RETHINKING THE CARBON ECONOMY:
COMBINED ELECTROCHEMICAL AND BIOLOGICAL REDUCTION FOR CARBON
DIOXIDE CAPTURE AND VALORIZATION

by

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A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Doctor of Philosophy
Department of Civil, Environmental, and Architectural Engineering
2019

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Rethinking the carbon economy: Combined electrochemical and biological reduction for carbon
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Rethinking the carbon economy: Combined electrochemical and biological reduction for carbon dioxide capture and valorization

Thesis directed by Professor Jason Ren

Abstract

Carbon is a primary component of most present-day commodities and can be viewed as the backbone of our global economy. Carbon dioxide is considered one of the most abundant forms of carbon on earth and its continued buildup in the atmosphere has reached concentrations of around 410 ppm (ca. 32000 gigatons) [1]. Though many fear the impending effects of this accumulation on global climate patterns, others have viewed this plentiful compound as a cheap and largely untapped resource. Engineered carbon dioxide reduction to high energy density fuels and chemicals presents an economically sound approach to mitigate climate change and yield tremendous environmental and societal benefits. Due to the rapid decrease in the cost of renewable energy, it is practical to design devices that may use solar or wind power to simultaneously drive CO₂ sequestration and transformation. The injection of an electron into CO₂, however, requires highly negative potentials (large energy investment) and the kinetics are quite sluggish under standard conditions. Coupling CO₂-fixing autotrophic microorganisms with electrochemical systems is a sustainable approach for engineered (non-photosynthetic) CO₂ reduction, however, this is a nascent field and only a few approaches have been presented to date. In this work, I conducted multi-disciplinary research in the fields of microbiology, material science and electrochemistry to develop a two-stage biohybrid system that feeds electro-

generated syngas produced from a CO₂ electrolysis cell to a downstream bioreactor for highly efficient conversion of CO₂ to value-added carbon products. The specific studies presented in this dissertation consist of an investigation of tuning key syngas fermentation parameters of *Clostridium ljungdahlii* to create desired metabolites, development of a versatile carbon material from waste-grown fungal biomass, synthesis and characterization of novel CO₂ reduction catalysts, and design and optimization of a two-stage biohybrid system for engineered carbon dioxide reduction. My studies conclude with future research focused on developing electro catalysts for electrochemical hydrogen production to be used a feedstock for a downstream catalytic hydrogenation process as well as other work involving the creation of functionalized hollow-fiber membranes for dual CO₂ conversion and delivery.

Acknowledgements

I would sincerely like to express that I only take a small fraction of credit for the great accomplishment of completing this dissertation and other doctoral requirements, as I could not have done it without all of the amazing and wonderful people who have relentlessly showed their support along the way. Firstly, I would like to thank my advisor Dr. Jason Ren for his guidance, encouragement and confidence in allowing me to pursue my research ideas and for always keeping my best interests in mind. I feel very lucky to have had you as my advisor and as a friend. Moreover, I would like to thank everyone in the Ren research group for their technical and life advice along the way. I would also like to thank Pin-Ching Maness who made NREL feel like a home to me and graciously welcomed me into her lab as well as supported me throughout my graduate studies. In addition, I am extremely grateful for all the help and encouragement I received while working at NREL and am glad I got the opportunity to know many of the scientists and establish life-long friendships. I would also like to thank and acknowledge Eunsol Park for her help with material characterization described in Chapters 4 and 6. To all my committee members, Dr. JoAnn Silverstein, Dr. Angela Bielefeldt, and Dr. Sherri Cook, thank you for all your time, welcomed feedback, and advice. Lastly, I would like to thank all of my family and close friends who have always showed me love and support. I am truly blessed to have you all in my life.

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CHAPTER 1

INTRODUCTION

1.1 Rational

Carbon dioxide (CO₂) is considered one of the most abundant sources of carbon on earth. The current CO₂ concentration in the atmosphere is approximately 410 ppm, which corresponds to ca. 3,200 gigatons of CO₂ [1]. In addition, more than 40 billion tons of CO₂ are released from human activities every year, leading to a concentration increase of ca. 2 ppm per year [2]. Although this rapid accumulation of CO₂ has prompted various global environmental and societal concerns, it may also be considered a renewable source of a low-cost building block. Carbon is the primary component of fuels, chemicals and materials that are used everyday and thus compose a large section of the global economy. By 2050, the human population is expected to increase from ca. 7.6 to 9.8 billion people, creating a huge increase in demand for such commodities [3].

The utilization of CO₂ as a renewable carbon feedstock for fuels and chemicals could simultaneously help satisfy this demand while actively mitigating global CO₂ emissions. Technologies that can accomplish this task could pave the way for a closed-carbon economy while yielding tremendous social and environmental benefits. Furthermore, such devices can achieve excellent economic profits by leveraging the low-cost of CO₂ as a feedstock for high value products and by taking advantage of governmental carbon capture incentives [4].

In the past, the majority of techniques that could convert CO₂ into valued carbon products have involved photosynthetic carbon assimilation via plants, algae or cyanobacteria followed by biomass deconstruction and upgrade processes [5]. The commercialization of such systems is, however, fundamentally limited by low-energy to biomass conversions as well as large water, land, and nutrient requirements that will undoubtedly become more precarious with growing populations [6-9].

With that in mind, incredible efforts have been made to develop other engineered carbon reduction platforms to accomplish conversion of CO₂ into products. These include non-biological processes such as thermocatalysis, photocatalysis and electrocatalysis, as well as non-photosynthetic biocatalytic processes. To date, the bulk of these devices have been challenged by the large energy requirement to reduce CO₂ to organic molecules [10,11]. In recent years, cheap and abundant renewable energy sources such as solar or wind have become an attractive supply of reducing power for CO₂ reduction. As photocatalytic and thermocatalytic techniques directly convert sunlight to either free electrons or heat, a greater abundance of electrons does not directly benefit them [11]. In contrast, the increased accessibility of cheap (renewable) electrons has greatly improved the viability for scale-up of approaches that involve electrocatalytic or non-photosynthetic biocatalytic CO₂ reduction.

Abiotic electrocatalytic CO₂ reduction utilizes the electrons from an external source (i.e. solar or wind) as the reducing power to transform CO₂ to a variety of products on the electrode surface. Since CO₂ is extremely stable at standard conditions, an electrocatalyst is typically employed at the electrode to mediate the reaction [12]. Present approaches for electrochemical CO₂ reduction have been able to demonstrate excellent conversion rates and efficiencies for products like carbon monoxide (CO). For example, Peng et al. (2018) recently showed that their preferentially oriented silver nanoparticles could reduce CO₂ to CO with a faradic efficiency of 96.7% at a low operating potential of -0.69V vs. RHE [13]. Although promising, products such as CO are of limited value as they require further processing to be used as fuels or upgraded into other value-added commodities. As a result, contemporary studies have focused on the formation of reduction products requiring four or more electrons (i.e. ethylene, oxalate) [14,15]. This work inherently poses additional challenges associated with higher over potentials and complex

parallel reactions schemes that lead to decreased product specificity [16]. Presently, multi-electron reduction of CO₂ to products such as acetate, methane (CH₄) or ethanol (C₂H₅OH) has shown to be very energy inefficient and can only generate these products at low rates and yields [17,18].

In contrast, non-photosynthetic biocatalytic approaches for CO₂ reduction have demonstrated great overall energy efficiencies and can generate multi-reduction compounds with excellent product selectivity [19]. In the past decade, an approach known as microbial electrosynthesis (MES) has received a lot of attention [20-22]. In this process, chemolithoautotrophs directly take electrons from an electrode using extra-cellular transfer (EET) mechanisms and use it as the reducing equivalent to convert CO₂ into organic products. MES reactors can produce an extensive portfolio of compounds ranging from volatile fatty acids (formic acid, acetic acid, propionic acid, butyric acid) to alcohols (i.e. ethanol) [19,22]. Though encouraging, the production rates of MES technologies are orders of magnitudes lower than abiotic electrocatalytic systems. This is largely because catalysis in MES technologies is limited by usable electrode surface area, mass transfer of nutrients, and rates of microbial metabolic processes (i.e. EET) [19-23].

Combining the current understanding of electrocatalytic and non-photosynthetic biocatalytic CO₂ conversion techniques, it seems that their limitations are quite complementary. As the EET rate in MES seems to be a limiting factor, circumventing these processes via an intermediate electron donor may be an effective solution. Accordingly, the most efficient approach for CO₂ reduction to products may be a hybrid technique that combines high rates of electrochemical CO₂ reduction to electro-generated intermediates followed by highly efficient biological upgrade of the intermediates to value-added products. The goal of this doctoral

dissertation was to investigate abiotic and biological techniques for CO₂ reduction to develop such a novel biohybrid platform. In the proposed two-stage design, microorganisms are separated from the electricity source, which enables optimized processes for both steps. In the first stage, CO₂ is electrochemically converted to syngas (mixture of CO/H₂/CO₂) in an innovative gas diffusion CO₂ electrolysis setup. The electro-generated syngas mixture is then delivered to the second stage where autotrophic bacteria utilize these components to synthesize biocommodities at high yields.

Figure 1: Two-stage biohybrid system for CO₂ valorization

1.2 Formal Hypothesis

The following hypotheses can be examined stand-alone but were developed and challenged to investigate the feasibility of creating a biohybrid system that uses renewable energy to power electrochemical CO₂ reduction to syngas as an intermediate feedstock for biological conversion to fuels and chemicals.

Hypothesis I. The metabolite generation by *Clostridium ljungdhalii* can be directed via tuning the H₂/CO ratio of the syngas feedstock.

Hypothesis II. Fungi grown in wastewater can create a sustainable source of biomass that can be converted to a multi-functional carbon material.

Hypothesis III. CO₂ reduction catalysts can be deposited on carbon support material and generate CO and H₂ at high efficiencies. A) Increasing local proton sources of cobalt porphyrins can enhance CO₂ reduction capabilities. B) Bulk electrolyte pH and cation solvation effect CO₂ reduction conversion efficiencies

Hypothesis IV. A two-stage biohybrid system can electrochemically reduce CO₂ to syngas as an intermediate feedstock for biological conversion into fuels and chemicals via a pure culture of *Clostridium ljungdahlia*.

1.3 Dissertation guideline

This thesis has been designed such that each chapter can be read independently, while also addressing the outlined hypotheses. Chapter 2 tested hypothesis I, that the metabolite generation by *Clostridium ljungdhalii* can be directed by tuning the H₂/CO ratio of the syngas feedstock. As CO and H₂ are viable candidates to act as electron mediators, determining their influence on

product formation was crucial. The results from Chapter 2 were published in *Biomass and Bioenergy*. Chapter 3 tested hypothesis II, that fungi grown in wastewater can create a sustainable source of biomass that can be converted to a multi-functional carbon material. This hypothesis helped build necessary material synthesis and characterization skills used in the following chapters. The results from Chapter 3 were published in the *Journal for Cleaner Production*. Chapter 4 investigated hypothesis III, that increasing the cation availability of CO₂ reduction catalysts can improve CO production at high efficiencies. Hypothesis IIIa focused on accelerating the electroreduction of CO₂ via addition of proton donating ligand groups to cobalt porphyrins. Hypothesis IIIb focused on the effects of bulk electrolyte pH and solvating ions on CO₂ reduction performance. The results will be submitted for publication to the *Journal of CO₂ Utilization*. Chapter 5 tested the hypothesis IV that a two-stage biohybrid system can electrochemically reduce CO₂ to syngas as an intermediate feedstock for biological conversion to fuels and chemicals. The results from Chapter 5 are being prepared for submission to *Green Chemistry*. Future research directions are discussed in Chapter 6. Work describing the integration of platinum nanoparticles attached to covalent organic frameworks for electrochemical hydrogen production is discussed. These results are a portion of a manuscript being submitted to the *Journal of American Chemical Society*. Moreover, biohybrid research involving the use of functionalized hollow fiber membranes is also presented.

CHAPTER 2

DIRECTING CLOSTRIDIUM LJUNGDAHLII FERMENTATION VIA H₂/CO SYNGAS RATIO

2.1 Introduction

Synthesis gas (syngas) is a gaseous mixture containing mainly carbon monoxide (CO), hydrogen (H₂) and carbon dioxide (CO₂) generated from the gasification of biomass or other carbon-rich resources such as coal. It has been demonstrated that syngas can be used to generate a variety of valuable chemicals such as acetate, ethanol and 2,3-butanediol (2,3-BD) at high yields and specificities [24]. The low cost and versatility of the syngas feedstock makes it a very attractive approach for chemical production. In general, the conversion of syngas into commodities is performed using either metal-based catalysts (known as Fischer–Tropsch process) or microbial catalysts (known as syngas fermentation). Compared to metal-based catalysis, microbes offer several advantages, such as higher product selectivity, greater product titers, lower energy costs, and greater resistance to poisoning [25-28].

Presently, several anaerobic bacteria have been recognized to grow autotrophically on syngas components. Due to its ability to efficiently utilize both H₂ and CO, *C. ljungdahlii* has been researched as a promising microbial catalyst for syngas fermentation. The unique ability for these microbes to grow autotrophically on these substrates relies on the Wood-Ljungdahl pathway (WLP) that has been reviewed in detail [Fig. A.1; [29-31]]. Briefly, when CO is available it can provide reducing equivalents via CO oxidation catalyzed by the carbon monoxide dehydrogenase (CODH) enzyme in a biological water-gas shift reaction (Eq. A.1). If CO is absent, reducing equivalents can be provided by H₂ via hydrogenase enzymes (Eq. A.2) and CO₂ must be used as the carbon source instead. Both mechanisms (using CO or H₂/CO₂)

ultimately produce acetyl-CoA that can be hydrolyzed to acetate or ethanol. If enough energy is available to the cell, acetyl-CoA can be used to generate pyruvate (Eq. A.3). Pyruvate is a central intermediate for anabolic processes and can lead to the production of more reduced products including 2,3-BD (Fig. A.2).

The microbial conversion of gaseous substrates to products can be viewed macroscopically as a two-step process involving first the transport of the gas to the liquid/cell surface followed by the utilization of the gas by the cell [32]. As a result, bioconversion is in part limited by the physical and thermodynamic attributes of the gaseous substrates available. Examining the properties of the electron donors (H_2 and CO), it can be noted that they differ greatly in thermodynamic and mass transfer capabilities (Table A.1). For instance, CO delivers electrons at a much lower half-cell potential and releases about 120.18 kJ/mol more Gibbs free energy during its conversion to ethanol than H_2 (Table A.1; Eq. A.5, [31]). Furthermore, CO ($K_H = 7.4$ mM/atm) has a much greater Henry's law constant than H_2 ($K_H = 0.78$ mM/atm) and thus can more readily partition into the liquid state for cellular uptake [33]. Due to differences in physiochemical properties such as these, the composition of products generated via fermentation is greatly affected by the mixture of the syngas present.

Although the amount of CO_2 amongst syngas blends is relatively consistent (typically between 5-15%), the relative H_2 and CO concentrations can vary extensively depending on the feedstock, gasifier type, and processing conditions used [34]. The National Energy Technology Laboratory (NETL) recently found H_2 and CO concentrations of coal-derived syngas ranges between 2-34% and 1-59%, respectively [35]. As a result, syngas H_2/CO ratios can range between 0.03-2.0, however, most coal-derived syngas contains H_2/CO ratios between 0.4-1.0

[11]. Similarly biomass-derived syngas typically contains H_2/CO ratios between 0.4-1.0 but can range between 0.2-4.6 [35].

