Optimum Co-product Utilization from Hydrothermal Liquefaction of Microalgae

by

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A Thesis Presented in Partial Fulfillment
of the Requirement for the Degree
Master of Science

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ABSTRACT

The project aims at utilization of hydrothermal liquefaction (HTL) byproducts like biochar to grow microalgae. HTL is a promising method to convert wet algal biomasses into biofuels. The initial microalgae liquefaction at a temperature of 300 °C for 30 minute, converted 31.22 % of the *Galdieria sulphuraria* and 41.00 % of the *Kirchneriella cornutum* into biocrude. Upon changing the reactor from a 100 ml to a 250 ml reactor, the yield in biocrude increased to 31.48 % for *G. sulphuraria* and dropped to 38.05 % for *K. cornutum*. Further, energy recoveries based on calorific values of HTL products were seen to drop by about 5 % of the 100 ml calculated values in the larger reactor.

Biochar from HTL of *G. sulphuraria* at 300 °C showed 15.98 and 5.27 % of phosphorous and nitrogen, respectively. HTL products from the biomass were analyzed for major elements through ICP-OES and CHNS/O. N and P are macronutrients that can be utilized in growing microalgae. This could reduce the operational demands in growing algae like, phosphorous mined to meet annual national demand for aviation fuel. Acidic leaching of these elements as phosphates and ammoniacal nitrogen was studied. Improved leaching of 49.49 % phosphorous and 95.71 % nitrogen was observed at 40 °C and pH 2.5 over a period of 7 days into the growth media. These conditions being ideal for growth of *G. sulphuraria*, leaching can be done *in-situ* to reduce overhead cost.

Growth potential of *G. sulphuraria* in leached media was compared to a standard cyanidium media produced from inorganic chemicals. Initial inhibition studies were done in the leached media at 40 °C and 2-3 vol. % CO₂ to observe a positive growth rate of 0.273 g L⁻¹ day⁻¹. Further, growth was compared to standard media with similar composition in a 96 well plate 50
\( \mu L \) microplate assay for 5 days. The growth rates in both media were comparable. Additionally, growth was confirmed in a 240 times larger tubular reactor in a Tissue Culture Roller drum apparatus. A better growth was observed in the leached cyanidium media as compared to the standard variant.
DEDICATION

To my beloved family Mathew K. Joseph, Jessy Mathew and Merlyn Mathew.
ACKNOWLEDGMENTS

I would like to sincerely acknowledge and thank my advisor and panel chair Dr. Shuguang Deng, for his constant support and supervision throughout my master’s program. His adept guidance, prompt inspiration, apt suggestions and continual guidance in every step of my efforts helped me succeed in the completion of my degree.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>COMMON ABBREVIATIONS</td>
<td>xiv</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1. Microalgae - A Source of Bio-Energy</td>
<td>3</td>
</tr>
<tr>
<td>1.1.1. <em>Galdieria sulphuraria</em></td>
<td>3</td>
</tr>
<tr>
<td>1.1.2. <em>Kirchenella cornutum</em></td>
<td>4</td>
</tr>
<tr>
<td>1.2. Method to Convert Microalgae into Useable Energy</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1 Biochemical Processes</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1.1. Anaerobic Digestion</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1.2. Fermentation</td>
<td>6</td>
</tr>
<tr>
<td>1.2.1.3. Hydrogen Production</td>
<td>6</td>
</tr>
<tr>
<td>1.2.2. Transesterification</td>
<td>7</td>
</tr>
<tr>
<td>1.2.3 Thermochemical Processes</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3.1. Hydrothermal Liquefaction</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3.2. Pyrolysis</td>
<td>9</td>
</tr>
<tr>
<td>1.2.3.3. Gasification</td>
<td>9</td>
</tr>
<tr>
<td>1.2.3.4. Combustion</td>
<td>10</td>
</tr>
<tr>
<td>1.3. Products from Hydrothermal Liquefaction</td>
<td>10</td>
</tr>
<tr>
<td>1.3.1. Biocrude</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>1.3.2.</td>
<td>Biochar</td>
</tr>
<tr>
<td>1.3.3.</td>
<td>Aqueous Phase</td>
</tr>
<tr>
<td>1.3.4.</td>
<td>Non-Condensable gas</td>
</tr>
<tr>
<td>1.4.</td>
<td>Problem Statement</td>
</tr>
<tr>
<td>1.5.</td>
<td>Scope and Objectives of the Project</td>
</tr>
<tr>
<td>1.5.1.</td>
<td>Hydrothermal Liquefaction of two algal species</td>
</tr>
<tr>
<td>1.5.2.</td>
<td>Phosphorous and Nitrogen Recovery from Biochar</td>
</tr>
<tr>
<td>1.5.3.</td>
<td>Growth of <em>Galdieria sulphuraria</em> using Leached Macronutrients</td>
</tr>
<tr>
<td>1.6.</td>
<td>References</td>
</tr>
<tr>
<td>2.</td>
<td>HYDROTHERMAL LIQUEFACTION OF TWO ALGAL SPECIES</td>
</tr>
<tr>
<td>2.1.</td>
<td>Introduction</td>
</tr>
<tr>
<td>2.2.</td>
<td>Materials and methodology</td>
</tr>
<tr>
<td>2.2.1.</td>
<td>Microalgae and Materials</td>
</tr>
<tr>
<td>2.2.2.</td>
<td>Instruments</td>
</tr>
<tr>
<td>2.2.3.</td>
<td>HTL experimental procedure</td>
</tr>
<tr>
<td>2.3.</td>
<td>Analysis methods</td>
</tr>
<tr>
<td>2.4.</td>
<td>Results and discussions</td>
</tr>
<tr>
<td>2.4.1.</td>
<td>Analysis of the microalgae and HTL products</td>
</tr>
<tr>
<td>2.4.2.</td>
<td>Influence of volume increase on product distribution</td>
</tr>
<tr>
<td>2.4.3.</td>
<td>Influence of volume increase on energy recovery</td>
</tr>
<tr>
<td>2.4.4.</td>
<td>Effect of Strain on product yields</td>
</tr>
<tr>
<td>2.5</td>
<td>References</td>
</tr>
</tbody>
</table>
3. PHOSPHOROUS AND NITROGEN RECOVERY FROM BIOCHAR

