although the maximum hydrogen production rate was slightly lower for Ni ($Q=1.2-1.3 \text{ m}^3/\text{m}^3/\text{d}$; 0.6 V applied) than Pt (1.6 m$^3$/m$^3$/d). Nickel dissolution was minimized by replacing medium in the reactor under anoxic conditions. The stability of the Ni particles was confirmed by examining the cathodes after 12 MEC cycles using scanning electron microscopy and linear sweep voltammetry. Analysis of the anodic communities in these reactors revealed dominant populations of Geobacter sulfurreducens and Pelobacter propionicus. These

---

4 Material presented in this chapter is in press: Sellembo PA, Merrill MD, Logan BE. Hydrogen production with nickel powder cathode catalysts in microbial electrolysis cells. Int. J. Hydrogen Energy. Accepted on Nov. 3rd 2009.
results demonstrate that nickel powder can be used as a viable alternative to Pt in MECs, allowing large scale production of cathodes with similar performance to systems that use precious metal catalysts.
6.1. Introduction

Most current hydrogen production methods use processes such as steam reforming and coal gasification that rely on non-renewable energy sources [1]. Electrohydrogenesis using microbial electrolysis cells (MEC) is a promising approach for hydrogen production from organic matter, including wastewater and other renewable resources [2, 3]. In an MEC, exoelectrogenic bacteria oxidize organic matter into CO₂, electrons, and protons. The bacteria transfer electrons from the oxidation reaction to the anode and release protons to the solution. Hydrogen gas is formed by the reaction between electrons and protons at the cathode, achieved by adding a supplemental voltage (0.114 V in theory for acetate as the substrate) to that produced by the bacteria (≥ 0.2 V) to overcome the endothermic barrier of hydrogen formation (0.414 V).

Most MEC research has been done using cathodes containing a Pt catalyst, with the Pt accounting for the greatest percentage of the cost of the MEC electrodes [2, 4, 5]. The high cost of Pt and its susceptibility to poisoning has led researchers to examine non-precious metals and alternatives. MECs with stainless steel (SS) brush cathodes (type 304) produced hydrogen ($Q = 1.7 \text{ m}^3/\text{m}^3 \text{ d}$, applied voltage of $E_{ap} = 0.6 \text{ V}$) at rates similar to those containing platinum ($Q=0.5-2.0 \text{ m}^3/\text{m}^3 \text{ d}$) due to the catalytic activity of the SS and the increased surface area of the brush [6]. Electrodeposited nickel alloys (NiMo and NiW) were used in MEC cathodes that produced hydrogen at a rate of $Q= 2.0 \text{ m}^3/\text{m}^3 \text{ d}$ ($E_{ap}=0.6 \text{ V}$) [7]. An MEC with a cathode lacking a metal catalyst produced hydrogen at a lower rate of $Q= 0.57 \text{ m}^3/\text{m}^3 \text{ d}$ and higher applied voltage of $E_{ap}=1 \text{ V}$ [8]. MECs with
biocathodes have also shown promise, achieving $Q=0.63 \text{ m}^3/\text{m}^3 \text{ d}$ at $E_{ap}=0.7 \text{ V}$ [9].

Tungsten carbide was recently examined as a catalyst for hydrogen production under neutral pH conditions [10], but it was not evaluated in an MEC.

Nickel, nickel alloys, and SS are widely used as cathodes for alkaline water electrolysis due to their relatively good catalytic activity, high corrosion stability, and low cost [11]. Their performance has been well studied using various electrochemical techniques, such as cyclic voltammetry and linear sweep voltammetry (LSV), but only under alkaline conditions [12-18] or at high temperatures. High temperatures (450-700°C) are used for hydrogen production by the water-gas shift reaction from carbon monoxide [19] or steam reforming [20]. MECs typically operate at neutral pHs and at temperatures (20-30°C) suitable for microbial growth. Thus, the hydrogen evolution activity of metal catalysts needs to be better understood under these less optimum conditions.

Flat sheet metal cathodes made from stainless steel and Ni have been shown to perform similar to, or better than, Pt sheet cathodes based on LSV scans and MEC tests under neutral pH conditions and ambient temperatures [21]. Electrodeposition of nickel oxide onto the stainless steel and nickel alloy surfaces improved performance to $Q=0.76 \text{ m}^3/\text{m}^3 \text{ d}$ from $Q=0.01-0.1 \text{ m}^3/\text{m}^3 \text{ d}$ (no Ni oxide) at $E_{ap}=0.6 \text{ V}$. While these studies are useful for understanding the performance of materials under well defined conditions, it is not necessary to construct the electrode from one solid piece of material. For example, the Pt used in MEC cathodes consists of nano-sized particles in order to minimize total mass of the material, and thus material costs.
In this study, we constructed cathodes made with commercially available nickel and SS powders with different sizes at different metal loadings, and compared their performance to platinum powder cathodes. The various cathodes were screened for their performance in neutral pH solutions and at 30°C using LSV. The most promising cathodes, based on the lowest overpotentials in LSV scans, were then tested in single chamber MECs for hydrogen gas production.

6.2. Materials and methods

6.2.1. Cathodes

Commercially-available metal powders of nickel (2-10 µm), nickel oxide (≤ 74 µm), and stainless steel catalysts (≤ 140 µm) were obtained from Alfa-Aesar, MA. Filamentous nickel powders with smaller particle sizes were obtained from INCO specialty products, NJ (Ni 210: 0.5-1 µm, Ni 110: 1-2 µm and Ni 255: 2.2-2.8 µm; all >99% pure). Cathodes were made by mixing the metal powder with Nafion™ binder (Sigma-Aldrich, MO), and applying the mixture using a brush onto carbon cloth (surface area 7 cm², 30% wet proof, BASF Fuel Cell, NJ). Platinum catalyst was used as a control (0.002 µm) (10 wt% on Vulcan XC-72; BASF Fuel Cell, NJ).