Figure 2: *Clostridium ljungdahlii* syngas fermentation- Maximum reported ethanol to acetate concentration ratio (log scale) vs. H_2/CO ratio of the syngas used for culturing [36-46]. Vertical lines indicate boundaries of typical range for syngas H_2/CO ratios [34-35]. Batch cultures- blue diamonds; continuously stirred tank reactor (CSTR) culturing- downward facing green triangles; other culturing methods (i.e tubular reactor, immobilized cell bioreactor)- red triangles.

Due to the fact that syngas composition is quite variable, research studies that have focused on *C. ljungdahlii* syngas fermentation have used a wide variety of blends. Comparing published literature it can be seen, in general, when culturing with higher CO concentrations (relative to H_2) more electron-dense products are generated (e.g., ethanol) [Fig. 2]. For example, when growing *C. ljungdahlii* in a continuously stirred tank reactor (CSTR) under an H_2/CO ratio of 0.36, a maximum ethanol to acetate ratio of 16 was obtained. Conversely, when using a higher H_2/CO ratio of 1.2, a maximum ethanol to acetate ratio of only 2.5 was achieved under similar conditions [38,41]. Phillips et al. [32] made similar observations of product formation when comparing the growth of *C. ljungdahlii* on H_2/CO_2 vs. CO/CO_2 .

This relationship between syngas and product composition is, however, difficult to predict using present data as for the same H₂/CO ratio the product ratio can vary across orders of magnitude due to differences in important fermentation parameters such as culturing method, media composition, pH, and gas pressure used across published data. Currently, there is limited understanding on how syngas blends can effect relative product composition. This information could be useful in process design as it can aid in selecting for parameters that produce syngas blends tailored toward desired commodities (i.e. acetate, ethanol, 2,3-BD). Heiskanen et al. [47] acknowledged the importance of syngas compositions on product formation and noted dramatic shifts in acetic and butyric acid production by *Butyribacterium methylotrophicum* when adding H₂ or CO₂ to a CO filled culturing headspace. In the present study, the impact of syngas composition on growth and metabolite production of *C. ljungdahlii* was systematically investigated with the goal of deriving a clear relationship between syngas H₂/CO ratios and product composition. This was accomplished by culturing microbes with syngas blends across common H₂/CO ratios and fixing key parameters such as growth media composition, pH, initial gas pressure, and culturing method.

2.2 Materials and methods

All microbial cultures were maintained in 162.5 mL Wheaton serum bottles (Borosilicate glass, Fisher Scientific, UK) containing 50 mL of ATCC PETC 1754 liquid media [48, 49]. The initial pH of the media was adjusted to 7.0 using a concentrated 1M solution of potassium hydroxide [50]. Prior to autoclaving (121°C for 30 minutes), ultra high purity argon gas (General Air Service Supply- UN1006) was diffused into the liquid media for approximately 45 minutes to remove any oxygen. Gas impermeable butyl rubber septum-type stoppers and aluminum crimp

seals were then used to seal the bottles and the headspaces were purged with argon for another 10 minutes. After autoclaving, the bottles were cooled to room temperature (25°C).

The headspace pressure of each bottle was then normalized to 0.5 atm by removal of excess argon gas using sterilized needled syringes and a needled pressure gauge (Geothermal-k4517N41600). For all syngas cultures, 150 mL of filter-sterilized syngas composed of the following H₂/CO ratios; 0.5,1.0,1.5, 2.0 was then injected directly into the headspace using sterile needled syringes and verified with a gas chromatographer. In a similar manner, 10% of the final headspace was filled with filter sterilized high purity CO₂ (General Air Service Supply-UN1013) to create an initial headspace pressure of 1.6 atm as verified with a sterile needled pressure gauge. The media in each serum bottle were then reduced by addition of 0.5mL of the reducing agent to make final concentrations of 3.1mM L-cysteine-HCl and 1.7mM sodium sulfide hydrate.

Fructose was omitted from all syngas test cultures but was used to propagate a seed culture. The seed culture contained 5g/L fructose and was prepared using the same methods as the syngas cultures without the addition of H₂/CO or removal of argon. Prior to inoculation, the seed culture was grown for 48 hours to an optical density (OD) of 0.8. After the reducing agent was added to the syngas cultures, 2.5 mL of the seed culture was used as inoculum. All cultures were incubated horizontally on an orbital shaker incubator, set at 200 rpm and 37°C.

2.2 Gas analysis

Gas samples were obtained directly from the headspace of batch cultures using sterilized gas-tight syringes in a sterile laminar flow hood. H₂ and CO molar headspace concentrations were determined by manually injecting 250µl of sample into a gas chromatograph (Agilent

Technologies-7890A) equipped with a thermal conductivity detector (TCD) and packed column [19]. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 250°C, respectively. Helium gas was used as the carrier gas at a flow rate of 5 mL min⁻¹. CO₂ molar headspace concentrations were monitored by manually injecting 100µl of sample into a gas chromatograph (Hewlett Packard-5890) equipped with a thermal conductivity detector (TCD) and packed column [51]. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 190°C, respectively. Helium gas was used as the carrier gas at a flow rate of 30 mL min⁻¹. Molar concentrations were determined by fitting data to a five-point standard curve prepared for each gas.

2.3 Liquid analysis

Liquid samples (1mL) were withdrawn directly through the top of each culture bottle using sterile needled syringes in a laminar flow hood. The liquid samples were analyzed for optical density (600nm) as well as acetate, ethanol, and 2,3-BD content. Optical density measurements were made using a spectrophotometer (Beckman Coulter-DU800) at a fixed wavelength of 600nm. Metabolites were analyzed via high-pressure liquid chromatography [51]. Prior to analysis, liquid samples were passed through 0.22µm filters. Organic acids were separated on an Aminex HPX-87H X column with 4 mM H₂SO₄ as the eluent. Molar concentrations were determined by fitting peak areas to a standard curve prepared by diluting ethanol fermentation analytical standard solutions (Bio-Analytical-LQC-NREL-50) and 2,3BD HPLC standard mixes (Sigma Aldrich-42038).

2.4 Yield and conversion efficiency computations

To investigate the bioconversion of gases into metabolites, product yields ($Y_{p/s}$) and electron-mol (e-mol) recoveries were calculated for each culturing condition [52]. Yields were determined using Eq. 1 (below):

$$Y\left(\frac{mol-p}{mol-s}\right) = \frac{\sum_{i=1}^N n_{i produced}}{n_{H_2 consumed} + n_{CO consumed}}, \text{Eq. 1}$$

where $n_{(i)}$, n_{H_2} and n_{CO} are the number of moles of product i , hydrogen and carbon monoxide, respectively. Theoretical yields were determined directly from stoichiometry via Eqs. A.4-A.9 [52].

The efficiency of electrons converted from gas into products was evaluated using Eq. 2 (below)

$$\text{e-mol recovery (\%)} = \frac{\sum_{i=1}^N (n_{(i) produced} \cdot n_{e^-(i)})}{n_{H_2 consumed} \cdot n_{e^- H_2} + n_{CO consumed} \cdot n_{e^- CO}} \cdot 100, \text{Eq. 2}$$

where $n_{(e^- H_2)}$ and $n_{(e^- CO)}$ are the number of e^- molar equivalents per mol of hydrogen and carbon monoxide.

2.3 Results and discussion

3.1 Syngas consumption under different gas mixture ratios

C. ljungdahlii effectively utilized both CO and H₂ across the range of H₂/CO ratios tested. At a given ratio, mass transfer and thermodynamic favorability each played a key role in gas consumption and the products generated. In general, gas consumption rates showed linear relationships with relative gas concentrations likely due to mass transfer limitations of the gases to the liquid/cell surface. For instance, maximum CO consumption rates in the H₂/CO ratios range of 0.5 to 1.5 showed a near linear decrease with decreasing initial concentration of CO [Fig. 3]. Comparable CO consumption rates of ca. 0.45 mM/hr were observed using H₂/CO ratios between 1.5 and 2.0. This was likely because CO pressures were low enough for mass transfer to

not significantly affect consumption rates but instead uptake was limited by reaction kinetics at the cell surface.

Figure 3: *Clostridium ljungdahlii* gas consumption rates-Maximum CO consumption rates (green squares) averaged from duplicate batch cultures with varying initial syngas blends in headspace during first 240 hours of cultivation; Maximum H₂ consumption rates (blue circles) averaged from duplicate batch cultures with varying initial syngas blends in headspace during first 240 hours of cultivation; Standard deviation shown in error bars.

Analogous to CO consumption, H₂ utilization also seemed to be limited by gas-liquid mass transfer when using H₂/CO ratios in the range of 0.5 to 1.5 as a proximate linear trend was observed between H₂ consumption rates and the relative concentration of the gas. When using H₂/CO ratios in the range of 1.5 to 2.0, comparable H₂ consumption rates of ca. 0.55 mM/hr were observed, indicating that mass transfer was likely no longer limiting at these high concentrations. Furthermore, H₂ consumption rates were faster than CO consumption in this range [Fig. 3].

Thermodynamically CO is the preferred substrate as its oxidation delivers more Gibbs free energy to the cell than reactions with H₂. Comparable utilization of both H₂ and CO only occurred when using an H₂/CO ratio of 1.5 likely because the higher relative concentration of H₂

adequately enhanced gas-liquid mass transfer to outcompete the thermodynamic favorability of CO as a substrate. In contrast, CO was consumed at greater rates than H₂ when using H₂/CO ratios of 0.5 and 1.0. The affinity for CO over H₂ at these ratios likely stems from a combination of superior gas/liquid mass transfer abilities (larger Henry's law constant) and because more Gibbs free energy is available to the cell when it is used as a substrate.

It is important to recognize that CO₂ is both a reactant in the reactions of H₂ and a product of CO reactions. Therefore, the free energy of these reactions and thus the relative affinity of *C. ljungdahlii* for each substrate (H₂ and CO) are influenced by the concentration of CO₂. Figure 4 shows CO₂ concentrations after 48, 120, and 240 hours of culturing under each initial H₂/CO ratio. During the first 48 hours, CO₂ was produced in all cultures. This indicates that CO was being utilized, producing CO₂ as a by-product of cell growth.

Figure 4: Comparison of *Clostridium ljungdahlii* CO₂ production-Average CO₂ concentration of batch cultures with varying initial syngas blends in headspace; Initial values shown as green Xs, concentrations after 48 hours as yellow diamonds; after 120 hours as purple squares; after 240 hours as blue stars; Standard deviation shown in error bars.

After 120 hours, CO was fully consumed in H₂/CO ratios above 1.0 and cultures began to consume H₂ with CO₂ as the carbon source. This is best exemplified by cultures grown on H₂/CO ratios of 2.0 that consumed more than 5mM of CO₂ during the 48-120 hour period of cultivation. As H₂ consumption continued concurrently with CO₂ fixation, a near linear trend developed after 240 hours of culturing between CO₂ concentrations and initial headspace H₂ content. Specifically, cultures with higher initial H₂/CO ratios (≥ 1.5) ultimately consumed CO₂ to final concentrations lower than the initial concentration, while cultures with lower initial H₂/CO ratios (< 1.5) produced CO₂ to final concentrations greater than the initial [Fig. 4]. This is likely because at higher H₂/CO ratios, more hydrogen is consumed than CO, increasing the demand for CO₂ as a carbon source.

3.2 Product generation under different gas mixture ratios

C. ljungdahlii synthesized chemicals directly from syngas components. A spectrum of products was generated dependent on the relative concentration of each gaseous substrate. Acetate and ethanol were the major products produced under all initial H₂/CO ratios tested. Figure 5 shows the maximum product concentrations achieved during cultivation using each initial H₂/CO ratio. Acetate formation was favored by increased H₂ concentrations in the headspace with a maximum acetate concentration of 35.21 ± 0.84 mM achieved using an H₂/CO ratio of 2.0. Acetate production gradually dropped along with decreasing H₂/CO ratios as the lowest concentration (22.64 ± 0.55 mM) was obtained at a ratio of 0.5. This trend is in agreement with previous work that shows increased H₂ concentrations lead to increased acetate formation in pure and mixed consortia of carboxydutrophs [53, 54].

Figure 5: Impact of H₂/CO ratio on product formation- Maximum concentration of acetate, ethanol, and 2,3BD achieved during first 240 hours of cultivation under each initial H₂/CO ratio tested; Acetate-blue circle; Ethanol- orange square; 2,3-BD- green triangle; Averaged from duplicate batch cultures; Linear regression shown as dashed lines with coefficients of determination; Standard deviation shown in error bars.

In contrast, the production of more reduced metabolites was favored by increased concentrations of CO in the headspace. For example, the maximum ethanol concentration increased from 5.39 ± 0.52 mM to 7.44 ± 0.67 mM when the H₂/CO ratios decreased from 2.0 to 0.5. Similarly, the production of 2,3-BD was also favored by increased CO in the headspace reaching a maximum concentration of 5.20 ± 0.79 mM using an H₂/CO ratio of 0.5. Interestingly, almost no 2,3BD was formed under the highest H₂ concentrations (i.e., using H₂/CO ratios of 1.5-2.0), suggesting that the additional free energy from CO reactions was necessary for the formation of this more reduced metabolite.

Complementary to acetate formation, a near linear relationship between H₂/CO ratio and both ethanol and 2,3-BD production was observed across the range of H₂/CO ratios tested [Fig. 5]. This relationship between product formation and syngas compositions can be expected

thermodynamically. The higher Gibbs free energy released with CO reactions stems from the ability of CO to directly become the carbonyl moiety in the eastern branch of the WLP. When CO is absent, CO₂ has to be reduced to CO consuming additional electrons (from H₂), yielding less free energy and electron-dense by products. This phenomenon is exemplified in Figure 5 that shows the lowest ethanol concentration of 5.39 ± 0.52 mM occurred under the highest H₂ concentration.

Figure 6: Comparison of *Clostridium ljungdahlii* acetate and ethanol production -Maximum molar acetate to ethanol ratios observed under varying initial syngas blends in headspace; Averaged from duplicate batch cultures; Linear regression shown as dashed line; Standard deviation shown in error bars.

This relationship is further demonstrated by the proximate linear trend obtained from plotting the maximum ethanol to acetate ratio achieved by each culture vs. the initial H₂/CO ratio [Fig. 6]. As expected, the greatest ethanol to acetate ratio of 1.06 ± 0.04 was observed under the highest CO concentrations (i.e. using an H₂/CO ratio of 0.5).

3.3 *Clostridium ljungdahlii* growth on different syngas mixtures

C. ljungdahlii effectively grew across the range of H₂/CO ratios tested. All cultures grew within 48 hours of cultivation, indicated by increases in optical density. Figure 7 shows optical densities of liquid samples measured by a spectrophotometer at a fixed wavelength of 600nm after 48, 120, and 240 hours of cultivation under each initial H₂/CO ratio tested.