3.1. Introduction .............................................................................................................. 43
3.2. Materials and Methodology ..................................................................................... 46
   3.2.1. ICP-OES protocol for analysis of algal biomass ................................................. 46
   3.2.2. Preparation of leaching media and materials ....................................................... 50
   3.2.3. Design of experiments and preliminary optimization ......................................... 50
      3.2.3.1. Study of effect of temperature on leaching .................................................. 51
      3.2.3.2. Study of effect of pH in leaching .................................................................. 52
   3.2.4. Optimized leaching of phosphates and ammonia from biochar ....................... 52
   3.2.5. N and P measurements ....................................................................................... 53
3.3. Results and discussions ........................................................................................... 53
   3.3.1. Effect of temperature on leaching ................................................................. 53
   3.3.2. Effect of pH on leaching ..................................................................................... 55
   3.3.3. Optimized leaching of phosphates from HTL biochar ...................................... 58
   3.3.4. Mass balance of products from HTL and leaching studies ................................ 64
3.4. References ............................................................................................................... 66

4. GROWTH OF GALDIERIA SULPHURARIA IN LEACHED NUTRIENTS RECOVERED
   FROM BIOCHAR .......................................................................................................... 70
4.1. Introduction ............................................................................................................... 70
4.2. Materials and Methodology ...................................................................................... 74
   4.2.1. Algae Strain collection and maintenance .......................................................... 74
   4.2.2. Growth study with leached nutrients ............................................................... 74
<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.3. Experimental conditions for growth studies</td>
<td>75</td>
</tr>
<tr>
<td>4.2.3.1. Growth in a 250 µL microplate assay</td>
<td>75</td>
</tr>
<tr>
<td>4.2.3.2. Growth in a 6 ml photo bioreactor</td>
<td>76</td>
</tr>
<tr>
<td>4.2.4. Growth and optical density measurements</td>
<td>76</td>
</tr>
<tr>
<td>4.2.5. N and P measurements</td>
<td>76</td>
</tr>
<tr>
<td>4.3. Results and discussions</td>
<td>77</td>
</tr>
<tr>
<td>4.3.1. Toxicity study of biomass in leached media</td>
<td>77</td>
</tr>
<tr>
<td>4.3.2. Comparative growth of <em>G. sulphuraria</em> in standard and leached media</td>
<td>79</td>
</tr>
<tr>
<td>4.4. References</td>
<td>84</td>
</tr>
<tr>
<td>5. SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH</td>
<td>86</td>
</tr>
<tr>
<td>5.1. Summary</td>
<td>86</td>
</tr>
<tr>
<td>5.2. Recommendations for future work</td>
<td>88</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>89</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table | Page
--- | ---
2.1: Chemical Analysis for two strains and HTL products: *G. sulphuraria* and *K. cornutum* | 32
2.2: Product ratio from hydrothermal liquefaction of biomass: *G. sulphuraria* and *K. cornutum* on increasing reactor volume from 150 to 250 ml. | 34
2.3: Energy recovery for two strains: *G. sulphuraria* and *K. cornutum* on increasing reactor volume from 150 to 250 ml. | 36
3.1: Microwave digestion protocol for biomass, biocrude, biochar and aqueous phase... | 48
3.2: Wavelength used to ascertain concentration of elements through inductively coupled plasma optical emission spectrophotometer (ICP-OES) | 49
3.3: Effect of leaching temperature on phosphorous recovery from HTL biochar, as phosphates | 54
3.4: Effect of leaching pH on phosphate recovery from HTL biochar, as phosphates | 56
3.5: Mass Balance from hydrothermal liquefaction (* recovered as bio-crude, bio-char or aqueous phase) | 64
4.1: Growth data in terms of ash free dry weight for *Galdieria sulphuraria* grown at 40 °C and 2-3 vol. % CO₂ in: a) Inorganically mixed cyanidium media and b) Cyanidium media with ammonia and phosphates leached from HTL biochar | 79
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1: Methods for biofuels production from microalgae</td>
<td>05</td>
</tr>
<tr>
<td>2.1: United States Consumption of Energy for 2015</td>
<td>23</td>
</tr>
<tr>
<td>2.2 Variation in products from HTL as the reactor volume is increased from 150 ml to 250 ml for two strains of microalgae</td>
<td>35</td>
</tr>
<tr>
<td>3.1: Increase in phosphates leached over a period of four days in a 50 ml PP centrifugal tube</td>
<td>55</td>
</tr>
<tr>
<td>3.2 Increase in phosphates leached over a period of seven days in a 50 ml PP centrifugal tube</td>
<td>58</td>
</tr>
<tr>
<td>3.3: Leaching study results for: a) Phosphates over a period of 7 days and b) Ammoniacal nitrogen over a period of 7 days</td>
<td>61</td>
</tr>
<tr>
<td>3.4: Study of effect on leaching of nitrogen and phosphorous from G. sulphuraria HTL biochar at varying pH at the end of 7 days</td>