Nickel oxide was electrodeposited on carbon cloth by applying 20 V at ~1.5 A for 40 s (1696 power source, B&K Precision, USA) with an anode stainless steel brush (SS type Cronifer 1925 HMo, made in house) in a solution containing 18 mM NiSO₄ and 35 mM (NH₄)₂SO₄ at a pH = 2.0 (adjusted by adding H₂SO₄) [21, 22]. Carbon cloth
cathodes were prepared before electrodeposition by applying a base coat of carbon black (CB, 5 mg/cm²) and Nafion™ (33 µL/cm²).

### 6.2.2. Electrochemical evaluation of catalysts

Performance of the cathodes was evaluated by LSV using a potentiostat (PC4/750TM, Echem Analyst, v. 5.5, Gamry Instruments, PA). The cathodes were placed in electrochemical cells (4 cm long by 3 cm diameter) with an Ag/AgCl reference electrode and platinum wire counter electrode in 2 mM phosphate buffer solution (pH 7.0). LSV scans from -0.4 to -1.4 V with IR compensation (to compensate for the ohmic drop between the working and reference electrode) were repeated three times, at a scan rate of 2 mV/s.

### 6.2.3. MEC reactor construction

Single-chamber MECs made of Lexan were 4 cm long containing 3 cm diameter cylindrical-shaped chambers [23]. Anodes were ammonia-treated graphite fiber brushes (25 mm diameter × 25 mm length, 0.22 m² surface area) made with a titanium wire twisted core [24, 25]. The anodes were first enriched with bacteria in microbial fuel cells (MFCs) containing conventional Pt-catalyst air cathodes [26] that were inoculated using a solution from an acetate-fed MFC reactor that had been running for over two years [27]. Duplicate reactors were operated in fed-batch mode using acetate (1 g/L) and a 50 mM phosphate buffer nutrient medium (pH 7) [28] in a 30°C temperature room. After at least
three repeatable cycles, the MFCs were modified to function as MECs by replacing the cathodes and sealing the end of the reactors from air, providing an oxygen-free environment. The voltage needed for MECs was supplied via an external power source (3645A; Circuit Specialists, Inc, Arizona). After each fed batch cycle (when gas production stopped), the reactors were drained, exposed to air for 15 minutes to minimize growth of methanogens [23] (except as noted), refilled with substrate solution, and sparged with ultra high purity nitrogen gas for five minutes. For tests done under complete anaerobic conditions, the reactors were drained and refilled inside an anaerobic glove box (N₂/H₂ volume ratio of 95/5). In this case, it was not necessary to sparge the reactors with nitrogen.

**6.2.4. Analysis after MEC cycles**

Continuous gas production was measured using a respirometer (AER-200, Challenge Technology, AZ), with the gas collected in gas bags (100 ml capacity, Cali-5 bond, Calibrated Instruments Inc., NY). The composition of the gas in the MEC headspace and gas bags was analyzed using two gas chromatographs (models 8610B and 310, SRI Instruments, CA) with molesieve columns (5A 80/100, Alltech, IL) and thermal conductivity detectors. Argon was used as the carrier gas for H₂, O₂, N₂ and CH₄ analysis, and helium was used as the carrier gas for CO₂ analysis.

Cathodes were examined using scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDS) at 20 kV (Quanta 200, FEI, OR). Soluble nickel was analyzed via inductively coupled plasma atomic emission spectroscopy (ICP-AES;
Optima 5300DV, Perkin-Elmer, MA) at a detection limit of 0.01 ppm. Surface area was obtained by multipoint BET (Brunauer, Emmett, and Teller) based on nitrogen adsorption (ASAP 2020, Micromeritics, GA).

6.2.5. Calculations

The calculated total geometric surface area of the catalyst particles in the cathodes, $A_{b,p}$ (m$^2$), is:

$$A_{b,p} = \frac{A_p m_p}{V_p \rho_{b,p}} = \frac{4 \pi r^2 m}{4/3 \pi r^3 \rho_{b,p}} = \frac{3m}{\rho_{b,p} r}$$  \hspace{1cm} (1)

where $A_p$ is the surface area of a single particle; $V_p$ the volume of particles calculated using the average particle radius, $r$; $\rho_{b,p}$ the bulk density of the particle (provided by the manufacturer); and $m$ the mass of catalyst added to the cathode.

The performance of the MEC reactors was evaluated as previously described [2, 21] in terms of: Coulombic efficiency (CE) (%) based on total Coulombs recovered compared to the initial mass of substrate; cathodic hydrogen recovery ($r_{H2,cat}$) (%) or the recovered electrons as hydrogen compared to the current transferred; overall hydrogen recovery ($r_{H2,COD}$) (%), defined as the percentage of hydrogen recovered compared to the theoretical maximum based on added substrate; volumetric current density ($I_v$) (A/m$^3$), calculated from the maximum current production over a 4-hr period normalized to the volume of solution; volumetric hydrogen production rate ($Q$) (m$^3$ H$_2$/m$^3$ d) based on hydrogen gas produced normalized to the reactor volume; energy recovery relative to
149
electrical input ($\eta_E$) (%); and overall energy recovery ($\eta_{E+S}$) (%) based on both electrical input and heat of combustion of the substrate ($\Delta H_{\text{facelet}}=870.28$ kJ/mol).

6.2.6. Community Analysis

Community analysis of the anode biofilm was performed at the end of 12 cycles for an MEC containing either a Ni or Pt catalyst cathode. Community analysis was not performed on the cathode community as there was no visible biofilm on the electrode surface. Bacteria were extracted from the anode by cutting fibers from the brush using sterile scissors, and vortexing them in sterile buffer with glass beads. The DNA was extracted using Power Soil DNA isolation kit (MoBio laboratories, CA). The 16S rRNA gene fragment of the extracted DNA was amplified by PCR using 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1541R (5’-AAGGAGGTGATCCAGCC-3’) universal primers. Amplified fragments were purified with a QIAquick PCR purification kit (Qiagen, JAPAN), and cloned into *Escherichia coli* competent cells (TOPO TA cloning kit, Invitrogen, MD). Plasmids were extracted using the spin protocol of the EZ96 Fastfilter kit (Omega Bio-tek, GA). Forty-six plasmids from each sampled reactor were sequenced with the T7 primer on the TOPO plasmid (ABI 3730XL DNA Analyzer, Applied Biosystems, CA). Database searches for related gene sequences were conducted through the GenBank nucleotide sequence database using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences of 16S rRNA genes were aligned and used to generate a phylogenetic tree using the MEGA 4.0.2 program, neighbor-
joining method with bootstrap test (500 replicates). Only closest matches (≥90% similarity) were included.