Thermodynamically, it would be expected that anabolic processes would be favored by greater levels of CO as more free energy is available to the cell when it is used as the electron donor. Cell growth is, however, dictated by ATP content. When CO is used as the primary electron donor, more products such ethanol and 2,3-BD are generated, at the expense of ATP production. For example, when CO is utilized via the WLP an investment of one ATP is required in the western branch to convert formate into formyltetrahydrofolate (CHO-THF) [Fig. A.1]. CHO-THF is eventually used to generate acetyl-CoA, the building block for products such as acetate, ethanol and pyruvate. Since CO utilization results in the generation of more reduced products, less ATP/electrons is available for cell growth than when H₂ is the primary electron donor. A linear trend between cell growth and increasing initial H₂ content is then expected. This was observed in this study as after 48-120 hours of cultivation, growth was favored by increased H₂ in the headspace.

Figure 7: *Clostridium ljungdahlii* growth on syngas- Average optical density of batch cultures with varying initial syngas blends in headspace; After 48hrs of cultivation- orange circle; After 120hrs of cultivation-green square; After 240hrs of cultivations-blue diamond; Averaged from duplicate cultures; Standard deviation shown as error bar

Interestingly, cultures in the H_2/CO range of 1.0-1.5 grew slightly less than others after 240 hours. This phenomenon may be partly attributed to the unique chemical properties of CO. Although, CO is an excellent metabolite for its ability to serve as a carbon source and provide electrons at an extremely low potential, it can also potentially disrupt other cellular operations [30, 55]. CO contains a triple bond that makes it a weak Lewis base and an unsaturated soft ligand that can accept metal $d\pi$ electrons. As a result, CO acts as both a π acceptor and sigma donor that can form stable complexes with the low-valent states of the metal sites in enzymes. This property makes CO a strong inhibitor of metalloproteins that use low valent metals in their operations, such as hydrogenases that function to bind diatomic gases (i.e. H_2) [30].

Cultures in the H_2/CO range of 1.0-1.5 may have grew slightly less than others due to minor inhibition of their hydrogenase enzymes via high concentrations of CO. At an H_2/CO ratio

of 2.0 there was likely not enough CO to cause notable inhibition of hydrogen uptake as the highest optical density of ca. 0.45 was achieved after 240 hours [Fig. 7]. Similar optical densities were also achieved at H₂/CO ratios of 0.5. Although this may be explained by experimental error, it is likely that since CO was the dominant substrate any inhibition of hydrogen may have been too minimal to cause observable difference in cell growth.

3.4 *Clostridium ljungdahlii* product yields and efficiencies

Product yields and e-mol recoveries were calculated to further evaluate the bioconversion of gases into metabolites under each culturing condition [Fig. 8]. The highest yields of 0.188 ± 0.01 mol-p/mol-s and 0.203 ± 0.02 mol-p/mol-s were achieved under syngas ratios of 0.5 and 2.0, respectively, while cultures under ratios of 1.0 and 1.5 achieved similar yields ca. 0.161 mol-p/mol-s. All experimental yield values were comparable to those determined in similar *C.ljungdahlii* batch fermentation studies [56].

Figure 8: *Clostridium ljungdahlii* product yield and efficiencies; Experimental and theoretical yields: mol of products per mol of gas consumed (left y-axis); total electron-mol recovered in products (right y-axis)

As expected, product yields followed a similar trend as cell growth. It is likely that the generation of metabolites by 1.0 and 1.5 were partly limited by CO inhibition of hydrogenase enzymes as noted by others [57]. This was likely the cause of lower yields and electron recovery in products (70-77%). Interestingly, the yields of cultures under the 0.5 and 2.0 syngas blends were similar, despite having significantly different mixtures of products. This is also reflected in that both culturing scenarios achieved ca. 87% of theoretical yield values. These findings are parallel to those of Gaddy et al. [32] when culturing *C.ljungdahlii* on H_2/CO_2 and CO/CO_2 . Analogous to these results, e-mol recoveries for cultures using 0.5 and 2.0 syngas blends were in a similar range between 88-92%. It is expected that the remaining electrons were used to generate other minor metabolites or were needed to fulfill additional energy requirements for cell maintenance [43, 58]. Notably, the composition of the metabolites generated was dependent on the syngas blend used although conversion efficiencies were comparable under each culturing scenario.

4.0 Conclusions

The results of this study demonstrate that the H_2/CO ratios of syngas play a critical role in the spectrum of products generated via syngas fermentation by *C. ljungdahlii*. Production of electron-dense products was favored by CO in the headspace. Linear relationships were obtained that describe the impact of the syngas H_2/CO ratios in the range of 0.5-2.0 on acetate, ethanol, and 2,3-BD formation, respectively. This information on the relative formation of products in relation to H_2/CO ratios will be useful in selecting syngas production parameters that can now be

more accurately optimized for selecting syngas composition tailored toward desired product generation.

CHAPTER 3

PRODUCTION OF MAGNETIC BIOCHAR FROM WASTE-DERIVED FUNGAL BIOMASS FOR PHOSPHORUS REMOVAL AND RECOVERY

3.1 Introduction

Phosphorus is an essential element for life in aquatic ecosystems, however, excess amounts introduced from agricultural runoff or municipal wastewater can have extremely harmful effects such as eutrophication of receiving waters [59]. As such, development of efficient and sustainable methods that remove phosphorus from aqueous systems are needed to protect wildlife and meet increasingly rigid pollution regulations worldwide. To date, various techniques involving biological treatment, membrane separation, chemical precipitation, electrolysis and adsorption have been investigated [60,61]. Of these technologies, phosphorus adsorption onto biochar is considered to be one of the most promising due to its low cost, high selectivity, eco-friendliness, and ease of operation [62,63].

Michalekova- Richveisova et al. (2017) recently demonstrated maximum phosphate adsorption capacities of 0.036 mg/g, 0.132 mg/g, and 0.296 mg/g using corn-cob, garden wood waste, and wood chips biochars, respectively [64]. Others, however, have demonstrated far better phosphorus adsorption capabilities using biochars modified with iron particles. For example, Cai et al. (2016) reported a maximum phosphate adsorption of 5.07 mg/g using magnetic biochar derived from water hyacinth [65]. Notably, the modification of biochars with iron does not only make biochar a better adsorbent for some contaminants but also holds the added benefits of easy removal and recovery using simple magnets [66].

Though beneficial, the production of most magnetic biochars can be quite complicated and unsustainable. The majority of current methods involve a set of secondary processes in which a

magnetic medium is deposited on the surface of a char [67,68]. For instance, Wang et al. (2015) prepared magnetic pine wood biochar by mineralizing hematite and mixing the suspension with the feedstock material prior to pyrolysis [69]. Alternatively, Yang et al. (2018) impregnated iron directly into waste-activated sludge biomass prior to pyrolysis and found the phosphorus adsorption capabilities were greatly improved compared to samples fabricated using deposition methods [70]. Nevertheless, current methods such as these involve the use of expensive chemicals, complicated processing techniques, and generation of complex wastes.

In this work, we present a simple and novel bottom-up method for magnetic biochar production that alleviates these drawbacks and demonstrates excellent performance. Unique to our method, iron precursors are not impregnated in the char nor deposited onto the biochar post-pyrolysis, but rather accumulated and encapsulated within the living biomass feedstock prior to processing. The key to this innovative approach is the use of widely available municipal waste streams in combination with the robust fungus, *Neurospora crassa* (N. crassa), to generate biomass as the feedstock material for pyrolysis. N. crassa is capable of bio-accumulating high concentrations of many metals such as iron, cobalt, and nickel at high yields without affecting biomass growth [71-73]. Furthermore, N. crassa is easy to culture and exhibits a rapid growth rate on many minimal media. As such, N. crassa has been used in a wide variety of biological studies and its genome has been thoroughly mapped making it amenable to genetic manipulation [74]. The culmination of these unique qualities allow for a cheap, sustainable and adaptable source of biomass.

N. crassa is grown directly in iron-rich wastewater streams, which not only enables magnetic biochar production but also accomplishes wastewater treatment by removing metals and organics

from the waste stream. Typical waste streams can be from water treatment plants that use iron coagulants and primary effluent from wastewater treatment or industrial wastewater streams.

Here we demonstrated for the first time that *N. crassa* wild type could be grown on blends of waste streams, and the resulting biomass could be effectively processed into a magnetic biochar material. We further demonstrate that the physiochemical properties of the materials could be tuned by controlling the relative volumes of each of the waste streams used in the growth media. This technique allows for a low-cost, versatile, and sustainable approach to create magnetic biochar directly from municipal waste. The structure and morphology of the processed chars were investigated using a suite of tools including scanning electron microscopy equipped with an energy-dispersive X-ray analyzer (SEM-EDS), BET-N₂ adsorption, X-ray photoelectron spectroscopy (XPS), X-ray powdered diffraction (XRD) and Fourier transform spectroscopy (FTIR). To confirm the adsorbent properties of these materials, batch phosphate adsorption studies were conducted. Adsorption mechanisms were analyzed by fitting experimental data to Langmuir, Freundlich and Temkin isotherms and characterizing the P-loaded chars.

3.2 Materials and methods

3.2.1 Material production and processing

N. crassa was grown in waste streams consisting of primary effluent (PE) from Boulder wastewater treatment facility (Boulder, CO) and iron coagulation backwash (BW) from Boulder reservoir water treatment plant (Boulder, CO). Due to low organic content, BW alone was not able to sustain biomass growth so it was diluted with PE at ratios of 1:4 (PE-BW 1:4) or 3:4 (PE-BW 3:4). Prior to use, both waste streams were autoclaved at 121°C for 15 min. Glucose was then blended with waste streams at 1% (w/v) to stimulate the fungal acclimation. The major

constituents of each growth blend prior to fungal inoculation were determined by inductively coupled plasma mass spectrometry (Perkin Elmer SCIEX-ICP-MS; Fig. A.3).

N. crassa wild- type strain (FGSC #262) stock was stored on Vogel's minimal media slants at -20 °C [75]. New cultures were initiated by growing cells on agar slants at 30 °C for 3 days in complete darkness. Conidia (fungal spores) were isolated from slants using standard methods and inoculated into 1L flasks of media at a 1:100 dilution [76]. After inoculation, fungal cultures were stored on an orbital incubator/shaker (Barnstead Max Q4000) at 30°C and 90 rpm for 48 hours. The resulting biomass was then harvested using a vacuum pump and 0.45 µm filters and dried in a desiccator for 24 hours at 50°C. The dry biomass was carbonized by heating the biomass to 700°C at a rate of 10°C/min of using an isotherm muffle furnace (Fischer Scientific-550 series). The resulting granular fungal char was then processed into a powder using a ball mill for 20 minutes (Retsch-PM100). It is expected that the particle size after grinding is less than 325 meshes. Figure 9 summarizes the fabrication method of the magnetic biochar.

Figure 9: Flow diagram for magnetic biochar production- Fungal biomass derived from waste streams processed into powdered magnetic biochar material. 1) Growth media prepared by blending waste-streams and inoculating fungal spores. 2) Fungal cultures grown for 48 hours at 37 °C, agitated at 90 rpm. 3) Biomass filtered using 0.45 µm filter then dried in a desiccator at 50 °C for 24 hours 4) Dry biomass carbonized in a muffle furnace at 700 °C 5) Granular material processed to a powder using a ball mill.

3.2.2 Material characterization

The magnetic properties of the chars were determined using a simple magnet. To test the stability of the magnetic properties, both chars were put into aqueous solution (pure water; 0.1g/L) and rotated at 120 rpm for 14 days at 25 °C in the dark. The morphology and structure of the powdered biochars were investigated using SEM (Jeol JSM- 6480LV, 90 kV). The elemental composition of the materials were examined using EDS (Oxford Instruments Link ISIS) coupled to the SEM. Surface composition was further investigated using FTIR spectra recorded in the

4000–400 cm^{-1} region with a resolution of 4 cm (Bruker Vector 22 FTIR spectrometer). Further analysis of the materials surfaces were conducted using X-ray photoelectron spectroscopy (PE-5800 X-ray Photoelectron Spectroscopy) performed in the range of 0–1100 eV. The surface crystallinity of each material was analyzed to identify Fe-bearing minerals using an X-ray diffractometer (XRD; Rigaku Homelab) equipped with a stepping motor, a graphite crystal monochromator, and a CuK α radiation source. The total surface area, average pore size, and total pore volume were measured using N₂ sorption on a NOVA 1200 analyzer and calculated using the BET method.

3.2.3 Batch adsorption experiments and kinetic testing

Batch adsorption experiments were conducted in 55 mL polypropylene tubes using 100 mg of adsorbent and 50 mL phosphorus solutions prepared with sodium phosphate (Sigma Aldrich-342483). Initial phosphorus concentrations of 4, 8, 16, 50, 70 and 90 mg-P/L were tested. As determined by preliminary experiments, adsorption equilibrium was achieved after shaking the tubes for 24 hours in a rotary shaker (150 rpm) at room temperature (25 ± 2 °C) in the dark. The solutions were then centrifuged at 4000 rpm for 15 min. The supernatant was sampled using sterile syringes and passed through 0.22 μm filters. All adsorption experiments were run in duplicate. Blank solutions, without adsorbent, were used as controls. Phosphorus concentrations of the absorption solution supernatant were obtained using Hach TNT 844 or High range Molybdovanadate testing kits per the Hach method. Phosphorus loaded chars were collected from centrifuge tubes and allowed to dry for 24 hours at room temperature in the dark. The P-loaded chars were then analyzed using FTIR and EDS.

3.2.4 Phosphorus adsorption isotherm

The amount of adsorbed phosphorus per unit biochar mass was calculated using Equation 3 (below).

$$q_e = V(C_0 - C_e)/m, \text{ (Eq. 3)}$$

where q_e is the equilibrium adsorbent capacity, C_0 is the initial and C_e is the equilibrium concentration of the phosphorous (mg/L); V is the volume of the solution (L); m is the weight of the adsorbent (g)

To better understand the underlying reaction mechanisms, data was fit to three adsorption isotherm models (below).

$$q_e = \frac{Q_o K_L C_e}{1 + K_L C_e}, \text{ Langmuir (Eq. 4)}$$

$$q_e = K_f C_e^{\frac{1}{n}}, \text{ Freundlich (Eq. 5)}$$

$$q_e = (RT \ln A_T / b_t) + (RT \ln C_e / b_t), \text{ Temkin (Eq. 6)}$$

where: q_e = amount of phosphorus adsorbed per gram of the adsorbent at equilibrium (mg/g); C_e = the equilibrium concentration of adsorbate (mg/L); Q_o = maximum monolayer coverage capacity (mg/g); K_L = Langmuir isotherm constant (L/mg); K_f = Freundlich isotherm constant (mg/g); n = adsorption intensity; A_T = Temkin isotherm equilibrium binding (L/g) constant; b_t = Temkin isotherm constant; R = universal gas constant (8.314J/mol/K); T = Temperature (K)

To examine the fit of these models, commonly used adsorption error analysis parameters: sum square of errors (ERRSQ) and average relative error (ARE) were calculated using Eq. 7 and Eq. 8 [77]. ERRSQ is said to be one of the most widely used error functions, however, it has a major disadvantage that at higher end liquid-phase adsorbate concentrations it will illustrate a better fit for experimental data [78]. To mitigate bias, the ARE function which aims at minimizing the fractional error distribution across the entire concentration range was used in conjunction with ERRSQ [79].

$$ERRSQ = \sum_{i=1}^n (q_{e,i,calc} - q_{e,i,meas})^2, \text{ Sum square of errors (Eq. 7)}$$

$$ARE = \frac{100}{n} \sum_{i=1}^n \frac{q_{e,i,calc} - q_{e,i,meas}}{q_{e,i,meas}}, \text{ Average relative error (Eq. 8)}$$

where $q_{e,i,meas}$ is the experimentally measured adsorbed solid phase concentration of the adsorbate on the adsorbent as determined using Eq.3 and $q_{e,i,calc}$ is the theoretical concentration of adsorbate on the adsorbent, which has been calculated using one of the isotherm models (i.e. Eq. 4, Eq. 5, or Eq.6).