<td>63</td>
</tr>
<tr>
<td>4.1: Production of energy in the United States for 2015</td>
<td>72</td>
</tr>
<tr>
<td>4.2: Inhibition study of G. sulphuraria over a period of 15 days</td>
<td>78</td>
</tr>
<tr>
<td>4.3: Growth of Galdieria sulphuraria in: a) 96 well microplate assay and b) 16 mm tubular reactor</td>
<td>81</td>
</tr>
<tr>
<td>4.4: Pie chart depiction of biochar composition through ICP-OES</td>
<td>83</td>
</tr>
</tbody>
</table>
### COMMON ABREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄</td>
<td>Ammonium sulfate</td>
</tr>
<tr>
<td>10 N H₂SO₄</td>
<td>10 Normal solution of sulfuric acid</td>
</tr>
<tr>
<td>AFDW</td>
<td>Ash-free dry weight</td>
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<tr>
<td>Avg</td>
<td>Average</td>
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<tr>
<td>AzCATI</td>
<td>Arizona Center for Algae Technology &amp; Innovation</td>
</tr>
<tr>
<td>Btu</td>
<td>British thermal units</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>Calcium chloride dehydrate</td>
</tr>
<tr>
<td>CAPS</td>
<td>Cleaved amplified polymorphic sequence</td>
</tr>
<tr>
<td>CCMEE</td>
<td>Culture collection of microorganisms from extreme environments</td>
</tr>
<tr>
<td>CHNS/O</td>
<td>carbon hydrogen nitrogen sulfur/oxygen</td>
</tr>
<tr>
<td>CM</td>
<td>Cyanidium media</td>
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<tr>
<td>CO</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DI</td>
<td>deionized</td>
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<td>DREAM</td>
<td>Deng’s Renewable Energy and Advanced Materials</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>Ferric chloride</td>
</tr>
<tr>
<td>G. sulphuraria</td>
<td><em>Galdieria sulphuraria</em></td>
</tr>
<tr>
<td>H/C</td>
<td>hydrogen/carbon ratio</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>Chemical/Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>H₂</td>
<td>hydrogen molecule</td>
</tr>
<tr>
<td>H₂S</td>
<td>hydrogen sulfide</td>
</tr>
<tr>
<td>HF</td>
<td>hydrofluoric acid</td>
</tr>
<tr>
<td>HHV</td>
<td>higher heating value</td>
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<tr>
<td>HTL</td>
<td>hydrothermal liquefaction</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Inductively Coupled Plasma-Optical Emission Spectrophotometer</td>
</tr>
<tr>
<td>K. cornutum</td>
<td>Kirchneriella cornutum</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>Pottassium dihydrogen phophate</td>
</tr>
<tr>
<td>LCA</td>
<td>Life Cycle Analysis</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>Magnesium sulfate heptahydrate</td>
</tr>
<tr>
<td>mol/mol</td>
<td>mole/mole ratio</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NCG</td>
<td>non-condensable gases</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>ammoniacal nitrogen</td>
</tr>
<tr>
<td>NOₓ</td>
<td>oxides of nitrogen</td>
</tr>
<tr>
<td>O</td>
<td>oxygen atom</td>
</tr>
<tr>
<td>O/C</td>
<td>oxygen/carbon ratio</td>
</tr>
<tr>
<td>OD</td>
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</tr>
<tr>
<td>OH⁻</td>
<td>hydroxide ion</td>
</tr>
<tr>
<td>P</td>
<td>phosphorous</td>
</tr>
<tr>
<td>pH</td>
<td>pouvoir hydrogène / power of hydrogen</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>phosphate</td>
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<tr>
<td>PP</td>
<td>polypropylene</td>
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<tr>
<td>Press</td>
<td>Pressure</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>ROI</td>
<td>Return on Investment</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RuBisCo</td>
<td>Ribulose-1,5-bisphosphate carboxylase oxygenase</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Std Dev</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>TEA</td>
<td>Techno-Economic Analysis</td>
</tr>
<tr>
<td>U.S. EIA</td>
<td>United States Energy Information Administration</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet – visible</td>
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<tr>
<td>vol. %</td>
<td>volume percentage</td>
</tr>
<tr>
<td>vol./vol.</td>
<td>volume/volume ratio</td>
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<td>wt. %</td>
<td>weight percentage</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Humans have been an integral portion of the earth’s ecological system for about a million years. The presence of civilizations has been an overload on the biosphere especially due to the increased need for food sources, space and energy. The energy demand has been on the rise since the dawn of industrial revolution (S. B. Darling et. al.; 2013). The world we live in has been fossil fuel based for its power requirements. Conventional energy resources adversely affect the habitat that organisms exist in. Conventional fuels like fossil fuels release a huge amount of acid gases like oxides of carbon, sulfur and nitrogen. These gases have been proven to increase greenhouse effect on earth (de Richter et. al.; 2017). A fossil fuel based system to meet the energy demand on planet earth may have seemed to be a smart choice at that time. Present tides are shifting towards a more sustainable growth. Recently the use of renewable sources for energy has been shown to reduce the need for fossil based sources. Renewable sources in turn remove the effect of greenhouse effect.