6.3. Results

6.3.1. Cathode selection by LSV

An MEC with a Pt catalyst typically produces 4-6 mA, or 0.6-0.9 mA/cm² (7 cm² cathode projected surface area). Overpotentials for metal catalysts of different sizes and loadings, and with different amounts of binder, were compared at a current density in this range (0.63 mA/cm² = -3.2 log A/cm²) to better predict their performance relative to MEC conditions (Table 6.1). Both Ni 210 on carbon cloth (60 mg Ni, 267 µL Nafion) and the standard Pt cathode (10 mg Pt, 400 µL Nafion) had the same low overpotential of -0.500 V at this current density (Table 6.1). Current densities produced with these two materials were also very similar over the complete range of applied voltages (Figure 6.1).
Table 6.1. Overpotentials vs SHE at current density of -3.2 log A/cm² for cathodes during third LSV scan at 2 mV/s. Surface area calculated using equation (1), NA – not applicable, ND- not determined.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Particle size (µm)</th>
<th>Surface Area (m²)</th>
<th>Qty (mg)</th>
<th>CB (mg)</th>
<th>Nafion (µL)</th>
<th>Potential (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (CB)</td>
<td>NA</td>
<td>0.00</td>
<td>0</td>
<td>60</td>
<td>400</td>
<td>-0.970</td>
</tr>
<tr>
<td>Platinum</td>
<td>0.002</td>
<td>1.45</td>
<td>10</td>
<td>50</td>
<td>400</td>
<td>-0.500</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>0</td>
<td>267</td>
<td>-0.500</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>0</td>
<td>300</td>
<td>-0.583</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>0</td>
<td>325</td>
<td>-0.713</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>0</td>
<td>375</td>
<td>-0.713</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>0</td>
<td>400</td>
<td>-0.720</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.60</td>
<td>60</td>
<td>30</td>
<td>400</td>
<td>-0.668</td>
</tr>
<tr>
<td>Ni 110</td>
<td>1-2</td>
<td>0.17</td>
<td>60</td>
<td>30</td>
<td>400</td>
<td>-0.720</td>
</tr>
<tr>
<td>Ni 255</td>
<td>2.2-2.8</td>
<td>0.23</td>
<td>60</td>
<td>30</td>
<td>400</td>
<td>-0.721</td>
</tr>
<tr>
<td>Ni 10255</td>
<td>2.2-3</td>
<td>0.24</td>
<td>60</td>
<td>30</td>
<td>400</td>
<td>-0.760</td>
</tr>
<tr>
<td>Ni 10256</td>
<td>3-7</td>
<td>0.03</td>
<td>60</td>
<td>30</td>
<td>400</td>
<td>-0.739</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.45</td>
<td>30</td>
<td>0</td>
<td>400</td>
<td>-0.747</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>1.35</td>
<td>90</td>
<td>0</td>
<td>400</td>
<td>-0.727</td>
</tr>
<tr>
<td>Ni 210</td>
<td>0.5-1</td>
<td>0.45</td>
<td>30</td>
<td>30</td>
<td>400</td>
<td>-0.683</td>
</tr>
<tr>
<td>Ni 10255</td>
<td>2.2-3</td>
<td>0.23</td>
<td>60</td>
<td>0</td>
<td>400</td>
<td>-0.760</td>
</tr>
<tr>
<td>Ni 10256</td>
<td>3-7</td>
<td>0.03</td>
<td>60</td>
<td>0</td>
<td>400</td>
<td>-0.740</td>
</tr>
<tr>
<td>NiO 87302</td>
<td>74</td>
<td>0.001</td>
<td>60</td>
<td>0</td>
<td>400</td>
<td>-1.110</td>
</tr>
<tr>
<td>eNiOx</td>
<td>0.001</td>
<td>ND</td>
<td>ND</td>
<td>60</td>
<td>400</td>
<td>-0.800</td>
</tr>
<tr>
<td>SS 316</td>
<td>16</td>
<td>0.01</td>
<td>60</td>
<td>0</td>
<td>400</td>
<td>-1.140</td>
</tr>
<tr>
<td>SS 316</td>
<td>150</td>
<td>0.002</td>
<td>120</td>
<td>0</td>
<td>400</td>
<td>-0.863</td>
</tr>
<tr>
<td>SS 410</td>
<td>150</td>
<td>0.002</td>
<td>120</td>
<td>0</td>
<td>400</td>
<td>-0.913</td>
</tr>
<tr>
<td>SS 304</td>
<td>150</td>
<td>0.002</td>
<td>120</td>
<td>0</td>
<td>400</td>
<td>-0.813</td>
</tr>
<tr>
<td>SS 303</td>
<td>150</td>
<td>0.002</td>
<td>120</td>
<td>0</td>
<td>400</td>
<td>-0.953</td>
</tr>
</tbody>
</table>
Figure 6.1. Tafel plots for select experimental cathodes in 2mM phosphate buffer, scan rate 2 mV/s, third scan.

Increasing the amount of binder added to the cathode from 267 µL to 400 µL reduced performance, while changing the mass of Ni added to the cathode (60 to 90 mg, with 400 µL of binder) did not improve performance of Ni 210. Increasing the surface area was expected to improve current density [29]. This suggests that the effective Ni surface area was not increased when more Ni was added for tests with the higher volume of binder (400 µL). This also may explain why increasing the volume of Nafion binder increased overpotentials, as this likely resulted in less available surface area of the Ni due to increased coverage of the Ni by the binder.

The addition of 30 mg of CB lowered the overpotential of Ni 210 to -0.668 V from -0.720 V at the same Ni (60 mg) and Nafion loading (400 µL). LSV scans when CB was added to Ni with other particle sizes (Ni 10255 and 10256), however, did not show
any changes in the current densities across the range of potentials examined. CB may have affected the catalytic activity of the Ni 210 cathode by increasing surface area, improving the application of the metal/binder layer due to the carbon’s increased porosity, and increasing chemical stability. Addition of carbon did not change the conductivity of the prepared cathodes (Pt: 1.2 Ω, Ni: 1.1 Ω, Ni+CB: 1.1 Ω), based on measurements with a handheld multimeter (Fluke 87V True RMS Multimeter, Everett, WA) of the dry electrode before it was placed in the MEC.