3.3 Results and discussion

3.3.1 Fungal growth and nutrient uptake

Figure 10: Biochar production and characterization images- Fungal cultures grown for 48 hours at 37 °C, agitated at 90 rpm (A) Researcher demonstrating magnetic abilities of PE-BW 1:4 (B) SEM image (5000x magnification, 5μm scale bar) of PE-BW 1:4 (C) and PE-BW 3:4 (D)

N.crassa was successfully cultivated on both of the wastewater blends tested. Mature cultures were observed after 48 hours of incubation, indicated by the appearance of conidia (fungal spores) across the surface of the fungal mats [Fig. 10a]. *N.crassa* depleted large amounts of

metal from the growth solutions likely via bio-sorption and metabolic uptake pathways as reviewed by others [80,81]. Larger biomass yields were attained by fungi grown on the PE-BW 1:4 media due to greater accumulation of metal [Fig. 11]. ICP-MS analysis of the growth media showed fungi grown under the PE-BW 1:4 media consumed 601.34 mg/L iron, while fungi under the PE-BW 3:4 media consumed only 228.4 mg/L [Fig. 11].

Figure 11: N.Crassa growth and nutrient uptake-Biomass yield (BY), COD removal and iron uptake per liter of wastewater used (left y-axis); Ca, Mg, P, Si uptake per liter of wastewater media used (right y-axis)

In contrast, carbon uptake was favored by fungi grown on the 3:4 dilution (PE-BW 3:4) that consumed about 0.83 g COD /L more than those under the 1:4 ratio (PE-BW 1:4), likely due to the increased availability of supporting nutrients (i.e. nitrogen, phosphorus, etc.). Other constituents that were readily removed from solution during fungal growth included Si, Mg, P and Ca [Fig. 11]. These nutrients were likely used to augment the utilization of iron or were bio-mineralized in separate metabolic processes as examined by others [82]. The average dry weight

of biomass per liter of media used were 1.77 g for PE-BW 1:4 media and 1.04 g for PE-BW 3:4 media, respectively [Fig. 11].

3.3.2 Material characterization

Fungal biomass resulting from the PE-BW 1:4 and PE-BW 3:4 growth medias were ultimately processed into fungal chars PE-BW 1:4 and PE-BW 3:4. Both finished chars exhibited magnetic properties. PE-BW 1:4 demonstrated a very strong affinity for magnets while PE-BW 3:4 displayed limited attraction [Fig. 10b]. After 14 days of being agitated and submerged in aqueous solution (pure water; 0.1 g/L), both chars retained their magnetic properties and were easily recovered from solution using a simple magnet. SEM images of the biochars revealed morphologies unique to each of the materials [Fig. 10c,d]. The greater initial metal content of the blend used to generate PE-BW 1:4 led to increased crystalline formation during the pyrolysis process compared to PE-BW 3:4. EDS coupled to the SEM revealed distinct elemental composition of each material surface.

Only a very small amount of elemental carbon (2.1%) was detected in PE-BW 1:4 while PE-BW 3:4 was composed of 23.7% [Table 1]. Both materials retained similar amounts of iron (ca. 45-50%), however, PE-BW 1:4 had a much greater Fe/C ratio (21.4 vs. 2.1) and higher oxygen content (19% vs. 25%) than PE-BW 3:4 which suggested superior iron oxide formation on the material surface.

Table 1: Major elemental constituents of powdered chars (by weight %), BET-N₂ specific surface area (SA), average pore diameter (APD) and total pore volume (TPV) of biochars.

Material	C (%)	O (%)	Si (%)	Fe (%)	Fe/C	SA (m ² /g)	APD (nm)	TPV (cm ³ /g)
PE-BW 1:4	2.1	23.9	29.8	45.0	21.4	51.6	13.2	0.2

PE-BW 3:4	23.7	19.6	1.4	50.5	2.13	53.0	6.1	0.1
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This is line with PE-BW 1:4's enhanced magnetic properties compared to PE-BW 3:4 and the crystal arrangements observed in SEM images. The smaller surface area (51.6 m²/g) and greater average pore diameter (13.2 nm) of PE-BW 1:4 further proposed amplified iron oxide development as these formations have small surface areas and ample transitional pores (2-50nm). PE-BW 1:4 had about double the total pore volume (0.2 cm³/g) of PE-BW 3:4 (0.1 cm³/g) even though the carbon content of PE-BW 3:4 was much greater.

This may also be attributed to the superior iron oxide formation of PE-BW 1:4 but could also be due to large silica oxide developments in PE-BW 3:4 as implied by its much greater surface silica content.

To further investigate the crystalline structures, FTIR spectra, XPS and XRD patterns were obtained for each of the powdered chars [Fig. 12]. XRD analyses identified maghemite (γ -Fe₂O₃) as the major crystalline in PE-BW 1:4 as characterized by strong diffraction peaks at 30.2°, 35.5°, 43.2°, 57.3°, and 62.9° [83,84]. The presence of maghemite was likely the source of magnetic attraction as proposed in similar work [85]. As expected, PE-BW 3:4 showed much weaker maghemite diffraction peaks and presented patterns more similar to an amorphous carbon material. Similar observations were made from the XPS Fe 2p_{3/2} spectra that showed two peaks at 710.6 and 724.1 eV, corresponding to Fe 2p_{3/2} and Fe 2p_{1/2}, that are the characteristic peaks of the Fe³⁺ ion in maghemite. Furthermore, satellite peaks at 719 eV also indicate maghemite formation. In agreement with XRD data, each of the maghemite XPS peaks for PE-BW 1:4 were much stronger than PE-BW 3:4 indicating a greater development of maghemite.

Figure 12: Fungal biochar material characterization summary- XRD diffraction pattern of PE-BW1:4, PE-BW 3:4; ν indicates maghemite peaks (A) Zoomed XPS spectra of PE-BW 1:4, PE-BW3:4 (B) FTIR spectra of PE-BW1:4 and PE-BW3:4; zoomed inset of Fe-O peaks ($400\text{--}725\text{ cm}^{-1}$; all other peaks of interest boxed and labeled (C)

FTIR spectra recorded from 400 to 4000 cm^{-1} showed both materials exhibited peaks centered at 432 , 556 , and 634 cm^{-1} that suggest the presence of Fe-O bonds in the form of maghemite [86-88]. As expected, PE-BW 1:4 displayed much more pronounced bands than PE-BW 3:4 owing to its greater iron oxide formation. PE-BW 1:4 also showed strong peaks around 831 cm^{-1} that indicate C-O-Fe surface bonds while PE-BW 3:4 displayed broader peaks centered at 1060 cm^{-1} that suggested silica oxide formation in the form of silicone (Si-O-Si). Both materials retained some organic structures as signified by the presence of transitional metal carbonyl groups with peaks centered at 1974 cm^{-1} and 2100 cm^{-1} and broad carboxylate peaks centered at 1600 cm^{-1} [67,89].

3.3.3 Phosphorus adsorption isotherms

Both biochars exhibited strong affinities for phosphorus across the range of initial concentrations tested. The highest equilibrium adsorption density of 23.9 mg/g was obtained using PE-BW1:4

and an initial phosphate concentration of 90 mg/L [Fig. 13]. This high performance demonstrates the practical use of this material in agricultural and environmental applications. For example, municipal wastewaters commonly contain 5-20 mg/L total phosphorus [90]. In this range, PE-BW 1:4 achieved an adsorption density of 2.42 mg/g using an initial phosphate concentration of 16 mg/L. In comparable studies, Huggins et al. (2016) reported an adsorption capacity for phosphate of 1.0 mg/g under an initial phosphate concentration of 18 mg/L using a granular biochar prepared from lodgepole pine wood [60]. Moreover, Chen et al. (2011) reported an adsorption capacity of 0.44 mg/g under a under an initial phosphate concentration 12 mg/L using a magnetic biochar orange peel and magnetite deposition [67].

Others have reviewed the affinity for iron particles to complex phosphorus in detail [67,91]. It is likely the greater phosphorus adsorption ability of PE-BW1:4 stems from its superior maghemite development. Moreover, the higher average pore diameter (13.2nm vs. 6.1nm) of PE-BW 1:4 may have further augmented surface interactions with the phosphorus in solution compared to PE-BW 3:4.

To better understand the underlying sorption behaviors of the phosphorus to the chars, data was fitted to the Freundlich, Temkin, and Langmuir isotherm models. All of the models reproduced the isotherm data fairly well with coefficients of determination (R^2) above 0.90 [Fig. 13]. Both PE-BW 1:4 and PE-BW 3:4 fit the Freundlich adsorption isotherm the best. This is best demonstrated by PE:BW 1:4 which exhibited an R^2 value of 0.99 and very low ERRSQ and ARE errors of 33.6 and 35.6, respectively.

Figure 13: Adsorption isotherms and error analysis of phosphorus on fungi derived biochars- PE-BW 1:4 adsorption isotherms; $\diamond$ =measured data (A) PE-BW 3:4 adsorption isotherms; $\star$ =measured data (B) Results of sum of square error analysis; Freundlich and Temkin error (left y-axis), Langmuir (Lang.) error (right y-axis) (C) Results of average relative error analysis for each isotherm fit to measured data (D)

The Freundlich model is often used to describe chemisorption on heterogeneous surfaces, which is in line with the composite nature of these materials and likely why the data fit this isotherm the best. This was also found by Lǔ et al. (2013) in similar studies using Fe–Al–Mn trimetal oxides for phosphate removal [92]. Interestingly, the Temkin model which is also commonly used for heterogeneous surfaces was the worst fit for both PE-BW 1:4 and PE-BW 3:4 with R^2 values of 0.91 and 0.94 and large ARE values of 81.2 and 92.9, respectively [Fig. 13]. The Temkin model assumes that adsorption is characterized by a uniform distribution of binding energies, which makes it better suited for homogeneous materials than the Freundlich model [93]. In comparison, it may be that the greater homogeneity of PE-BW 3:4, caused by less extensive iron oxide surface formation, made it a better fit to the Temkin model than PE-BW 1:4. This was further suggested by fitting the data to the Langmuir model that assumes that adsorption occurs at specific homogeneous sites on the adsorbent. PE-BW 3:4 fit the Langmuir

model almost as well as the Freundlich model with an R^2 of 0.98, ERRSQ of 122.7 and ARE of 27.2 [Fig. 13]. PE-BW 3:4 fit the Langmuir model much better than PE-BW 1:4, further indicating that its distinct adsorption mechanisms stem from its greater homogeneity.

3.3.4 Phosphate retention and surface complexes

To validate the phosphorus recovery capabilities of these materials, EDS and FTIR analyses were conducted on the chars post-adsorption [Fig. A.4; Fig. 14]. These analyses have been effectively used in many adsorption studies to elucidate interaction mechanisms between adsorbates and adsorbents [94,95]. At grander scale, EDS confirmed the presence of absorbed phosphorus on the chars. For example, after adsorption with a initial phosphate concentration of 50 mg /L the phosphorus surface atomic percent and weight percent of PE-BW 1:4 rose by 1.52% and 1.76%, respectively. This is complemented by FTIR analyses that showed noteworthy changes even after adsorption with initial concentrations as low as 10 mg/L [Fig. 14].

Figure 14: Phosphorus loaded biochar FTIR analysis summary- PE-BW 1:4 iron peaks (left plot) and phosphorus peaks (right plot) for raw material and after equilibrium in batch study with 10 mg P/L and 20 mg P/L (A) PE-BW 3:4 iron peaks (left plot) and phosphorus peaks (right plot) for raw material and after equilibrium in batch study with 10 mg P/L and 20 mg P/L (B)

For both materials, the Fe-O bands centered at 442 cm^{-1} and 634 cm^{-1} seem to lessen and even disappear post adsorption. Conversely, new bands at 865 cm^{-1} and 1025 cm^{-1} appeared after adsorption likely owing to new P-O and P-OH bonds, similar to findings of others [91, 92]. The combined results imply a substitution reaction between Fe-O groups with adsorbed P-ions. Interestingly, PE-BW 3:4 also showed the sharpening of a peak around 1380 cm^{-1} suggesting P-C bonds [Fig. 14]. This may demonstrate a second binding mode that is more pronounced in PE-BW 3:4 than PE-BW 1:4 which is expected because PE-BW 1:4 contains much less carbon (2.1% vs. 23.7%). Furthermore, both materials showed elevated peaks around 1600 cm^{-1} which are likely linked to C=O bonding associated with phosphate dissociation on the surface [89,96].

4.0 Conclusions

A novel bottom-up approach for biofabrication of magnetic biochar has been established. During cultivation, fungi can accumulate large amounts of metals and nutrients from municipal wastewaters allowing for a cheap and sustainable method for generation of biomass feedstock. The resulting fungal biomass can be easily processed into magnetic biochar and the physiochemical properties of the materials can be tuned by controlling fungal growth parameters. The magnetic biochars had viable surface areas ($51.6\text{-}53\text{ m}^2/\text{g}$), average pore sizes ($6.1\text{-}13.2\text{ nm}$), and magnetic capabilities. High phosphorus adsorption capacities of 23.9 mg/g endorsed the use of these materials in environmental applications. Phosphate adsorption on the chars best fit Freundlich isotherms and the results of FTIR and EDS analyses suggested that substitution of surface oxide groups by phosphate were primarily responsible for adsorption. The fungi derived

biochars have demonstrated good performance for phosphorus removal and may have numerous applications for adsorption of anionic species such as arsenic from drinking water and chromate for soil remediation. More applications of these magnetic chars are still be explored.

CHAPTER 4

ELECTROCHEMICAL CO₂ REDUCTION TO SYNGAS

5.1 Introduction

Selective and efficient conversion of carbon dioxide (CO₂) to high energy density fuels is paramount for a closed-carbon energy future. Ideally, renewable energy from solar or wind sources will be used to simultaneously drive CO₂ sequestration and transformation into fuels at ambient temperatures without the generation of complex wastes. The injection of an electron into CO₂, however, requires highly negative potentials (large energy investment) and the kinetics are quite sluggish under standard conditions [97-99]. As such, synthetic catalysts for the electrocatalytic reduction of CO₂ have been studied for several decades [100]. This work has resulted in numerous catalysts (primarily precious and non-precious metals) capable of producing two-electron products such as carbon monoxide (CO), formic acid (HCOOH) and oxalate (C₂O₄²⁻) at high yields and efficiencies [101,102]. For example, Peng et al. (2018) recently demonstrated that their preferentially oriented silver nanoparticles could reduce CO₂ to CO with a faradaic efficiency of 96.7% at a low operating potential of -0.69V vs. RHE [13].