Non-renewable resources that can be utilized to meet the energy demand of humans include hydro-electric, wind, solar, tidal and biomass. Many countries have been advocates of a new energy system based on such renewable resources (K. Zaman et. al.; 2017). Biomass through photosynthesis absorbs solar energy. Using this energy, they produce energy required for their sustenance. Not all the energy produced in the life of a plant is consumed by it. A portion of this energy is stored for future use. The use of this excess energy is the basic idea of energy production from biomass. In order to reduce the consumption of non-renewable natural resources, the use of renewable ones should
increase. The replacement of fossil fuels by biomass is a promising option for power technologies as well as for synthetic fuels production (W. Piekarczyk et al., 2013).

Biomass is renewable and definitely less polluting than present petroleum based energy sources, further they do not add to environmental levels of greenhouse gases like carbon dioxide (Subahana et al., 2015). There have been many ideas put forth for the conversion of biomass into useful biofuels. Recent strides in the field of bio-energy have algae based technologies at the forefront. Feedstock used for energy production from biomass is generally classified into four generations. The first generation includes edible seeds as feedstock that may cause ethical dilemmas due to food shortages. The ethical issue furthered the use of second generation sources, mostly non-edible seeds and agricultural wastes. The inconsistency in such feedstock availability led to great thought for a better source of biomass. Recent feedstocks of interest are algal that has a higher growth rate, considerable potential for bio-fuel production and is non-edible (S. Tamilarasan et. al.; 2014 and K. T. Lee et. al.; 2014).