In general, a decrease of particle size lowered the cathode overpotential for the smaller particle sizes (0.5 to 3 µm) but not for the larger particles (3-7 µm). This lack of direct relationship between particle size and current densities was also observed by others using cyclic voltammetry for nickel powders in alkaline solutions [30] as the catalytic activity can also be affected by other factors such as porosity and shape of the particles.

We were not able to obtain the same size particles for all metals. On the basis of the same weight of materials, however, SS 316 (16 µm) and NiO 87302 (74 µm) had higher overpotentials than nickel (0.5-7 µm), electrodeposited nickel (0.001 µm), or plain CB (Table 6.1). On the basis of similar calculated surface areas (0.001-0.002 m²), NiO had higher overpotentials than any of the stainless steels (150 µm) tested and CB. SS 316 -16 um had higher overpotentials than SS 316 - 150 µm (lower surface area) and Ni 10256 (similar surface area).

Stainless steel 304 (140 µm) had lower overpotentials at -3.2 log A/cm² than the other stainless steel materials tested, with overpotentials increasing in the order SS 304, SS 316, SS 410 and SS 303. This finding is consistent with our previous MEC study where sheet metal cathodes made from SS 304 performed better than other stainless
steels and nickel alloys [21]. In that study, however, overpotentials of MEC cells with used cathodes made of SS 304 were similar to overpotentials of SS 316 (SS 410 and SS 303 were not tested). The difference in overpotentials observed between these two studies could be due to particle geometry, interactions with the binder, effect of microbes and use.

6.3.2. MEC performance

The two metal powder and binder combinations that produced the lowest overpotentials in LSV scans (Ni 210 with 267 µL Nafion, and Ni 210+CB with 400 µL Nafion) (Table 6.1) were used as cathodes in MECs, and their performance was compared to the same reactors with Pt cathodes. Electrodeposited nickel oxide (eNiOx) was also chosen as an MEC cathode due to its good performance in a previous study [21]. The resulting BET total surface areas were 4.31 m²/g (Ni 210), 11.8 m²/g (Ni210+CB), 17.3 m²/g (eNiOx), and 11.2 m²/g (Pt).

6.3.2.1 Volumetric gas production and composition

The MECs with two nickel cathodes (Ni 210 and Ni 210+CB) produced gas similar in volume and composition to that obtained with Pt cathodes, but the performance was different for the eNiOx cathode (Table 6.2, Figure 6.2). Although the volume of gas produced was initially similar for all these electrodes, the volume of gas decreased for reactors with the eNiOx catalyst after 12 cycles compared to the Ni and Pt catalysts.
Gas composition was always very similar for all cathodes, with 92% H₂ for the Ni and Pt catalysts, and 94% H₂ for eNiOx (Table 6.2).

**Table 6.2.** Summary of MEC results for Ni210, Ni210+CB, eNiOx and Pt catalyst cathodes at an applied voltage of 0.6V, eighth cycle of operation.

<table>
<thead>
<tr>
<th>Metal</th>
<th>H₂  (±) (%)</th>
<th>Iv (±) (A/m³)</th>
<th>Q (±) (m³/m³ d)</th>
<th>CE (±) (%)</th>
<th>rₖ₂,cat (±) (%)</th>
<th>rₖ₂,COD (±) (%)</th>
<th>η (±) (%)</th>
<th>ηₑ+S (±) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni210</td>
<td>92±0</td>
<td>160±31</td>
<td>1.3±0.3</td>
<td>92.7±15.8</td>
<td>79±10</td>
<td>73±3</td>
<td>210±40</td>
<td>65±2</td>
</tr>
<tr>
<td>Ni210+CB</td>
<td>92±1</td>
<td>139±2</td>
<td>1.2±0.1</td>
<td>83.8±1.2</td>
<td>94±5</td>
<td>79±5</td>
<td>252±12</td>
<td>73±4</td>
</tr>
<tr>
<td>eNiOx</td>
<td>94±0</td>
<td>103±4</td>
<td>0.9±0.1</td>
<td>87.1±2.3</td>
<td>86±1</td>
<td>75±1</td>
<td>215±8</td>
<td>67±0</td>
</tr>
<tr>
<td>Pt</td>
<td>92±2</td>
<td>186±4</td>
<td>1.6±0.0</td>
<td>85±6.4</td>
<td>89±7</td>
<td>75±0</td>
<td>239±21</td>
<td>70±2</td>
</tr>
</tbody>
</table>
Figure 6.2. (A) Total gas production and (B) maximum current for MECs with Ni210, Ni210+CB, eNiOx or Pt catalyst cathodes, as a function of cycle number at an applied voltage of 0.6 V. Gas production for cycles 1-6 were not recorded for Pt and eNiOx due to equipment malfunction.
6.3.2.2 Current production

The maximum current produced with Pt cathodes (5.6 to 6.3 mA) (Figure 2B) was larger than with the other cathodes, and performance was consistent over 12 MEC cycles. The length of the cycle time increased in the following order: Pt, Ni210 (with and without CB) and eNiOx. For example, the time to reach the same low voltage of 0.02 V was 5-10 hours longer for Ni210 than Pt depending on the specific cycle. The maximum current increased for the two Ni cathodes over the first 6 cycles by as much as 4.7 mA for Ni 210, and 4.1 mA for Ni 210+CB. The current decreased by 3.3 mA, however, for eNiOx over the same 6 cycles (Figure 2B). The change in these maximum current densities indicated that the systems had not reached stable and steady conditions. There are a number of factors that could affect changes in the current densities over these cycles, including the possibility that the characteristics of the bacterial biofilm or microbial community was changing, or changes in cathode surface area due to metal oxidation or to separation of particles from the carbon cloth support. However, following the sixth cycle all the cathodes showed repeatable maximum current densities.