Although promising, products such as CO are of limited value as they require further processing to be used as fuels or upgraded into other value-added commodities. Consequently, further investigation into the formation of reduction products requiring four or more electrons is necessary. This work inherently poses additional challenges associated with higher over potentials and complex parallel reactions schemes that lead to decreased product specificity [103]. For instance, Hori and co-workers discovered that metallic copper could reduce CO₂ to significant amounts of multi-electron products such as methane (CH₄), ethylene (C₂H₄) and ethanol (C₂H₅OH) [104-106]. Nevertheless, a lack of specificity was evident as more than 14

different products could be generated by CO₂ reduction on copper and improving the precision of product generation has been an prodigious challenge [107].

Recently it was found that cobalt protoporphyrins immobilized on graphite could produce CH₄ as one of just three major products [108]. These findings are noteworthy as CH₄ can be readily used as a fuel and indicate that an electron transfer involving 8 electrons to CO₂ is viable with good precision. Several models based on first principal calculations have since been presented that describe the reaction pathways from CO₂ to CH₄ on cobalt porphyrins [109,110]. The culmination of these works suggests that optimizing fundamental parameters such as bulk electrolyte pH, local cation availability and electrolyte cation solvation effects may enhance the overall multi-electron reduction of CO₂. For example, an extensive density functional theory (DFT) model developed by Tripkovic et al. proposed that the production of CH₄ on cobalt porphyrin is a proton-concerted reaction pathway following the formation of an [CoP-CO₂.]⁻ adduct (Eq. A.11) [111]. It is then natural to expect that lowering the bulk electrolyte pH could accelerate the proton-concerted pathway and improve CH₄ yields. Though an experimental pH dependence on CH₄ production was noted by others between pH 1-3, it is yet to be expanded to more viable operating conditions (i.e. near neutral conditions)[108]. It is important to note that operating under lower pH may allow for the hydrogen evolution reaction (HER) to outcompete the CO₂ reduction reaction (CO₂-RR), decreasing faradaic efficiencies of carbon products [112]. Although an optimal pH that can balance the affinity of both HER and CO₂ reduction pathways could be determined, increasing the local proton availability (via modified ligand structures) in neutral or alkaline pH may circumvent these issues. Though this has not been verified using cobalt porphyrins, analogous concepts have been applied to iron porphyrins in aprotic solvents [113]. For instance, a high local concentration of protons via the addition of phenolic hydroxyl

groups in all phenyl group ortho positions of an iron tetraphenyl porphyrin (FeTPP) monomer greatly enhanced activity and stability for CO₂ reduction to CO and allowed for CO faradaic efficiencies above 90% through 50 million turnovers over 4 hours of electrolysis in N,N-Dimethylformamide (DMF) [113]. In-line with this discovery, more drastic ligand modifications of FeTPP have also been attempted and have yielded excellent CO faradaic efficiencies (95%) and cyclability (TOF = 4300 s⁻¹) [114].

The core of these strategies are based on studies that showed the catalytic efficiency and stability of iron porphyrins could be enhanced by the presence of Lewis acids such as Mg²⁺ or Ca²⁺ [115,116]. These works proposed a push–pull mechanism where the electro-produced reduced species pushes an electron pair to the CO₂ molecule and the electron-deficient acid synergist promotes the separation of one of the C–O bonds [117,118]. Although a comparable mechanism has not been established with cobalt porphyrins, the presence of solvating cations has been proposed to enhance CO₂ reduction on cobalt porphyrins. For instance, Miyamoto et al. (2018) recently expanded on Koper’s quantum chemistry model for water facilitated CO₂ reduction mechanisms on a cobalt porphine (CoP) to suggest that additional cation availability in the bulk solution could stabilize the [CoP-CO₂]⁻ intermediate leading to improved electrocatalytic CO₂ reduction efficiencies and lower overpotentials (Eq. A.23 vs. Eq. A.24) [119].

All things considered, first principles calculations such as these deliver useful insight to direct multi-electron reduction of CO₂ on metallic porphyrins but few models have been verified experimentally. The aim of our work was to use information from theoretical models to experimentally improve the electrocatalytic efficiency and yield of multi-electron reduction of CO₂ to CH₄ on immobilized cobalt porphyrins. An innovative examination of important factors

such as local proton availability, bulk electrolyte pH, and cation solvation effects was conducted. This included the synthesis and characterization of new catalysts cobalt (II)-5,10,15,20-tetrakis(2',6'-dimethoxyphenyl)-21H,23H-porphyrin (Co(o-CH₃)TPP) and cobalt(II)-5,10,15,20-tetrakis(2',6'-dihydroxyphenyl)-21H,23H-porphyrin (Co(o-OH)TPP). The results presented, hereto, demonstrate high yields and efficiencies for CO and CH₄ under optimized aqueous-based conditions. Furthermore, this work provides valuable experimental insight on existing theoretical models and helps identify key parameters that improve multi-electron reduction under realistic operation conditions.

5.2 Materials and methods

2.1 Catalyst synthesis

To examine the impact of local proton availability on CO₂ reduction, metalloporphyrins Co(o-OCH₃)TPP and Co(o-OH)TPP were synthesized according to the reactions schemes in Figure 15 and compared to the performance of Cobalt(II)-5,10,15,20-Tetraphenyl-21H,23H-porphine(CoTPP) purchased from Sigma Aldrich. Porphyrin structures 5,10,15,20-tetrakis(2',6'-dimethoxyphenyl)-21H,23H-porphyrin and 5,10,15,20-tetrakis(2',6'-dihydroxyphenyl)-21H,23H-porphyrin were produced using comparable techniques to others [120]. Full details of the synthesis reactions are provided in the appendix methods section [A.1]. Briefly, to synthesize Co(o-OCH₃)TPP, solid H₂(o-OCH₃)TPP (0.1453g, 0.170mmol) and anhydrous CoCl₂ (II) (0.441g, 3.40mmol) were dissolved in anhydrous tetrahydrofuran (THF, 30mL) under inert N₂ gas and 2,6-lutidine (0.060mL, 0.51mmol) was added. The solution was then refluxed for two hours. After removing THF solution, the mixture was dissolved in dichloromethane (DCM) and washed with deionized water three times. Organic solvent was concentrated down and the

product was collected. Co(o-OH)TPP was prepared in the same fashion except ethyl acetate was used to dissolve the mixture after removing the THF solution.

Figure 15: Summary of synthesis reactions for Co(o-OH)TPP and Co(o-OCH₃)TPP

2.2 Material characterization

The as-synthesized porphyrins and metalloporphyrins were characterized by proton nuclear magnetic resonance spectroscopy (¹H NMR), ultraviolet–visible spectroscopy (UV-Vis), and Fourier-transform infrared spectroscopy (FTIR). ¹H NMR spectra were recorded on a Bruker Avance-III 300 NMR spectrometer. Fourier transform infrared (FT-IR) spectra were measured

with an Agilent Technologies, Cary 630. UV-Vis Absorption spectra were recorded on an Agilent 8453.

2.3 Electrochemical testing

2.3.1 Preparation of working electrodes

Glassy carbon (GC) disks (0.3 cm diameter rod, BASi-MF-2012) were used as working electrodes for all homogenous testing conditions. GC disk electrodes were polished using alumina pastes followed by sonication in acetone for 10 minutes prior to use. For heterogeneous testing, a mixture of 5mg of catalyst and 5mg of carbon black was sonicated for 30 min in 2mL of DMF. 200 μ L of the resulting suspension was then drop casted on two sides of carbon paper (Toray-50% wet-proofed) and dried for 48 hours at room temperature (25 ± 0.1 °C) in a laminar flow hood.

2.3.2 Cyclic voltammetry

Cyclic voltammetry (CV) was carried out using a Pine Research (WaveDriver- 200 series) potentiostat connected in a three-electrode setup. A platinum wire (BASi-MF-1033) was employed as counter electrode along with an Ag/AgCl reference electrode and the appropriate working electrode (GC disk or carbon paper). Homogenous testing in DMF was conducted with 2mM of catalyst and 0.1M tetrabutylammonium hexafluorophosphate (NBu₄PF₆) as the supporting electrolyte; heterogeneous testing was conducted using catalyst coated-carbon paper and 0.5 M NaHCO₃ as the supporting electrolyte. In some experiments, DMF was mixed with deionized H₂O in varying concentrations (2M and 10M) with 0.1M NBu₄PF₆ as the electrolyte. Prior to analysis, the electrolyte solutions were saturated with either pure Argon or CO₂ gas.

2.3.3 Controlled potential electrolysis

Controlled potential electrolysis were conducted using the aforementioned potentiostat in combination with a standard Pine Research electrochemical cell (AKCELL1) containing two chambers separated by a glass frit. The cathodic compartment contained catalysts coated-carbon paper as the working electrode (partly submerged in the electrolyte solution) and an Ag/AgCl reference electrode with ceramic porous frit (BASi-MW-2030). The anodic compartment had a large graphite electrode (OD= 0.250", L= 12"). The supporting electrolyte in both chambers contained 0.5M NaHCO₃ dissolved in deionized water. In some experiments, the initial pH was adjusted using 1M potassium hydroxide or 1M hydrochloric acid solution and/or NaCl or CaCl₂ were added to the electrolyte. Prior to electrolysis, all electrolytes were saturated with either pure CO₂ or argon and the cell was sealed using gas tight rubber seals (Pine Research-14/20; 24/25).

2.3.4 Gas analysis

Gas samples were obtained directly from the headspace of the electrochemical cell using a 250 μ l gastight syringe (Hamilton-1700 series) and were immediately analyzed on an Agilent Technologies 7890A gas chromatography system equipped with a thermal conductivity detector (TCD) and packed column. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 250°C, respectively. Argon gas was used as the carrier gas at a flow rate of 5 mL min⁻¹. CO and H₂ molar concentrations were determined by fitting data to a five-point standard curve prepared for each gas using standard calibration gases.

5.3 Results and discussion

3.1 Material synthesis and characterization

The as-synthesized porphyrins and metalloporphyrins were characterized by ¹H NMR, UV-Vis, and FTIR. The culmination of results indicated successful synthesis of H₂(o-OCH₃)TPP and

$\text{H}_2(\text{o-OH})\text{TPP}$ and subsequent metalation reactions to produce $\text{Co}(\text{o-OCH}_3)\text{TPP}$ and $\text{Co}(\text{o-OH})\text{TPP}$, respectively. ^1H NMR demonstrated the disappearance of the dimethoxy group at 3.49 ppm indicating the production of $\text{H}_2(\text{o-OH})\text{TPP}$ from $\text{H}_2(\text{o-OCH}_3)\text{TPP}$ [Fig. A.5, A.6]. This was further supported by FTIR that showed the appearance of a broad peak around $3,300\text{cm}^{-1}$, indicating the presence of the hydroxy group [Fig. A.7, A.8]. Successful metalation of the porphyrins was indicated by a shift in the UV-vis Soret absorbance peak from 417nm to 413nm after metalation [Fig. A.9, A.10]. This was further suggested by FTIR spectra that showed the disappearance of the N-H stretching peak around $3,300\text{cm}^{-1}$ from both $\text{H}_2(\text{o-OCH}_3)\text{TPP}$ and $\text{H}_2(\text{o-OH})\text{TPP}$, indicating the substitution of cobalt into the carbon matrixes [Fig. A.7, A.8].

3.1.2 Impact of local proton source in aprotic solvent

CVs of $\text{Co}(\text{o-OCH}_3)\text{TPP}$, $\text{Co}(\text{o-OH})\text{TPP}$, and CoTPP were obtained in a DMF solution containing 0.1M NBu_4PF_6 saturated with CO_2 gas to assess the fundamental impact of the local proton source on electrocatalytic CO_2 reduction [Fig. 16]. Notably, an assortment of responses in catalytic behavior were observed between the catalysts upon introducing varying quantities of protons (2M, 10M H_2O) to the initially aprotic solvent [Fig. 16]. CoTPP seemed to be unaffected by the addition of water with a $\text{Co}^{2+}\text{--Co}^{+1}$ activation peak around -0.80V vs. Ag/AgCl and the start of a major reduction event stable around -1.85V vs. Ag/AgCl across each scenario. These findings are similar to those of Hu et al. (2017) when introducing protons via phenol to CoTPP immobilized on GC disk electrodes in DMF [121]. In contrast, the functionalized catalysts (i.e. $\text{Co}(\text{o-OCH}_3)\text{TPP}$ and $\text{Co}(\text{o-OH})\text{TPP}$) seemed to show enhanced catalytic activity upon the addition of water. For example, the onset potentials for the major reduction event of both $\text{Co}(\text{o-OH})\text{TPP}$ and $\text{Co}(\text{o-OCH}_3)\text{TPP}$ seemed to shift from ca. 300mV to more positive potentials upon adding 10M H_2O . Remarkably, the reduction current of $\text{Co}(\text{o-OCH}_3)\text{TPP}$ was much higher than

others upon the addition of 10M H₂O reaching about twice that of CoTPP and Co(o-OH)TPP at -2.5V vs. Ag/AgCl.

Figure 16: CO₂ reduction capabilities of modified cobalt porphyrin structures; Pt anode, Ag/AgCl ref. electrode, glass carbon working electrode; 1mM catalyst and 0.1M NBu₄PF₆ dissolved in DMF; Various amounts of H₂O added; saturated with CO₂

The culmination of these results are in-line with the findings of others using modified FeTPP catalysts for CO₂ reduction under homogenous conditions [120]. It is important to note that at the potentials near where the activation peaks start (ca. -800mV vs. Ag/AgCl), the current can be attributed to CO₂ reduction, HER or a mixture of both. Consequently, controlled potential electrolysis experiments were required to investigate the product selectivity of each catalyst and elucidate the affinity for each possible reaction mechanism.

3.1.3 Aqueous controlled potential electrolysis

To maintain the practicality of these findings, electrolysis was conducted under heterogeneous conditions with an aqueous electrolyte (at room temperature) at moderate potentials (-0.85V to -

1.5V vs. Ag/AgCl). At each operating voltage it was apparent that the functionalized catalysts (i.e. Co(o-OCH₃)TPP and Co(o-OH)TPP) favored the HER over the CO₂-RR in comparison to the bare catalyst, CoTPP. For instance, the faradaic efficiencies (FEs) of CoTPP for HER was only about a tenth of that of Co(o-OCH₃)TPP or Co(o-OH)TPP after an hour electrolysis at -1.2V vs. Ag/AgCl [Fig. 17].