All methods used presently can be grouped based on the energy source for conversion into: mechanical, thermochemical and biochemical processes. These methods are not mutually exclusive. There are a few hybrid-processes used for the conversion of biomass into biofuels (J. T. Claypool et. al.; 2016). While biochemical processing is typically very discerning as it gives distinct products in high yield. Thermochemical conversion provides multiple and often intricate products in short response times using inorganic catalytic agents. Inorganic catalysts employed in thermochemical methods increase the product quality or spectrum.
1.1. Microalgae - A Source of Bio-energy

Microalgae are diverse eukaryotic organisms predominantly present in an aquatic ecosystem. They employ photosynthetic pathways to meet metabolic demands. This makes them perfect candidates for biofuel production. Microalgae and macroalgae come under the broad classification of fourth generation feedstock for biofuel production (Jing Lü et al.; 2011). They have been present on earth before the inception of humankind and will continue to exist even after our death. There are more than ten thousand reported species of microalgae under eight phyla. The rate of diversity in this ancient organism is astounding and they can be found in almost all kinds of aquatic ecosystems. There are species that grow in extreme temperatures, extreme pH, and variety of salinity levels and even under huge pressure loads.

The interest in microalgae and their growth, harvesting and utilization for applications started after the 1940’s. During the World War II, Germany investigated the possibility of farming microalgae to meet the liquid fuel demand (G. H. Huang et. al.; 2010). Some of the species of interest in that work are still a topic of interest in present works.

1.1.1. *Galdieria sulphuraria*

*G. sulphuraria* is unicellular red alga that is most commonly found to exist in volcanic hot sulfur springs, solfatara soils and extremely hostile environments. Habitats with high concentration of arsenic, cadmium, mercury and other toxic metals can be spots where *G. sulphuraria* thrive (G. Schönknecht et. al.; 2013). It is an extremophile with the capability to be metabolically active through mixotrophic mechanisms. Many other
species of microorganisms fall short in the extreme conditions of growth preferred by this strain of microalgae making it the perfect strain for growth in unfavorable ecosystems. Also, it is rarely successively attacked by common parasites. Since this strain is capable of mixotrophism, greater culture depths and higher biomass densities than in raceways can be maintained to minimize footprint. The optimized conditions for growth of this species are reported to be between the pH of 1.0 and 4.0 and a temperature ranging between 25 and 56 °C (S.M. Henkanatte-Gedera et. al.; 2015). It is specifically tuned to grow well in warm environments. It has been reported to grow well in sterilized wastewater reducing its biological oxygen demand (T. Selvaratnam et. al.; 2014).

1.1.2. *Kirchneriella cornutum*

It is a fairly unheard of species that has only been a topic of interest in the last few years. In fact, no or very little reported literature is present for this strain of microalgae. In 2017, a report was published concluding a project to grow microalgae which featured this strain (P. J. Lammers et. al.; 2017). This thesis will try to elucidate some more into the composition of this strain and its products from hydrothermal liquefaction.
1.2. Method to Convert Microalgae into Useable Energy

Figure 1.1: Methods for biofuels production from microalgae.

1.2.1. Biochemical Processes

Methods employing the use of metabolic processes in organisms or bio-catalysts formed by microbes have been termed as biochemical processes. They vary in many respects, but the products are mostly singular and high in purity. The only visible drawback in most of these processes is the greater processing time and strict process conditions.

1.2.1.1. Anaerobic Digestion

Anaerobic Digestion has been one of the most extensively employed processes among biochemical methods. It is a well-documented method with low capital investments. The usual operation temperatures are about 35 – 55 °C. A neutral pH is proven to be optimum for anaerobic digestion (G. Kim et. al.; 2017). The actual process is completely driven by enzyme kinetics at the above mentioned optimum operational conditions. The rate of the net reaction is defined by the hydrolysis step in the net
reaction. The hydrolysis is to breakdown the lignocellulose in cell walls. The lower lignocellulosic content in microalgae makes them a better feedstock. The hydrolysis is followed by fermentation steps that produce the required products. These products are based on the feedstock and microbe used in the actual process. The process of anaerobic digestion is restricted due to the metabolism of the organism used. The average optimized process yields a conversion from 25 to 40 % after operation (A.E. Maragkaki et. al.; 2017).

The gaseous products from anaerobic digestion can be scrubbed to remove non-combustible components. The gases after being scrubbed may be used as fuels. They can be used directly to run an internal combustion to sufficiently effective burn.

1.2.1.2. Fermentation

Here, the microbes employed fall into the class of yeasts or bacteria. Both are different in their operational conditions. Yeasts are aerobic while bacteria used for fermentation are not. Yeasts are less stable as the mode of fermentation due to the possibility of ethanol inhabitation while, bacteria cannot be employed for many of the feedstock. The overall efficiency in converting biomass into useable products is 50 - 65 % when yeasts in used in fermenting microalgae (P. J. Slininger et. al.; 2016). The molar efficiency of conversion in fermentation is about 150 % for every mole of glucose.