6.3.2.3 Coulombic efficiency, recoveries and hydrogen production rates at 0.6 V

Coulombic efficiency, cathodic recovery, hydrogen recovery, energy recovery based on electrical input or overall energy recovery were the same (within ± 1 S.D.) for all cathodes used in MECs. These parameters are not directly correlated to each other because they are calculated for different parts of the cycle. For example, $I_v$ and $Q$ were based on the initial maximum current (averaged over a period of 2 hours), while
Coulombic efficiency was calculated from the measured current over a complete cycle. As cycle times were longer with Ni210 cathodes, it was possible to obtain higher values for CE\textsubscript{s} but lower I\textsubscript{Cs} for MECs with Ni210 compared to those with Pt cathodes. The only MEC performance parameter that varied among the tested cathodes was the hydrogen production rate. The production rate was slightly lower for Ni 210 (Q=1.2-1.3 m\textsuperscript{3}/m\textsuperscript{3}/d) than for Pt (Q=1.6 m\textsuperscript{3}/m\textsuperscript{3}/d) even though gas production and composition was similar for Pt and Ni 210 cathodes (Figure 2A). This lower production rate follows the lower maximum current density produced with Ni 210 (139-160 A/m\textsuperscript{3}) than with Pt (186 A/m\textsuperscript{3}) (Table 2). Q was lower for eNiOx than the other cathodes due to both a lower gas production rate and lower maximum current density (103 A/m\textsuperscript{3}).

6.3.2.4 MEC performance with Ni210 cathodes as a function of applied voltage

Hydrogen production rates with the two Ni cathodes increased with applied voltage and were not significantly different from each other, with the largest rates produced at the highest applied voltage of 0.8 V (Q = 1.85 m\textsuperscript{3}/m\textsuperscript{3}/d, Ni 210) (Figure 3A). There was no hydrogen production with the Ni catalysts at an applied voltage of 0.3 V, compared to 0.2 V previously observed with a Pt catalyst [23]. Coulombic efficiencies (CE) decreased slightly with applied voltage (89% at 0.8 V, to 81% at 0.4 V) (Figure 3B). Cathodic hydrogen recovery reached a maximum at 0.7 V (Ni 210=93%, Ni 210+CB=91%). Similarly, energy recovery based on electrical input (\(\eta_E\)) and overall energy recovery (\(\eta_{E+S}\)) increased with increasing applied voltage, with the maximum values for \(\eta_E\) at 0.8 V of 240%, and for \(\eta_{E+S}\) at 0.7 V of 74%.
\[ y = 3.7768x - 1.2627 \quad R^2 = 0.9828 \text{ Ni} \]

\[ y = 3.6272x - 1.1708 \quad R^2 = 0.9919 \text{ Ni+CB} \]
Figure 6.3. MEC performance for Ni210 catalyst cathodes at different applied voltages. (A) hydrogen production rate (B) cathodic recovery and Coulombic efficiency (C) energy recovery based on electrical input and overall energy recovery.

6.3.3. Cathode catalytic activity and surface analysis of used cathodes

The performance of the cathodes decreased after use in an MFC. Following 12 MEC cycles of operation, the “used” cathodes were again analyzed by LSV (Figure 6.4) and by SEM (Figure 6.5). LSV scans for both Ni 210 and Pt decreased in performance to similar overpotentials. For example, the overpotentials at -3.2 logA/cm² increased to -0.733 V for used Ni 210 and -0.713 V for used Pt, compared to -0.500 V for the new cathodes. The largest change in overpotential was observed for the used eNiOx cathodes, which increased to -1.043 V compared to -0.800 V for new cathodes. The LSV for the
used Ni 210+CB cathodes exhibited the least change following use in an MEC, with final overpotentials between those of Ni210 and eNiOx.

![Tafel plots for cathodes before and after use in 12 MEC cycles. Test conditions: 2mM phosphate buffer, scan rate 2 mV/s, third scan.](image)

**Figure 6.4.** Examples of Tafel plots for cathodes before and after use in 12 MEC cycles. Test conditions: 2mM phosphate buffer, scan rate 2 mV/s, third scan.

The surfaces of the Ni210-based cathodes observed using SEM showed very similar structures before and after 12 MEC cycles, both with ([Figure 6.5A and 6.5B]) and without CB (not pictured). eNiOx had finer structures before use ([Figure 6.5C]) but broader geometries and less material after use ([Figure 6.5D]), suggesting a loss of the nickel oxide material.
6.3.4. Nickel stability

It was hypothesized that the decreased performance of the nickel cathodes was due to corrosion and Ni dissolution. Ni concentrations in solution were therefore measured over 12 cycles (Figure 6.6A). The Ni concentration with the Ni 210 cathode was 4.9 ± 0.8 ppm, with lower concentrations for the Ni 210+CB (4.1±1 ppm) and eNiOx (1.67 ± 0.29 ppm). These values are substantially larger than that expected based on Ni
added into the medium (0.01 ppm) or the concentration measured in medium with the Pt cathodes (0.115 ± 0.007 ppm). A loss of Ni due to corrosion was also indicated by changes in BET surface areas, with decreases from 4.31 (new) to 3.84 m²/g (used) for the Ni 210 cathodes, and 11.83 (new) to 7.81 m²/g (used) for the Ni 210+CB cathodes. BET areas are not only affected by the catalyst, however, but also by the presence of CB, binder, or other materials that accumulated on the catalyst surface.