Figure 17: Cobalt porphyrin catalyst preparative scale electrolysis; Faradaic efficiencies of each catalyst at -0.85v vs. Ag/AgCl (A) -1.2v vs. Ag/AgCl (B) -1.5v vs. Ag/AgCl (C) Graphite anode electrode, catalyst deposited on carbon paper working electrode 0.5M KHCO₃ electrolyte; Hydrogen evolution reaction (HER) in dark blue; CO₂ reduction reaction (CO₂-RR) in light blue

This finding is significant as in comparable studies with iron porphyrins (under homogenous conditions) enhanced catalytic waves were attributed to only CO₂ reduction [114]. Using highly regarded DFT models, it was anticipated that the local proton availability would accelerate Eq. A.13 or Eq. A.15 as part of the proton-concerted reaction pathway (Eq. A.9, A.11, A.13, A.15) of CO₂ to CO. As the equilibrium potential for Eq. A.15 is high (-0.37mV vs. Ag/AgCl) it is likely that this reaction is spontaneous and non-limiting in the potential range tested, thus the impact of increased proton availability was not observed [109]. The local proton source could

have been used for Eq. A.13 but it seems that it was outcompeted by initial HER pathways (Eq. A.21 and Eq. A. 22). Interestingly, heterogeneous testing conditions allowed for CO₂ reduction to occur at very low over-potentials (ca. 50mV vs. Ag/AgCl) as noted by others in comparable studies [121,122]. At potentials close to the onset (ca. -0.8V vs. Ag/AgCl), H₂ pathways were limited and allowed for excellent CO₂ reduction efficiencies. For example, CoTPP achieved 98% CO₂-RR FEs compared to Co(o-OH)TPP which was about 34% [Fig. 17]. The immobilization of the catalysts on the porous carbon paper may have further exacerbated these efficiencies. Others have also remarked on the importance of strong electrode-catalyst interactions for multi-electron CO₂ reduction [123,124]. Interestingly, the CO₂ reduction capabilities of the bare catalysts (CoTPP) were not improved by addition of local proton sources. This suggests that improving proton-concerted pathways may not be effective in enhancing CO₂ reduction on cobalt porphyrins. As far as CH₄ production, several reduction pathways are currently under review and the pH dependence is not yet clear in neutral or alkaline conditions [110,125,126].

3.2. Bulk electrolyte pH

The role of the bulk electrolyte pH was examined in the range of 6.5-9.5 to examine the impact on CO₂ reduction on CoTPP in heterogeneous alkaline and neutral pH conditions [Fig. 18]. It seemed the pH of the solution did not greatly impact the CO₂ reduction efficiencies of CoTPP as the CO₂ -RR efficiencies were all between 95%-99% throughout the range tested (6.5-8.5). This suggests that H₂O is a suitable reactant in reduction pathways, such as Eq. A.24, in place of H⁺ as suggested by the first principle calculations of others [108,127]. Notably, the onset potential at pH 9.5 shifted to lower potentials and only about half the current was drawn at -0.85V vs. Ag/AgCl compared to the other samples [Fig. 18]. This may suggest that the presence of anions can actually interrupt CO₂ reduction pathways and extreme alkaline operating conditions should

be avoided. This is likely because at higher pH the speciation of a key intermediate CoP-COOH^- ($\text{pK}_a = 8.3$) changes and favors sequential proton–electron transfer rather than concerted [110]. The sequential proton–electron transfer reaction steps are likely more sluggish and lead to decreased observed current uptake and gas production.

Figure 18: Bulk electrolyte pH effect on CoTPP CO_2 reduction; Cyclic voltammetry at various pH (A) Current vs. time plot; potential at -0.85V vs. Ag/AgCl (B)

3.3 Influence of alkali cations on CO_2 reduction

3.3.1 Electrolyte cation effects

As the availability of protons showed to have little improvement on CO_2 reduction, an investigation of the presence of other alkali cations was conducted to improve CO_2 reduction on CoTPP. Controlled potential electrolysis of CoTPP on carbon paper in 0.5M NaHCO_3 were compared to 0.5M KHCO_3 . In theory, both potassium (K^+) and sodium (Na^+) would be absorbed to the negatively charged electrode through electrostatic attraction. K^+ , however, is a larger

molecule than Na^+ (atomic radii: 138pm vs. 102pm) and thus has a smaller hydration number (7 vs. 13) [128-130]. As a result, Na^+ has a larger geometric configuration that would potentially make it more difficult for H^+ to interact with the electrode, leading to decreased H_2 production. Nevertheless, this seemed to have little effect as samples containing both 0.5M NaHCO_3 and KHCO_3 achieved similar CO and CH_4 production [Fig. 19]. To further examine solvation effects, addition of monovalent and divalent cations were also compared by addition of 0.5M Na^+ and 0.5M Ca^{+2} to an electrolyte solution containing 0.5M NaHCO_3 . Interestingly, the addition of Ca^{+2} lead to very poor CO_2 reduction efficiencies and slow deactivation of the catalyst was observed likely via precipitation of CaCO_3 on the electrode surface. In contrast, the addition of Na^+ significantly improved the yield of both CO and CH_4 .

Figure 19: Solvating cation effect on CoTPP CO_2 reduction; 0.5M NaHCO_3 alone or with 0.5M NaCl or 0.5M Ca^{+2} added; 0.5M KHCO_3 electrolyte; Cyclic voltammetry 0.1 v/s (A) Carbon monoxide production (B) Methane production (C) Graphite anode electrode, catalyst deposited on carbon paper working electrode

For example, almost 1.6 times more mmoles of CO were produced in the electrolyte containing

0.5M NaHCO₃ upon the addition of 0.5M Na⁺. This was likely due to a combination of steric and electronic effects at the electrode surface. First principle calculation models developed by Miyamoto et al. (2018) suggest that Na⁺ can act as a Lewis acid stabilizing the key intermediate [CoP-CO₂]⁻ in CO₂ reduction to CO [119] This manifests itself as a higher equilibrium reaction potential (-0.74 vs. -1.33 V vs. RHE) for the solvated H₂O (Eq. A.23) vs. the bare molecule (Eq.A.24) when participating in reaction Eq. A.13 or A.14. Furthermore, the hydration of Na⁺ may have also inhibited H⁺ diffusion to electrode surface by the steric hindrances.

To further evaluate these effects, the impact of the sodium was further examined by testing concentrations in the range of 0.0-1.0M Na⁺ added to 0.5M NaHCO₃ solutions [Fig. 20]. As expected, increased Na⁺ concentration lead to increased product yield. Interestingly, CO production seemed to drop in favor of methane production. This may indicate that the Na⁺ stabilization effects are more important for the CO adduct stabilization than following reactions. Ultimately, the highest yield of .0082 mmols CH₄ were achieved after addition of 1.0M Na⁺. This presents excellent performance compared to copper(II)-5,10,15,20-tetrakis-(2,6dihydroxyphenyl)porphyrin at -1.1V vs. Ag/AgCl under heterogeneous conditions [131].

Figure 20: Sodium stabilization effect on CoTPP CO₂ reduction; 0.5M NaHCO₃ alone or with 0.5M or 1.0 M Na⁺ added; Cyclic voltammetry 0.1 v/s (A) Carbon monoxide production (B) Methane production (C) Graphite anode electrode, catalyst deposited on carbon paper working electrode

4.0 Conclusions

Cobalt porphyrins immobilized on carbon paper can produce CO and CH₄ in an aqueous electrolyte at near neutral pH and at a low over-potential of ca. 50mV. For the first time, the impact of increased local proton availability via modified CoTPP was examined under heterogeneous conditions. This led to accelerated H₂ production and suggested CO and CH₄ production pathways are not pH sensitive in near neutral pH conditions. This was supported by bulk electrolyte pH optimization that showed little effect on CO₂ reduction activity. Sodium solvation increased CO₂ reduction and high yields and almost 98% carbon products were achieved with addition of 1.0M Na⁺. The culmination of these findings has helped identify key operational parameters to facilitate multi-electron transfer to CO₂ under realistic and scalable conditions.

CHAPTER 5

TWO STAGE BIOHYBRID SYSTEM FOR ELECTROCHEMICAL SYNGAS PRODUCTION AND MICROBIAL CONVERSION

5.1 Introduction

Carbon dioxide (CO₂) is a major contributor to atmospheric “green-house” gases that are fundamentally accountable for global climate change. Currently, the atmospheric CO₂ concentration is about 410 ppm (3,200 gigatons) and continues to rise by ca. 2 ppm per year [132]. Carbon is the primary component of many everyday fuels, chemicals, and materials and sectors such as energy, agricultural, and transport account for more than 27 billion tons of CO₂ emissions each year [133]. As such, a platform that utilizes CO₂ as the primary feedstock for the synthesis of carbon commodities would be ideal for a closed carbon economy. In addition to generating important environmental and societal impacts, such techniques will also yield incredible economic benefits by taking advantage of impending governmental carbon tax incentives as well as the large margin of upgrading a cheap and abundant carbon source to high value commodities [134].

In the past, approaches for converting CO₂ to carbon products has involved the use of photosynthetic carbon assimilation via plants, algae or cyanobacteria [135,136]. The scale-up of such techniques is, however, inherently limited by low energy to biomass conversion efficiencies as well as large water and land use requirements [137]. Alternatively, tremendous efforts have been made to develop non-biological catalytic processes for the conversion of CO₂ into products, however, most of these devices have demonstrated substantial drawbacks such as low product selectivity, high temperature requirements, or generation of toxic wastes [138,139]. In recent years, some have shifted their focus to the integration of non-biological and biological

conversion techniques that aim to pair the advantages of both processes [140]. This work has coincided with considerable advances in solar and wind power that can now offer sources of low-cost and sustainable electricity to drive such devices.

One area that has received a lot of attention in the past decade is microbial electrosynthesis (MES) in which electrons are transferred to microbes via extra-cellular electron transfer (EET). Though several MES designs have demonstrated high-energy efficiencies (up to 90%), this technology has been fundamentally challenged by EET mechanisms that can only accommodate low current densities (at most $10\text{mA}/\text{cm}^2$) which result in low production rates (i.e. $< 1\text{g}/\text{l}/\text{day}$) [19,141]. To by-pass these limitations, other biohybrid approaches have been developed that involve the electrochemical generation of an electron donor that is fed to the microbe rather than using the electrode itself. For example, in a single-pot system H_2 produced by a NiMoZn alloy catalyst was fed to *Ralstonia eutropha* and aerobically converted to isopropanol at high yields ($216\text{mg}/\text{L}$) [142]. Though promising, high overpotentials were needed to maintain microbial growth as toxic reactive oxygen species were generated at potentials near optimal H_2 production [142]. Furthermore, the energy efficiencies of approaches that rely on aerobic bacteria, like *Ralstonia eutropha*, can only achieve limited energy efficiencies as a large portion of the feedstock electrons must end up reducing oxygen (O_2) to water rather than in the product. Alternatively, anaerobic bacteria such as acetogens can offer very high conversion of substrates to product (70-90%) as they utilize a reductive acetyl-CoA pathway that provides energy to the cell while assimilating and reducing CO_2 or C1 carbons [143]. An example of such a microbe is *Clostridium ljungdahlii*, which has the unique capability to convert CO or CO/CO_2 into high value products such as ethanol with excellent conversion efficiencies via the Wood-ljungdhali pathway [144]. This is advantageous because CO is a preferred substrate to H_2 as it

can partition into liquid phase easier for microbial uptake and releases more Gibbs free energy when used, leading to generation of more reduced products [144]. From an electrochemical standpoint, CO is also a preferred intermediate to H₂ as it directly utilizes CO₂ and can be produced at high efficiencies and selectivity. For example, Pan et al. (2018) recently demonstrated that their atomically dispersed Co–N5 site anchored on polymer-derived hollow N-doped porous carbon spheres could produce CO at faradaic efficiencies of almost 99% at low overpotentials (–0.73 vs. RHE) [145].

In this work, we propose a two-stage biohybrid system for CO₂ conversion into carbon commodities. In the first stage, CO₂ is fed to a gas diffusion CO₂ electrolysis cell that electrochemically converts CO₂ to CO. The effluent gas mixture is then fed to a downstream bioreactor containing *Clostridium ljungdahlii* that converts the feed gas into value-added commodities. The results presented, hereto, describe the development and optimization of this novel biohybrid system.

5.2 Materials and methods

5.2.1. CO₂ electrolyzer construction and operation

The CO₂ electrolyzer consisted of a membrane electrode assembly (5cm²) mounted in fuel cell hardware purchased from Dioxide Materials (Boca Raton, FL). Both the anode and cathode electrodes were prepared by depositing nanoparticle inks onto carbon diffusion layers (Sigracet 35 BC GDL, Ion Power). The cathode ink was synthesized by adding silver nanoparticles (30 mg) to distilled water (0.1 mL) and isopropanol (0.2 mL). The mixture was then sonicated for 1 min and spray-painted onto a 2.5 cm x 2.5 cm square cut out of carbon gas diffusion layer. The anode electrode was prepared in an similar fashion except it was coated with iridium oxide (30mg) rather than silver and 5% PTFE solution was added to the ink as a binder. A Sustainion

imidazolium-functionalized polymer membrane was sandwiched between the as prepared electrodes and the entire membrane-electrode assembly was mounted between two gaskets. The anode (titanium) and cathode (stainless steel) flow fields were then attached on the respective sides using bolts and ring terminals were screwed into both outer plates. For all tests, an anolyte solution (pH =7) consisting of 10mM KHCO_3 was circulated at 3mL/min with a peristaltic pump through the anode chamber. On the cathode side, a gas cylinder attached to a mass flow controller delivered CO_2 (99% purity) at 35 mL/min to a CO_2 humidifier bottle containing 100mL of deionized water. The effluent of the humidifier was connected directly to the inlet of cathode chamber. The electrochemical cell was connected to a DC-power supply [Circuit Specialists-3600series] that was operated under constant current conditions while the voltage was carefully monitored [Fig. 21].

Figure 21: Detailed schematic of CO_2 electrolyzer and associated reactions

5.2.2 Microbial culturing experiments

All microbial cultures were maintained in 162.5 mL Wheaton serum bottles (Borosilicate glass, Fisher Scientific, UK) containing 30 mL of rich YTF media [144, 146]. The initial pH of the media was then adjusted to 5.8 using a concentrated 1M solution of potassium hydroxide [144]. Prior to autoclaving (121°C for 30 minutes), ultra high purity argon gas (General Air Service Supply- UN1006) was diffused into the liquid media for approximately 45 minutes to remove any oxygen. Gas impermeable butyl rubber septum-type stoppers and aluminum crimp seals were then used to seal the bottles and the headspaces were purged with argon for another 10 minutes. After autoclaving, the bottles were cooled to room temperature (25°C). Bottles were then purged with a filtered (PTFE 0.2µm) gas mixture of H₂/CO₂ (25/75%) for approximately 35 minutes. In some cultures, fructose (5g/L) was also added from a stock solution. Prior to inoculation, cultures were then reduced by addition of 0.5mL of the reducing agent to make final concentrations of 3.1mM L-cysteine-HCl. Inocula (OD =0.5) from a previous culture were then added to the bottles in a 1 to 10 ratio. All cultures were incubated horizontally on an orbital shaker incubator, set at 200 rpm and 37°C.

5.2.3 *Clostridium ljungdahlii* genetic engineering

A CRISPR-Cas9 tool tailored for *C. ljungdahlii*, was used to generate a mutant lacking phosphotransacetylase (*pta*). Pta converts acetyl-CoA to acetyl-phosphate, the deletion of which will increase the pool of acetyl-CoA toward products such as ethanol or pyruvate derived metabolites. The CRISPR-Cas9 plasmid specifically targeted the *pta* gene with its deletion verified using PCR. Further details of the methods are provided elsewhere [147].