1.2.1.3. Hydrogen production

Many methods have been discussed over the past few years to convert microalgae into hydrogen fuel. The most practical ones are: sulfur deprivation of algae to produce hydrogen and lactic acid fermentation.
Anoxic conditions and sulfur deprivation is an efficient method of producing hydrogen gas from microalgae (K. Skjånes et. al.; 2013). This is also predicted to be a part of self-fermentation of microalgae into ethanol. During photorespiration, algae decompose stored carbohydrates and proteins stored during photosynthesis. This process is still not completely understood or well-documented. It is assumed that oxygenic photosynthesis is controlled to 10 % of the optimum before the process of hydrogen productions begins. About 50 % of the carbon dioxide to be produced from complete oxygenation of proteins and starch is converted into useable hydrogen using the following reaction:

\[ H_2O \rightarrow \text{Photosystem II} \rightarrow \text{Photosystem I} \rightarrow \text{Ferredoxin} \rightarrow \text{Hydrogenase} \rightarrow H_2 \]

Another method that may be utilized to produce hydrogen gas from microalgae is the fermentation of lactic acid. The carbon component in lactic acid is consumed with release of hydrogen. The yield from this method is similar to that in anoxic sulfur deprivation methods. Even though the collection of useable fuel is easier in this process, it is capital intensive due to the need for more than a single microbial species.

1.2.2. Transesterification

This technology has been extensively used to convert the lipids in microalgae into biofuels. It is a widely accepted and utilized method for the production of biodiesel. Biodiesel is used a replacement for conventional diesel in vehicles. Lipids contain about 21 % of the mass of microalgae and contain about 40 % of the possible energy output (S. Ponnusamy et. al.; 2014 and W. Sitthithanaboon et. al.; 2015). Lipids are present in
microalgae as triglycerols which can be converted into alkyl esters. These esters are what are termed as biodiesel. Here, the process of transesterification produces byproducts of very little value like glycerol. This process is efficient in itself, but it seldom is a holistic approach comparing the amount of lipids present and the net biofuel producible from a unit weight of dried microalgae.

**1.2.3. Thermochemical Processes**

Thermochemical processes use heat to aid in conversion of biomass. They can be classified on basis of increasing temperature and pressure into: carbonization, hydrothermal liquefaction, pyrolysis, gasification and combustion. Each of these will be discussed separately in the following paragraphs.

**1.2.3.1. Hydrothermal Liquefaction**

Thermochemical process that breaks biological polymers into constituent chemicals; at low temperature and high pressures. The operation temperature is between 180 °C and 330 °C and pressures above 240 bar (H. K. Reddy et. at.; 2016). The process of liquefaction necessitates the use of water-soluble slurry as the feed hence; this process is preferred in the conversion of animal manure and human wastes. The presence of water at elevated temperatures leads to a greater dielectric constant; this in turn helps increase the destruction of biomass cell walls. The moisture in feedstock act as catalysts at the operational conditions defined above (H. K. Reddy et. al.; 2013). The hydrocarbon-rich phase produced from liquefaction of biomass can be termed biocrude. Biocrude from liquefaction are of a high quality due to the low oxygen content (<16 wt. %); this comes at the expense of a liquid yield lesser than 40 wt. % (G. W. Huber et. al.; 2006). In general, liquefaction has been less investigated than other thermochemical processes.
This is lesser in the case of microalgae. Algae grow in water rich conditions and most other methods of biomass conversion require a pre-drying step. Hydrothermal liquefaction (HTL) prefers the lack of a completely dried feedstock. Applications of biocrude are similar to applications for biocrude, and will be discussed later.

1.2.3.2. Pyrolysis

Pyrolysis is a thermo-chemical decomposition of organic material at elevated temperatures in the absence of oxygen and is composed of various gases such as CO₂, CO, NOₓ, SOₓ, H₂S, H₂, aldehydes, ketones, volatile carboxylic acids and gaseous hydrocarbons (M. Verma et. al.; 2012). The process of pyrolysis usually occurs at 400 - 600 °C and at atmospheric pressure. Both the liquefaction and pyrolysis methods produce a liquid product. The amount of liquid product formed varies based on the temperature of the pyrolysis and feed quality. Pyrolysis can be separated into two groups based on the temperature of reactor, vapor residence and cooling time. Fast Pyrolysis is done at high temperatures with a small vapor residence time and quick vapor cooling time. Slow Pyrolysis or Conventional Pyrolysis works at a lower temperature, with greater vapor residence time (R. Volpe et. al.; 2017).