The standard procedure for changing the medium consisted of exposing the electrodes to air for 15 minutes (to reduce methanogen growth), but we reasoned this procedure led to Ni corrosion. To test whether this process was responsible for the loss of Ni in solution, after each cycle we replaced the MEC medium in an anaerobic glove box, thus avoiding exposure of the cathodes to air. Under these conditions of continuous anaerobic operation, there was a decrease of Ni in solution over successive cycles, from 5.7 - 6 ppm in the first cycle to 0.2 ppm (±0.4 ppm, Ni 210; ±0.3 Ni 210+CB) for the last 6 cycles (Figure 6.6B). Some of this dissolved nickel does not originate from the Ni 210 cathode, but likely originates from the water as Ni was also present in the used media from the MECs with Pt cathodes (0.115 ppm Ni). Based on the low concentrations of Ni in solution, we concluded that the primary mechanism of Ni corrosion was due to air exposure during replacement of the medium at the beginning of a fed-batch cycle.
Figure 6.6. Nickel content via ICP-AES analysis in MEC solution after (A) aerobic feeding and (B) anaerobic feeding.
6.3.5. Microbial community

The closest matches to sequences obtained from the anode biofilm from the MEC with a Ni cathode (Figure 6.7) were *Pelobacter propionicus* (54.3%) and *Geobacter sulfurreducens* (21.7%), both members of the $\delta$-proteobacteria (76.0% total). Other bacteria included *Dechloromonas aromatica* (4.35%), *Chromobacterium violaceum* (6.52%), *Thauera sp MZ1T* (8.69%), *Diaphorobacter sp TPSY* (2.17%) and *Polaromonas naphthalenivorans* (2.17%), all $\beta$-proteobacteria (23.9%). These communities were similar to those found in the MEC with a Pt cathode, with *P. propionicus* (56.5%) and *G. sulfurreducens* (39.1%) again dominant in the community (95.6% total) (data not shown). *D. aromatica RCB* (2.2%) was also present, along with *Alkaliphilus oremlandii OhILAs* (2.2%, Firmicutes). These results show that the cathode did not appreciably impact anodic community structure.

*Figure 6.7*. Microbial community and phylogenic distances to closest match based on 16S rRNA sequences recovered from the MEC anode with Ni210 catalyst cathode.
6.4. Discussion

6.4.1. Cathode Performance

Ni powder cathodes produced results similar to those obtained with Pt cathodes in MEC tests in terms of Coulombic efficiency, cathodic, hydrogen and energy recoveries. The Ni powder cathodes had a smaller calculated surface area (0.60 m²), measured BET surface area (4.3 m²/g) and particle sizes of the Ni (0.5 to 1 µm) than the Pt cathodes (1.45 m², 11.2 m²/g, 0.002 µm). Calculated surface areas, however, do not take into account catalyst interaction with the carbon black (if present) and carbon cloth, coverage by the binder, or particle agglomeration during application which would reduce the electrochemically active surface area. The similar performance of the Ni and Pt cathodes in MEC tests suggests that current densities were limited by factors other than the intrinsic catalytic activity of these metals. LSVs were useful in indicating general trends in MEC performance but cathodes ultimately need to be tested in MECs due to additional effects that are not measured during electrochemical testing, such as microbial interference or catalyst degradation.

There was loss of nickel when the MEC was aerated between cycles, as shown by elevated nickel concentrations in solution compared to the medium, and decreased BET surface areas. When exposed to air, a nickel oxide layer forms that is 3-5 monolayers thick [31]. Outer layers of nickel can become hydroxylated, forming Ni(OH)₂ in water, and may dissolve unless the metal is kept under alkaline conditions. If the MEC reactor was operated in batch mode, it would take 444 fed-batch cycles under the conditions examined here to dissolve the Ni on the cathode (60 mg) assuming an average loss of 4.5
ppm Ni per cycle in a 30 ml reactor. Based on fed-batch cycle times, this would mean that the cathode would need to be replaced after about a year. Nickel is naturally present in the environment, it is an essential micronutrient, and it is not listed as a toxic chemical by the EPA as there is no maximum level set for drinking water. Nickel is primarily a respiratory toxic metal [32] and can produce allergic reactions, chronic toxicity and respiratory tract cancer. One study showed no cancer tumors in mammalians which received Ni(II) compounds in drinking water [32], but another study showed gill damage in juvenile trout at waterborne Ni concentrations of 15.3 ± 2.6 ppm [33]. These concentrations are 3.4 times higher than the average Ni value measured in this study (4.5 ppm).

Nickel powder was relatively stable with respect to corrosion when MECs were continuously maintained under anaerobic conditions. Constant gas and current production over 12 cycles of MEC operation, a similarity of overpotentials between Ni 210 and Pt cathodes both before and after use, and a similarity of SEM images before and after use also support the stability of the metal in the Ni 210 cathode. Ni dissolution would not be an issue during large scale applications if the MEC was operated continuously, especially as the localized pH of the cathode could be alkaline [23, 34, 35]. However, a lack of aeration could increase methane production. During this study, the methane in the gas increased from 0 % to 15.2 ± 3.9 % for Ni 210, and to 4.4 ± 3.9 % for Ni 210+ CB by the eighth cycle when the MECs were not exposed to air between feedings. Techniques to decrease methane production, other than aeration, need to be explored to minimize corrosion of cathodes made with nickel powders (and potentially other metals).
There was a linear increase in hydrogen production rate with applied voltage, consistent with previous MEC studies using Pt catalysts [4, 23, 26]. There was no measured hydrogen production, and only a very small current, with the Ni 210 catalysts at an applied voltage of 0.3 V, compared to the lower value of 0.2 V previously observed with a Pt catalyst in a similar setup [23]. Hydrogen evolution may not be occurring at this low potential with Ni. Ni has a lower exchange current density and binds hydrogen atoms less strongly than Pt [36]. This suggests that lower current densities (and therefore lower hydrogen production) would occur with Ni than Pt at the same applied potential, based on predictions using the Tafel expression

\[
\log J = \log J_0 + \frac{\alpha_c n_e F}{2.303RT} (E - E_0)
\]

where \( J \) (A/cm\(^2\)) is the current density, \( J_0 \) (A/cm\(^2\)) the exchange current density, \( \alpha_c \) the cathodic transfer coefficient, \( n_e \) the number of electrons per reaction (2 for hydrogen), \((E - E_o)\) (V) the overpotential, \( F \) Faraday’s constant (96,485 C/mol electrons), and \( R \) the gas law constant (8.3145 J/mol K) (assuming that \( \alpha_c \) is independent of the metal). As the applied potential increases, the reaction rate at the surface increases and the transport of the species to or from the surface can become the rate limiting step. The type of metal used as the catalyst will not affect the rate of hydrogen evolution once it becomes mass transfer limited. Mass transfer limited hydrogen evolution likely occurred at an applied voltage of 0.6 V, as the performance of the MECs was the same with either Pt or Ni cathodes at this voltage. Mass transfer limited transport could also be the reason that the amount of Ni 210 metal loading (between 60 and 90 mg) did not affect overpotentials in LSV tests. It has previously been observed that potentials produced with cathodes
prepared similarly to the ones used here (carbon cloth, Nafion, catalyst) did not vary significantly with Pt loading (0.1-2 mg/cm²) in chronopotentiometry tests (measure cathode potential by applying a constant current) [37]. This is a different effect than in hydrogen fuel cells where the performance is greatly affected by Pt loading [38]. The overpotentials needed to transition from kinetic limiting conditions to mass transport limiting conditions are system dependent.