5.2.4 Two-stage biohybrid system operation

Figure 22 describes the full setup of the biohybrid system. The effluent gas from the CO₂ electrolyzer was passed through an empty condensation bottle (100mL) with a gas tight lid to dry the gas. The gas was then passed through a filter (2μm PTFE) and directed through the lid of a bioreactor bottle (100mL) that was submerged in a water bath controlled with a heating jacket and module set to 37 (deg. C) [51]. The water bath and bioreactor were placed on a magnetic stir plate that agitated the media in the reactor bottle at 375 rpm. The headspace of the bioreactor was pumped via peristaltic pump into a condensation collector at 15 mL/min and the final effluent was released to atmosphere in a standard fume hood.

Figure 22: Schematic of two-stage biohybrid system operating process

5.2.5 Gas and liquid analyses

For electrochemical performance analyses, the CO₂ electrolyzer was operated for at least 2.5 hours to reach steady state before sampling. After this time tedlar bags (Supel-Inert Foil) were attached directly to the effluent of the cathode side and filled for 33 minutes to fill the bag. Gas tight syringes (Hamilton-250µl) were then used to pierce the septa of the bags to obtain gas samples. H₂ and CO molar headspace concentrations were determined by manually injecting 250µl of sample into a gas chromatograph (Agilent Technologies-7890A) equipped with a thermal conductivity detector (TCD) and packed column [144]. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 250°C, respectively. Helium gas was used as the carrier gas at a flow rate of 5 mL min⁻¹. CO₂ molar headspace concentrations were monitored by manually injecting 100µl of sample into a gas chromatograph (Hewlett Packard-5890) equipped with a thermal conductivity detector (TCD) and packed column [144]. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 190°C, respectively. Helium gas was used as the carrier gas at a flow rate of 30 mL min⁻¹. Molar concentrations were determined by fitting data to a five-point standard curve prepared for each gas.

During fermentation experiments, liquid samples (1mL) were withdrawn using a sample tube submerged in the media. The liquid samples were analyzed for optical density (600nm) as well as acetate and ethanol content. Optical density measurements were made using a spectrophotometer (Beckman Coulter-DU800) at a fixed wavelength of 600nm and converted to dry cell densities using a standard calibration curve. Metabolites were analyzed via high-pressure liquid chromatography [144]. Prior to analysis, liquid samples were centrifuged at 14000rpm for 10 minutes and the supernatant was passed through 0.22µm filters. Organic acids were separated

on an Aminex HPX-87H X column with 4 mM H_2SO_4 as the eluent. Molar concentrations were determined by fitting peak areas to a standard curve prepared by diluting ethanol fermentation analytical standard solutions (Bio-Analytical-LQC-NREL-50).

5.3 Results and discussion

5.3.1 Electrochemical performance evaluation

Galvanostatic CO_2 electrolysis tests were conducted under various current densities to evaluate the performance of the gas diffusion electrolysis module. At 80 mA/cm^2 , only a small amount of CO was produced while the faradaic efficiency for hydrogen reached about 60%. This amounted to a hydrogen generation rate of 4.2 mmol/hr [Fig. 23]. As current density was increased, the faradaic efficiencies for CO as well as the CO production rates increased dramatically. For instance, at a current density of 200 mA/cm^2 almost no hydrogen was evolved and the faradaic efficiency for CO was over 80%. This translated to a CO production rate over 15.0 mmol/hr .

Figure 23: Gas production and faradaic efficiency at variable current densities; 10 mM KHCO_3 electrolyte anolyte; gas samples obtained after steady state achieved (2.5hrs) indicated by stable voltage

As the CO faradaic efficiency seemed to plateau around 78-80%, an operating condition of 200mA/cm² was chosen as it produced viable CO production rates for bioconversion [144]. A series of test were then conducted by controlling the maximum voltage of the electrolysis cell while operating at constant current [Fig. 24]. It was then determined that a minimum voltage of 3.2V was necessary to maintain the CO₂ electrolysis at 200mA/cm².

Figure 24: CO₂ electrolysis current density vs. time-CO₂ electrolysis at 3.2 V and 2.9 V; Maximum current set to 1000mA, 10mM KHCO₃ electrolyte solution

5.3.2 Two-stage biohybrid system bioconversion

Effluent gas from the CO₂ electrolysis module was continually bubbled into a downstream bioreactor containing *Clostridium ljungdahlii*. Throughout fermentations, the CO₂ electrolysis voltage was very stable between 3.6-3.8V and maintained a current density of 200mA/cm² [425]. The operating voltage was a bit higher than the voltage (3.2V) determined in performance experiments due to increased resistance of the system associated with added pressure from the

bioreactor attachment. As a baseline for performance, the system was run under mixotrophic conditions (5g/L fructose added and substantial growth was observed after 24 hours as dry cell densities approached 1.3 g/L [Fig. 25]. Under autotrophic conditions (0 g/L fructose added), cell densities were about 0.4 g/L after 24 hours of culturing. This is expected as much more reducing power is available under mixotrophic conditions leading to higher cell densities. Under autotrophic conditions, cells continued to slowly grow and after 120 hours of culturing reached a cell density of 0.54 g/L.

Figure 25: Biohybrid system microbial growth and system stability-*C. ljungdahliae* cultured with CO_2 electrolysis conducted at $200\text{mA}/\text{cm}^2$; microbes grown with media amended with fructose A) and without B) Dry cell concentration determined from optical density (600nm)

Acetate and ethanol were the major products under both mixotrophic and autotrophic conditions. After 48 hours of culturing, autotrophic fermentations yielded approximately 3.0 g/L and 28 mg/L of acetate and ethanol, respectively [Fig. 26]. As expected, mixotrophic fermentations achieved around 3.4 g/L and 153 mg/L more acetate and ethanol due to great availability of substrates. This computed to a rate of ca. 2.7 g/l/day of acetate and 100 mg/l/day of ethanol.

Interestingly, the rate of acetate production under autotrophic conditions was comparable reaching around 1.5 g/l/day. It is worth noting that this acetate production rate is a significant improvement compared to state-of-the-art MES designs [19]. Ethanol production rate was around 14 mg/l/day but as this metabolite is associated with the non-growth phase it was likely much higher between later time points (i.e. between 48-120 hours).

Figure 26: *C.ljungdahlii* acetate (left axis) and ethanol production (right axis) in two-stage biohybrid system over 48 hrs- Electrolysis at constant current of 200mA /cm². *C.ljungdahlii* acetate (left axis) and ethanol production (right axis) rate comparison in two-stage biohybrid system over 48 hrs; Electrolysis at constant current of 200mA /cm²

5.3.3 *C.ljungdahlii* strain optimization for enhanced ethanol production

To improve the ethanol production rate and yield of *C.ljungdahlii* a mutant strain was developed using a Crisper-Cas 9 deletion. Wild-type and PtA mutant *C.ljungdahlii* strains were cultured with H₂/CO₂ and fructose (5g/l) to quantify the impact of the deletion. PtA mutant cultures produced 80mg/g-cell more ethanol than the wild type strain [Fig. 27]. In addition the molar ethanol to acetate ratio was 26% higher (.11 vs. .09). As the PtA enzyme is directly linked to growth via the Wood-Wjungdahl pathway, it is expected that this effect will be exacerbated in

cultures maintained under syngas alone (without fructose addition). Future characterization work with this PtA mutant is currently underway.

Figure 27: Comparison of *C.ljungdahlii* PtA mutant and wild-type control product generation-products generated per dry cell weight (left axis); molar product ratio (right axis)

Conclusions

A novel two-stage biohybrid system for CO₂ conversion into carbon commodities has been developed. The CO₂ gas diffusion electrolyser showed excellent stability at a high current density of 200mA/cm² and produced CO at an outstanding faradaic efficiency of over 80%. This yielded a CO production rate of 15mmol/hr. When paired with a bioreactor containing a pure culture of *C.ljungdhalii*, exceptional cell densities of 1.3g/L and 0.44g/L were achieved after 48 hours of mixotrophic and autotrophic culturing, respectively. This amounted to high acetate and ethanol production rates of 1.5g/l/day and 14mg/l/day under autotrophic conditions. Work with PtA mutant strains of *C.ljungdhalii* showed great potential to improved ethanol production as 80 more mg ethanol/g cell were achieved compared to the wild-type. Further optimization of the biohybrid system are on-going including exploration of ways to increase CO generation rates,

enhance mass transfer in the bioreactor and refine the *C.ljungdhalii* mutant strains towards desired products.

CHAPTER 6

FUTURE RESEARCH

6.1 Platinum nanoparticles dispersed in novel covalent organic frameworks for electrochemical hydrogen production and conversion

6.1.0 Introduction

The portfolio of compounds that can be electrochemically generated and used as electron carriers in a biohybrid system is presently very limited. Currently the only viable candidates include compounds such as H₂, CO and formate that can be produced at high faradaic efficiencies and current densities [148,149]. It is not a surprise that each of these compounds only requires a two electrons transfer as electroreduction of CO₂ involving greater than two electrons has been a tremendous challenge due to a lack of product selectivity and low energetic efficiency [150]. Breakthroughs in direct multi-electron CO₂ reduction could allow for high efficiency generation of biocompatible electron carriers such as methanol, methane, ethylene, and acetate, in-turn opening the door to new types of microbes that can be integrated into biohybrid systems and products that can be synthesized. Catalytic hydrogenation provides an alternative to direct electrochemical CO₂ reduction to efficiently synthesize new feedstock compounds for biohybrid conversion. In catalytic hydrogenation processes, electrochemically produced hydrogen is reacted with CO₂ to produce chemicals such as CO, methane, methanol, or ammonia (via haber-bosch process with nitrogen) [151-153]. Recently, this field has gained a lot of momentum and the production of methane and methanol using catalytic hydrogenation has already reached energetic efficiencies up to 70% [154,155].

The key to the overall efficiencies of these processes, however, lies in the efficiency of the primary hydrogen evolution reaction (HER, $2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$). HER is sluggish and requires

electrocatalysts to achieve efficient water splitting [156-158]. Platinum (Pt) is the most well-known and efficient electrocatalyst that outperforms many other noble or non-noble metals. Recently, it was found that dispersing ultrafine Pt nanoparticles (PtNPs) in a carbon matrix could significantly improve the catalytic capability and the stability of the catalysts [159]. Various supporting substrates have been utilized to increase the dispersion of metal-nanoparticles, including carbon substrate, graphene, porous polymers, mesoporous silica, covalent organic frameworks (COFs), and metal-organic frameworks (MOFs) [159-167]. Amongst these, COFs are promising templates to prepare nanocomposites because of their high porosity, crystallinity, stability, and functional group diversity [159, 168-171]. In this work, a novel porphyrin-bipyridine-based COF (COF-bbyTPP) was synthesized and PtNPs were well dispersed within its matrix. The as-prepared materials were characterized using a suite of tools including Fourier transform infrared resonance (FTIR), nuclear magnetic resonance (NMR), Brunauer-Emmett-Teller analysis (BET), inductively coupled plasma mass spectrometry (ICP-MS), transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy (XPS). The electrochemical performance of these catalysts was then evaluated using cyclic voltammetry and controlled potential water electrolysis. Future research will focus on optimization of the HER reaction using these catalysts and integration of the electrochemically produced H₂ for catalytic hydrogenation of intermediates to generate feedstock compounds for bioconversion.

6.1.1 Material and methods

Synthesis of COF-bpyTPP

A pyrex glass tube was charged with porphyrin tetraamine (20 mg, 0.0296 mmol) and bipyridine-dialdehyde (16 mg, 0.074 mmol). A mixture of solvents, butanol/1,2-

dichlorobenzene/6M acetic acid (1/1/0.25, total volume 2.25 mL) was added. The glass tube was then frozen at 77 deg. K and sealed under vacuum using an open flame. It was then placed in an oven at 120 °C for 3 days. Violet precipitates were formed, which were collected, washed with butanol and acetone, and dried to give the product 17.0 mg (80 %).

Synthesis of Pt@COF-bpyTPP

COF-bpy-TPP (4 mg) was dispersed in methanol (3 mL). An aqueous solution of K_2PtCl_4 (5 mg, 2 mL) was added to the above COF suspension. After removing the solvents, the remaining solid was re-dispersed in MeOH/H₂O (5 mL, v/v= 3:2). A solution of sodium borohydride in methanol (0.25 M, 2 mL) was added. The mixture was stirred at room temperature for 2 days. The solid was collected, washed with ethanol and dichloromethane, and dried to give the product (3.2 mg).

Material characterization

¹H NMR spectra were recorded on a Bruker Avance-III 300 NMR spectrometer. FTIR spectra were measured with a Agilent Technologies, Cary 630. N₂ adsorption isotherm was measured by a Quantachrome Autosorb ASiQ automated gas sorption analyzer. TEM was performed on a FEI Tecnai T12, 120kV TEM. ICP-MS was recorded on a Perkin Elmer Optima 7300DV. XPS was measured by PE-5800 X-ray photoelectron spectroscopy.

6.1.1.4 Electrochemical HER performance

A mixture of 10mg of catalyst and 10mg of carbon black was sonicated for 30 min in 4mL of DMF. 100μL of the resulting suspension was then mixed with 5% nafion (1:100), drop casted on two sides of carbon paper (Toray-50% wet-proofed) and dried for 48 hours at room temperature

(25 $\pm$ 0.1 °C) in a laminar flow hood. Cyclic voltammetry and controlled potential electrolysis were conducted in a Pine Research (WaveDriver- 200 series) potentiostat in combination with a standard Pine Research electrochemical cell (AKCELL1) containing two chambers separated by a glass frit. The cathodic compartment contained catalysts coated-carbon paper as the working electrode (partly submerged in the electrolyte solution) and an Ag/AgCl reference electrode with ceramic porous frit (BASi-MW-2030). The anodic compartment had a large graphite electrode (OD= 0.250", L= 12"). The supporting electrolyte in both chambers contained 0.5M NaHCO₃ dissolved in deionized water. Prior to electrolysis, all electrolytes were saturated with either pure CO₂ or argon and the cell was sealed using gas tight rubber seals (Pine Research-14/20; 24/25). Gas samples were obtained directly from the headspace of the electrochemical cell using a 250 μ l gastight syringe (Hamilton-1700 series) and were immediately analyzed on an Agilent Technologies 7890A gas chromatography system equipped with a thermal conductivity detector (TCD) and packed column. The oven temperature was a constant 60°C. Injector and detector temperatures were stable at 150°C and 250°C, respectively. Argon gas was used as the carrier gas at a flow rate of 5 mL min⁻¹. CO and H₂ molar concentrations were determined by fitting data to a five-point standard curve prepared for each gas using standard calibration gases.

6.1.2 Preliminary results

Material synthesis and characterization

Imine-linked COF-bpyTPP was obtained by a Schiff-base reaction between a free-base porphyrin and bipyridine-dialdehyde via an solvothermal synthesis method in a mixed solvent of butanol/*o*-dichlorobenzene/6M acetic acid [Fig. 28]. The formation of the imine linkages in COF-bpyTPP were confirmed by FTIR and solid state ¹³C NMR. For example, FTIR spectra of the COF-bpyTPP showed an absorbance peak around 1650 cm⁻¹ owing to C=N bonding in the

imine linkage [Fig. A.11]. Furthermore, the absence of the C=O peak at 1685 cm^{-1} and N–H at 1602 cm^{-1} indicates that the starting materials were consumed to synthesize the COF material [Fig. A.11]. The presence of imine bonding was further supported by solid state ^{13}C NMR that revealed a characteristic carbon atom peak around about 155 ppm [Fig. A.12, 172].