1.2.3.3. Gasification

It is the endothermic conversion of biomass into useful syngas at the temperature range of 750 – 1000 °C. There, a part of the energy required for the process of biomass gasification is produced by combustion of the feed itself. The composition of the gas produced is dependent on the feed quality, temperature and fluidizing gas. Syngas (gasification intermediate) is composed of carbon monoxide, carbon dioxide, hydrogen, nitrogen (if air is used as oxidizing agent), and small quantities of hydrocarbons such as
methane and ethane \textit{(M. Sarkar et al., 2014)}. Syngas can be directly combusted to produce energy or can be converted to normally used forms like, diesel and gasoline using the Fischer-Tropsch process. This is not a common process in the conversion of microalgal feedstock into useable biofuels.

1.2.3.4. Combustion

Combustion or aerial oxidation consumes biomass to produce energy by direct heating. It is undertaken at temperatures above 1000 °C. There is an excess supply of oxygen and hence, an almost complete conversion of the biomass can be expected. This process is pretty primitive and has been used since the medieval times to produce energy. Controlling the burning rate in the combustion equipment is difficult since raw biomass fuel may be inhomogeneous and have a remarkable burning rate \textit{(T. Nakahara et al., 2015)}. The higher moisture contents in microalgae leads to combustion not being a preferred method of biofuel production.

1.3. Products from Hydrothermal Liquefaction

Biomass type, structure and reactor operating conditions are detrimental in defining and predicting chemical and physical properties of HTL products. Also, the technology used and product retrieval machinery and time is important for the amount of product produced.

1.3.1. Biocrude

Biocrude or else known as bio-oil are the major produce from pyrolysis. It is usually dark brown in color and smells of charred biomass. Yields above 50 % are common for woody feedstock, this is much lower for microalgae \textit{(A. Dimitriadis et. al.; 2017)}. The conversion rate is determined by the feed’s bio-physical composition
especially the cellulose, hemicellulose and lignin content. Some other chemical compositions of interest in microalgae include proteins, carbohydrates and lipids.

Biocrude is a complex mixture of more than 50 carbon-based elements and compounds formed in the liquefaction reactions that are basically “confined” to the liquid form. Biocrude is extremely distinct from the conventional fossil-fuel based liquid products. The oxygen concentration is dependent on the feed and catalyst employed. It can vary up to about 12 % of the final product concentration (T. Muppaneni et. al.; 2017 and D. Chiaramonti et. al.; 2017). Reduction of oxygen is of utmost importance for increase in calorific value and fuel efficiency. It was extensively studied by a few researchers and they have come to observe that the fraction of oxygen in biocrude decreased appreciably with increasing pressure (P. Biller et. al.; 2015). Another important cause of variation from fossil crude oil is the high moisture content. The biocrude moisture content is reported to be below 25 % of the final product. It can be caused due to the residual moisture in the feed (that can be reduced by various drying mechanisms) or ‘reaction water’ that is produced from the combination of oxygen and hydrogen during HTL.

The chemical configuration of biocrude is reliant on many features, and comprises many types of oxygen containing species. In addition to water, major chemical ingredients of biocrude are ~4 % benzene, 5 - 12 % cyclic ketones, 6 - 18 % aliphatic hydrocarbons, 6 - 57 % aliphatic acids, 1 - 3 % aliphatic esters, 3 - 8 % phenols and 2 - 8 % amides (Y. Huang et. al.; 2016). Biocrude is highly unstable. It has an almost exclusive aging and constancy issues, and as it is not an equilibrium reaction process product, it is recognized to change over the course of time. Many researchers have designed various
methods for the efficient storage of the produced oil. Low temperature storing of the oil is a commonly employed measure to minimize these variations.

1.3.2. Biochar

A black solid carbonaceous material is produced from hydrothermal liquefaction as a byproduct. The amount of char produced is dependent on the operating conditions. Biochar product produced from HTL can range from 16.5 % to 55.2 % by weight (T. Muppaneni et. al.; 2017 and H. K. Reddy et. al.; 2016). The major element in char is found to be carbon (greater than 60%). The other major elements found are hydrogen, nitrogen, oxygen and sulfur. The raw biomass properties really influence the chemistry of char formation, with the operation temperature and the heating rate being the main operation parameters that have a strong influence on char’s structure (A. Paethanom and K. Yoshikawa; 2012). Further, the biochar produced in HTL is rich in metallic components and phosphorous. Generally, the large amount of ash constituents in the biomass feed material ends up in the biochar. Recent works have used biochar as a soil nutrient to replace nitrogen fertilizers (C. Yao et. al.; 2016), adsorption medium (L. Leng et. al.; 2015), water treatment method (L. Peng et. al.; 2015) and electrochemical capacitors (M. Sevilla et. al.; 2014).