### 6.4.2. Microbial community

The anodic microbial community was very similar for MECs with Ni 210 and Pt cathodes, indicating the cathodes did not affect development of the community on the anode. The communities of the MECs were mostly comprised of *Geobacteraceae* (21.7-39.1% *G. sulfurreducens* and 54.3-56.5% *P. propionicus*) and other anaerobes, except for *Polaromonas naphthalenivorans* which is an aerobe heterotroph. Other acetate-fed Pt-cathode MEC reactors with phosphate buffer were similarly found to be dominated by *Geobacteraceae* (16% *Geobacter* sp., 56% *P. propionicus*) [39], while 2-chamber MECs with carbonate buffer were dominated by *Shewanella* and *Pseudomonas* [40].

*G. sulfurreducens* was the only known exoelectrogen [41] present from the bacteria identified in these MECs, and therefore the role of the other microbes in the biofilm is unclear. The most abundant bacterium identified in the MECs was *P. propionicus* which is not known to oxidize acetate and has been shown to be incapable in electron transfer to metals or electrodes [42]. *Pelobacter* sp. may have a fermentative or syntrophic role with *Geobacter* sp. that has to be further studied, similar to syntrophic
interactions in microbial fuel cells between exoelectrogens and non-exoelectrogens [43, 44].

6.4.3. Cathode Costs

The cost of the nickel used in the cathode is orders of magnitude less than the cost of the platinum, resulting in a total cost for catalyst that would be small compared to the costs of other cathode materials. Ni 210 at a loading of 60 mg per 7 cm$^2$ would cost $2.82/m^2$ compared to $700/m^2$ for the platinum loading used here (calculations based on our purchase prices). At this low cost for the Ni catalyst, the cost of the Nafion binder becomes the main economic consideration as a loading of 267 µL per 7 cm$^2$ of Nafion costs $500/m^2$. Carbon cloth was also very expensive ($850/m^2$), but this cost could probably be reduced. For example, carbon mesh in bulk costs only $25/m^2$. While carbon mesh has been successfully used as an anode [45], it was indicated that it was too porous to be used for the cathode with current preparation techniques. Additional research is therefore needed to find alternatives for the other cathode components as the cost of the Ni catalyst would be a small percentage of the total cathode cost.

6.5. Conclusions

Ni powders showed similar performance to Pt powders when used as catalysts in MEC cathodes made with carbon cloth and a Nafion binder. The stability of Ni powders was demonstrated by consistent current densities in LSV scans before and after use of
cathodes in MECs over 12 cycles (12 days), as well as by visual analysis of particles in SEM scans and measurements of nickel concentrations in MECs kept under fully anoxic conditions. Avoiding exposure of the Ni catalyst to air was essential for avoiding nickel corrosion and dissolution but increased methane production. Analysis of the anodic biofilms in the MECs showed that *Geobacter sulfurreducens* and *Pelobacter propionicus* were the most abundant bacteria on the anode surface in MECs and that this community was not affected by the use of either Pt or Ni cathode catalysts.

6.6. Acknowledgements

The authors thank R. Wagner, P. Kiely, T. Saito, M. Mehanna, S. Cheng and D. Jones for assistance with microbiology, electrochemistry and MEC experiments, and M.J. Janik, J.M. Perez and W.A. Lloyd for their advice and insight. This research was supported by Award KUS-I1-003-13 by King Abdullah University of Science and Technology (KAUST).

6.7. References


[33] Pane EF, Richards JG, Wood CM. Acute waterborne nickel toxicity in the rainbow trout (Oncorhynchus mykiss) occurs by a respiratory rather than ionoregulatory mechanism. Aquatic toxicology 2003; 63: 65-82.


Chapter 7

Conclusions and Future Research

Electrohydrogenesis via microbial electrolysis cells is an innovative technology that can generate hydrogen while simultaneously treating waste materials or other organic matter. Hydrogen is a highly efficient, clean, sustainable and renewable energy carrier that has the potential to replace fossil fuels in the near future. There were two main goals in this dissertation: 1) to investigate hydrogen production via fermentation and via electrohydrogenesis using the byproduct of biodiesel production (B-glycerol) as substrate and 2) to investigate first row-transition metals as cheaper alternatives for platinum in MEC cathodes.

Fermentation of pure glycerol (0.28 ± 0.01 mol-H₂/mol, P-glycerol) and fermentation of B-glycerol (0.31 ± 0.01 mol-H₂/mol) achieved yields lower than that achieved with fermentation of glucose (1.06 ± 0.15 mol-H₂/mol). 1,3-propanediol (PD) was a major byproduct during fermentation of glycerol but was not formed during fermentation of glucose. PD production lowers hydrogen production as the formation of PD requires 1 mol H₂ per mol PD formed. Higher hydrogen gas yields were obtained in this study than previously obtained with mixed culture fermentation (0.05 mol-H₂/mol), along with the maximum PD yields achieved so far (0.69 ± 0.09 mol-PD/mol-glycerol).