COF-bpyTPP has metal chelating bipyridine moieties in the backbone of the framework, making it is a good host for nanoparticles [171,173]. The COF-supported PtNPs (PtNPs@COF) were obtained through the initial chelating reaction between COF-bpyTPP and soluble Pt (II) salts, followed by further reduction with NaBH_4 to form the metal nanoparticles. Prior to incorporating PtNPs, the BET surface area of the COF-bpyTPP was estimated to be $333\text{ m}^2/\text{g}$.

Figure 28: Pt@COF compound and material characterization summary- Schematic representation of preparation of COF-bpyTPP and PtNPs@COF-bpyTPP (a); the TEM image of PtNPs supported on COF-bpyTPP (b) and their size distribution (c), the TEM image of PtNPs prepared in the absence of any COF materials

The dispersion and size distribution of the as-prepared PtNPs@COF were assessed by TEM. The TEM images show well-dispersed PtNPs@COF of sizes varying from 1 to 6 nm [Fig. 28 b, c]. The formation of well-dispersed PtNPs in such small sizes is likely attributed to the presence of the COF support with high porosity and orderly arranged bipyridine metal-chelating sites in the backbone of the framework structures. A control sample (PtNPs without the COF matrix) showed random aggregations of PtNPs indicating the critical role of the COF in the formation of well-dispersed ultrafine nanoparticles [Fig. 28d].

ICP-MS analyses of the PtNPs@COF revealed that the weight percent of Pt loading on the COF materials is 6.09 wt%. The oxidation state of the as-prepared PtNPs was confirmed by XPS measurement [Fig. A.13]. The analysis of the Pt 4f spectrum of the Pt@COF-bpyTPP shows two peaks with binding energies of 71.8 and 74.9 eV, corresponding to $4f_{7/2}$ and $4f_{5/2}$ of Pt^0 .

Electrochemical HER performance evaluation

The catalytic activity and stability of PtNPs@COF were evaluated under alkaline conditions. It is worthy pointing out that HER in alkaline solution is more difficult than acidic conditions as water must first be dissociated by the Volmer step to make adsorbed hydrogen on the surface of metals, leading to a higher overpotential [174]. As such, catalyst testing under alkaline conditions is a truer test of HER performance as under acidic conditions HER can occur at almost no overpotential via proton reduction [174]. Furthermore, testing under a higher pH is far more practical for industrial applications of water electrolyzers at large-scale [174]. Cyclic voltammetry (CV) was conducted at 1mV/s with 0.5 M $NaHCO_3$ dissolved in deionized water, saturated with CO_2 (pH 8.3). CV results revealed that the onset potential of Pt/C was ca. -500mV more negative than that of PtNPs@COF [Fig. 29a]. This was attributed to an improved kinetics barrier in the water dissociation step by the as prepared PtNPs@COF catalyst. To clearly

demonstrate the HER capabilities of the compounds, controlled potential electrolysis (-0.95 V vs. Ag/AgCl) were conducted for one hour [Fig. 29]. The reduction current of PtNPs@COF was very stable for over an hour of electrolysis and achieved a high current density of -70 mA/cm². This demonstrated excellent performance compared to the Pt/C that showed signs of slow decomposition over the electrolysis and achieved a current density of only -10 mA/cm² under the same conditions [Fig. 29b]. These differences in current densities were also reflected in the amounts of hydrogen evolved at each catalyst. After one-hour ca. 490 μ mol/mg of hydrogen gas was produced by the electrocatalysis using PtNPs@COF (6.09 wt% Pt). This amount is 13 times larger than that was produced in the presence of Pt/C (10 wt% Pt, 38 μ mol/mg) [Fig. 29c]. TEM image of PtNPs@COF after the electrolysis shows no signs of agglomeration of the nanoparticles, indicating its high stability under alkaline electrolysis conditions [Fig. 29d]. The excellent catalytic performance can be attributed to the porous structure of the COF-bpyTPP and the dispersity of PtNPs on the COF substrate.

Figure 29: Pt@COF electrochemical hydrogen evolution summary (a) Polarization curves of PtNPs@COF (red line) and Pt/C (blue line) in a 0.5 M NaHCO₃ at a 100 mVs⁻¹ scan rate; (b) Controlled potential measurements at -0.95V vs. Ag/AgCl for 60 minutes with PtNPs@COF (red line) and Pt/C (blue line); (c) The amount of hydrogen evolution by PtNPs@COF (red rectangular) and Pt/C (blue circle); (d) A TEM image of PtNPs@COF after one-hour bulk electrolysis under the alkaline condition

On-going and future work

The as-synthesized COF-bpyTPP are an excellent supporting material to deposit metal nanoparticles because of their high crystallinity, large porosity, and thermal stability in addition to ample nucleation sites. COF-bpyTPP successfully supported PtNPs (~2.95 nm) which were highly active and stable. The obtained PtNPs@COF shows the enhanced catalytic performance and excellent stability toward HER in alkaline media. PtNPs@COF outperformed commercial Pt/C in alkaline solution, generating 13 times more hydrogen with nearly 100% faradaic

efficiencies. Future work will focus on further characterizing the HER potential of this catalyst under different bulk electrolyte pH as well as in the presence of different solvation cations. Furthermore, this catalyst may be integrated into a new biohybrid system to generate H_2 that is catalytically hydrogenated to a product such as methanol for microbial conversion. More applications of this catalyst are still being explored.

6.2 Functionalized hollow fiber membrane for dual electrochemical CO_2 reduction and enhanced gas mass transfer

The bottleneck of syngas fermentation processes has often been the gas-liquid mass transfer of gaseous substrates ($H_2/CO/CO_2$) to the cell surface. A variety of reactor types including stirred tank reactors (STR), trickle bed reactors (TBR), packed bed reactors (PBR), monolithic biofilm reactors (MLBR), membrane-based reactors (MBR), and bubble column reactors (BCR) have been tested [175]. Orgill et al. (2013) compared the volumetric mass transfer coefficient ($K_{tot}A/V_L$) in 3 syngas fermentation configurations, and they found hollow fiber membrane reactors (HFMR) provided the highest $K_{tot}A/V_L$ (1062 h^{-1}) (more than doubled TBR (421 h^{-1}) and nearly 10-folds higher than STR (114 h^{-1}))[176].

Therefore, future research will aim to maximize syngas mass transfer and uptake by combining gas-delivery and utilization to a single chamber using an innovative HFMs coated with electro-catalysts for CO_2 conversion. The innovative catalyst coated HFMs will serve dual roles as both the site of CO_2 conversion and syngas dispersion into the surrounding media for bacterial uptake. This will greatly simplify the reactor design (as well as lower volumetric requirements) and allow for more rapid generation of desired products. The CO_2 feedstock will be fed directly through HFMs to the growth media of the bioreactor and will be converted to syngas via an electrocatalyst on the surface of the HFMs [Fig. 30].

The functionalized-HFMs will be constructed using coating methods similar to those used in Chapter 4. HFMs will then be characterized using techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), X-ray photo-electron spectroscopy (XPS) and in-situ raman spectroscopy. The electrocatalytic capabilities of the functionalized HFMs will then be quantified using electrochemical techniques such as CV, linear sweep voltammetry (LSV), electrochemical impedance spectroscopy (EIS). CO₂ conversion products will be defined under controlled current experiments. The functionalized HFM will then be optimized for the desired CO/H₂ composition for fermentation by adjusting parameters such as applied potential, CO₂ feed rate and operating temperature.

Figure 30: One-pot biohybrid system schematic -Single chamber reactor with functionalized HFM for simultaneous syngas generation and utilization; Feed CO₂ is electrochemically converted to syngas that is available for microbial uptake in the same reactor

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Appendix A

Appendix equations:

$(\Delta G_r^\circ \text{ (kJ/mol)}) = -175.04$

$(\Delta G_r^\circ \text{ (kJ/mol)}) = -224.75$

$(\Delta G_r^\circ \text{ (kJ/mol)}) = -306.17$

$(\Delta G_r^\circ \text{ (kJ/mol)}) = -94.92$

$(\Delta G_r^\circ \text{ (kJ/mol)}) = -104.57$

Appendix tables:

Table A.1: Henry's law constants and electrochemical half-cell potentials of reduced gas species

Gaseous Substrate	K_H^1 , (mM/atm)	E_0^2 , (mV)
CO	7.40	-581
H ₂	0.78	-420

- 1) Henry's Law constant under standard conditions (T= 298.15 K) [10]
- 2) Electrochemical half cell potentials under standard conditions (T= 298.15, P=1 atm, Conc.= 1M) calculated for Eq. A.7 and A.8, respectively

Appendix figures:

Figure A.1: Wood-Ljungdahl pathway- Formation of acetyl-CoA from syngas components in western and eastern branches used for acetate, ethanol, and pyruvate production [21]

Figure A.2: Extended Wood-Ijungdahl pathway- Conversion of Pyruvate to 2,3-Butanediol [22]

Figure A.3: Major constituents of growth blends used to generate fungal biomass as determined via ICP-MS analysis- right y-axis for total iron concentration only; Major constituents defined as initial concentration >1 mg/L.

Figure A.4: Phosphorus loaded biochar EDS summary- Atomic phosphorous % of PE-BW 1:4 and PE-BW 3:4 raw samples and after equilibrium in batch study with 20 mg P/L and 50 mg P/L (A) Phosphorous weight% of PE-BW 1:4 and PE-BW 3:4 raw samples and after equilibrium in batch study with 20 mg P/L and 50 mg P/L (B)

Figure A.5: ^1H NMR (300 MHz, CDCl_3) of $\text{H}_2(\text{o-OCH}_3)\text{TPP}$

Figure A.6: ^1H NMR (300 MHz, CD_3OD) of $\text{H}_2(\text{o-OH})\text{TPP}$

Figure A.7: FT-IR of $\text{H}_2(\text{o-OCH}_3)\text{TPP}$ (black) and $\text{Co}(\text{o-OCH}_3)\text{TPP}$ (red)

Figure A.8: FT-IR of $\text{H}_2(\text{o-OH})\text{TPP}$ (black) and $\text{Co}(\text{o-OH})\text{TPP}$

Figure A.9: UV-Vis in dichloromethane $\text{H}_2(\text{o-OCH}_3)\text{TPP}$ (black) and $\text{Co}(\text{o-OCH}_3)\text{TPP}$ (red)

Figure A.10: UV-Vis of $\text{H}_2(\text{o-OH})\text{TPP}$ (black) and $\text{Co}(\text{o-OH})\text{TPP}$ in methanol

Figure A.11: FTIR of TPP, bpy, COF-bpyTPP, and Pt/COF-bpyTPP

Figure A.12: Solid C NMR of COF-bpyTPP

Figure A.13: XPS of Pt/COF-bpyTPP

Appendix supplemental methods:

5,10,15,20-tetrakis(2',6'-dimethoxyphenyl)-21H,23H-porphyrin.

BF₃OEt₂ (0.13mL, 1.08mmol) was added to a mixture of 2,6-dimethoxybenzaldehyde (1.2760g, 7.70mmol) and pyrrole (0.53mL, 7.70mmol) in chloroform (750mL) under nitrogen. It was stirred at room temperature for 90 minutes in dark. After 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.31g, 5.78mmol) was added, it was stirred at 65°C for 90 minutes. Once it cooled down to room temperature, triethylamine was injected to remove the excessive acid. The mixture was concentrated down. Silica gel column chromatography was done by dichloromethane. To obtain pure product, further purification was done with recrystallization in ethyl acetate. 21% yield (0.3450g). ¹H NMR (300 MHz, CDCl₃) δ = 8.66 (s, 8H), 7.67 (t, J = 8.4 Hz, 4H), 6.96 (d, J = 8.4 Hz, 8H), 3.49 (s, 24H), -2.49 (s, 2H). IR (cm⁻¹): 3326, 2934, 2834, 1584, 1469, 1428, 1245, 962; λ_{abs} (CH₂Cl₂): 418, 514, 546, 589, 643.

5,10,15,20-tetrakis(2',6'-dihydroxyphenyl)-21H,23H-porphyrin.

A solution of H₂(o-OCH₃)TPP (0.2g, 0.234mmol) in dry dichloromethane was placed in ice bath while boron bromide (0.35mL, 3.74mmol) was added. The mixture was stirred at room temperature overnight. Ethyl acetate was added to suspension and it was washed with NaHCO₃ (aq). Organic layer was separated and washed with water twice. Solvent was removed and pure violet compound was obtained. 66% yield (0.1147g). ¹H NMR (300 MHz, CD₃OD) δ = 8.92 (s, 8H), 7.50 (t, J = 8.2 Hz, 4H), 6.84 (d, J = 8.2 Hz, 8H). IR (cm⁻¹): 3441, 2920, 2848, 1580, 1458; λ_{abs} (CH₃OH): 414, 513, 550, 587, 641; ESI-MS ([M+H]⁺) calcd for C₄₄H₃₀N₄O₈ 743.2142, found 743.2135.

Cobalt (II) - 5,10,15,20-tetrakis(2',6'-dimethoxyphenyl)-21H,23H-porphyrin.

Solid of $\text{H}_2(\text{o-OCH}_3)\text{TPP}$ (0.1453g, 0.170mmol) and anhydrous CoCl_2 (II) (0.441g, 3.40mmol) were dissolved in anhydrous THF (30mL) under inert gas. 2,6-lutidine (0.060mL, 0.51mmol) was added. The solution was refluxed for two hours. After removing THF solution, the mixture was dissolved in dichloromethane and washed with water three times. Organic solvent was concentrated down and product was collected. 85% yield (0.132g). IR (cm^{-1}): 2920, 2834, 1584, 1469, 1428, 1245; $\lambda_{\text{abs}} (\text{CH}_2\text{Cl}_2)$: 414, 536; ESI-MS ($[\text{M}+\text{H}]^+$) calcd for $\text{C}_{52}\text{H}_{44}\text{CoN}_4\text{O}_8$ 911.88, found 911.2484.

Cobalt (II) - 5,10,15,20-tetrakis(2',6'-dihydroxyphenyl)-21H,23H-porphyrin.

The same procedure to synthesize $\text{Co}(\text{o-OCH}_3)\text{TPP}$ was followed except ethyl acetate to dissolve the mixture after removing THF solution. $\text{H}_2(\text{o-OH})\text{TPP}$ (0.0573g, 0.0772mmol), anhydrous CoCl_2 (II) (0.200g, 1.54mmol), 2,6-lutidine (0.028mL, 0.2316mmol), and anhydrous THF (12mL) were used. 80% yield (0.0494g). IR (cm^{-1}): 2920, 2856, 1580, 1458; $\lambda_{\text{abs}} (\text{CH}_3\text{OH})$: 426, 538; ESI-MS ($[\text{M}+\text{H}]^+$) calcd for $\text{C}_{44}\text{H}_{28}\text{CoN}_4\text{O}_8$ 799.66, found 799.