Varying inoculum sources affected hydrogen yields while pretreatment methods were unsuccessful in improving hydrogen production. Wheat soil was a better inoculum probably due to a better natural acclimatization of bacteria for degradation of glycerols due
to the higher content of triglycerides in wheat. The maximum overall conversion efficiency was only 9.3% for glycerol (based on 3 mol-H₂/mol-glycerol with production of acetate). This suggests that further improvements in hydrogen yields are possible. Further research needs to be done to evaluate the relationship between triglycerides content in the inocula and gas production. A systematic approach should be done to evaluate alternative pretreatment methods to screen for hydrogen producers while minimizing PD producers. Some pretreatment methods that could be attempted include boiling + freezing, input of electrical current, and minimal aeration. The first step may be to identify the pure cultures that can metabolize glycerol with minimum production of PD and then determine an environment suitable for their growth. Additional research could be done to utilize the byproducts from glycerol fermentation in technologies that will consume non-fermentable organic material such as anaerobic digestion (methane), electrogensis (electricity) or electrohydrogenesis (hydrogen).

P-glycerol, B-glycerol and glucose produced higher hydrogen yields in MECs than by anaerobic fermentation. A hydrogen yield of 3.9 mol-H₂/mol was obtained using glycerol, at relatively high rates of 2.0±0.4 m³/m³d ($E_{ap}=0.9$ V). This is 56% of the maximum possible yield by oxidation (7 mol-H₂/mol-glycerol), and higher than the yield that could be achieved by fermentation. The hydrogen yield for glucose was similar to that of glycerol on a per carbon basis (7.2 mol-H₂/mol or 3.6 mol-H₂/mol-3C, 1.9±0.3 m³/m³d). Fermentation intermediates accumulated rapidly in the solution during electrohydrogenesis for both glycerol and glucose, but they were consumed within a few hours. The main intermediates were 1,3-propanediol during glycerol electrohydrogenesis, and acetate and propionate during glucose electrohydrogenesis. Electron recycling and
cell carbon storage occurred during glucose electrohydrogenesis, and to a smaller extent during glycerol electrohydrogenesis. The use of a higher applied voltage (0.9 V vs. 0.5 V) resulted in less methane production, more total gas production and less variability over successive cycles. Further research is needed to determine how to increase gas production and decrease methane formation of fermentable substrates at lower applied voltages.

Hydrogen was produced via electrohydrogenesis using B-biodiesel (0.41±0.1 m³/m³ d) but at a lower yield than when using P-glycerol, probably due to the presence of sodium sulfate, methanol and soaps in the mixture. Additional research is needed to determine the effect of the B-biodiesel components on MEC hydrogen production, including catalyst deactivation. It can then be determined if it is economical to remove or minimize that component from B-biodiesel or to look for catalyst alternatives that will not deactivate in the presence of that component.

Cathode materials (carbon cloth, platinum and Nafion™) are the most expensive components in MEC reactors. Their costs need to be reduced for MECs to be used in large scale applications. Nickel and stainless steel (SS) alloys were compared to platinum on a sheet metal geometry to minimize surface effects. The best MEC performance was obtained in a MEC with SS A286 sheet metal as the cathode (0.9 V applied voltage). The platinum sheet metal displayed average performance compared to other metals. The performance of the Pt cathode decreased with use probably due to metal poisoning by the sulfur and nitrogen in the MEC environment. The relative ranking of these metals in MEC tests was in agreement with cyclic voltammetry studies. The performance of the best MEC materials was further improved by electrodepositing a nickel oxide layer on the surface of the sheet metal. However, the performance of the nickel oxide cathodes
decreased over time due to a reduction in mechanical stability of the oxides. These results demonstrate that non-precious metals can be used as substitutes for platinum in MEC cathodes. Mechanical long term stability of the nickel oxide layer needs to be improved by evaluating combinations of different supports, catalysts, binding agents, electrodeposition formula compositions and deposition methods. Additional pure metals and alloys should be evaluated in electrochemical tests and MEC tests for their relative performance compared to platinum under MEC conditions (neutral pH, ambient temperatures, microbial population).

Commercially available nickel and stainless steel powders were evaluated as MEC cathodes. Ni 210 powder (0.5 to 1 µm) cathodes produced results similar to those obtained with Pt (0.002 µm) cathodes in terms of gas production, Coulombic efficiency, cathodic, hydrogen and energy recoveries during MEC tests. The production rate was slightly lower for Ni 210 ($Q=1.2-1.3$ m$^3$/m$^3$/d) than for Pt ($Q=1.6$ m$^3$/m$^3$/d) due to higher currents obtained with Pt even though gas production and composition were similar for both cathodes. Overpotentials in linear voltammetry scans were used to screen the various metal powders in neutral pH solutions and at 30°C to select cathodes prior to MEC testing. Ni 210 cathodes were stable under fully anaerobic conditions but some Ni dissolution occurred if there was air exposure in between batch cycles. The MEC systems were probably mass transfer limited, instead of kinetics limited, as the performance of the MECs was the same with either Pt or Ni cathodes at applied 0.6 V. The anodic microbial community consisted mainly of *Geobacteraceae* sp. (21.7-39.1% *G. sulfurreducens* and 54.3-56.5% *P. propionicus*) for both Ni 210 and Pt cathodes, indicating the cathodes did not affect development of the anodic microbial community. The cost of the nickel used to
manufacture these experimental cathodes were orders of magnitude less than the cost of the platinum. Further research is therefore needed to find alternatives for the other cathode components (binder and support). Additional metal powders should also be evaluated for performance in an MEC environment. MEC reactor design can be further improved to minimize mass transfer limitations.
VITA

Priscilla A. Selembo

EDUCATION
2006-2010 Ph.D. in Chemical Engineering, Penn State University.
1995-1997 M.S. in Chemical Engineering, Penn State University.

PROFESSIONAL EXPERIENCE
2000-2006 HPLC and SPE Production Supervisor, Sigma-Aldrich, Bellefonte, PA
  • Led new product development efforts in most production areas.
  • Identified and implemented opportunities for improvement by increasing capacity and reducing costs.
1998-2000 Chemical Engineering in Coolants Technology, Texaco Inc. Beacon, NY
  • Engineering and Applied Research (product and test development).
  • Technical and Marketing Support (solved field problems, US and Latin America).
  • Information Technology (managed U.S. research database).

TEACHING EXPERIENCE
2006 Teaching Assistant, Penn State University ChE 497A: Process Safety
1997 Teaching Assistant, Penn State University ChE 414: Kinetics and Industrial Chemistry
1993 Teaching Assistant, Lafayette College Chem 102: A Chemical Perspective

PUBLICATIONS