1.3.3. Aqueous Phase

Previous analysis of the aqueous phase has shown it to be highly complex with compound classes including small organic acids, phenols, cyclopentenones, alcohols, ethers, esters, and N-heterocyclic compounds (C. Gai et. al.; 2015 and E. Panisko et. al.; 2015). The aqueous phase produced during hydrothermal liquefaction of microalgae has been shown to have high contents of nitrogen (L. G. Alba et. al.; 2013a, P. Biller et. al.
The use of these macronutrients has a great topic of interest in the last decade. Many of these aqueous phases are also rich in toxic metals that would inhibit the growth of a viable culture in them. Any use of this water would reduce the cost of the energy generation using microalgae. Also, the aqueous phase contains hydrocarbons that may be assimilated by certain algae. The quality of the aqueous phase produced is dependent on the operational conditions and the algal strain employed (D. L. Barreiro et. al.; 2015).

Some biorefinery concepts have been presented that use the aqueous phase as the source of make-up nutrients especially for nitrogen (L. G. Alba et. al.; 2013b). To my belief, this concept hasn’t been much successful as such a system would call for the utilization of a strain that can utilize the hydrocarbons either mixotrophically or heterotrophically in extreme metal concentrated environments.

1.3.4. Non-condensable gas

Not all the vapors generated through HTL are volatile hydrocarbons that can be easily condensed into liquid form. The process of cooling the reactors to room temperature brings most of the hydrocarbons back to liquid form. The remaining oil and other diatomic gases like nitrogen, oxygen, hydrogen and some carbon dioxide, carbon monoxide escape out as gases. These gaseous products from HTL reaction are collectively referred to as non-condensable gas. The NCG fraction also carries the inert gases that had been used in deoxygenating the reactor, such as nitrogen gas. The nitrogen gas is used to vent out the oxygen in the reactor prior to liquefaction to reduce the chance of increased oxygen content in products. Also, nitrogen provides and inert atmosphere in the reactor. The production of char gradually decreased at higher working temperatures, similar to the liquid fraction, while the gas yield increased (N. Gómez et. al.; 2016).
1.4. Problem Statement

This work is done to study the possibility of utilizing biochar produced from hydrothermal liquefaction of microalgae in growing a new batch of algae. It aims at estimating the amount of nitrogen and phosphorous in HTL biochar and various methods to improve nutrient leaching. The process of HTL is studied in two strains in different reactors to predict variations and possible reasons. The works also looks at a scale-up study for final growth of microalgae in leached nutrients from biochar.

1.5. Scope and Objectives of the Project

This thesis works aims at experimentation and reporting of results from some of the headway made at Arizona Center for Algae Research and Technology (AzCATI) and the Deng Renewable Energy and Advanced Materials (DREAM) Lab. The works explained here try to study variation in the process of HTL within the experimental limits of a batch process and study the viability of optimum byproduct utilization. They are divided into the following chapters:

1.5.1. Hydrothermal Liquefaction of two algal species.

Hydrothermal liquefaction is one of the promising methods to convert wet algal biomass into biofuels. HTL was done for microalgae in two different reactors varying in volumes. Two strains of microalgae were liquefied in both conditions in triplicates to determine the effect of reactor changes on the energy recovery and product recovery through HTL. The two seasonal strains of interest were: *Galdieria sulphuraria* and *Kirchneriella cornutum*. All the strains were liquefied to produce bio-crude at 300 °C and reaction residence time of 30 minutes, under elevated pressure using water as solvent. Byproducts formed included biochar and a nutrient rich aqueous phase. A quantitative
analysis of the products in both reactors for each strain was studied to come at a conclusion.

### 1.5.2. Phosphorous and Nitrogen Recovery from Biochar

The process of hydrothermal liquefaction produces a considerable amount of biochar. Biochar is considered to be a byproduct of the process and does not have many viable applications. The biochar produced at the above mentioned conditions of 300 °C and 30 minutes were analyzed for phosphorous content using inductively coupled plasma optical emission spectrometry (ICP-OES) and for nitrogen content using a combustion elemental analyzer. It was observed that 1 gram of hydrothermal liquefaction (HTL) biochar has 15.98 mg (Fig. 4.4) of phosphorous and 5.27 mg of nitrogen (Table 2.1). It is investigated if these macronutrients can be successfully leached out to be used in various applications. This chapter studies the effect of process parameters like temperature and pH of the leaching media. It concludes with the enhanced leaching conditions for biochar produced from HTL.

### 1.5.3. Growth of *Galdieria sulphuraria* using Leached Macronutrients

In this study, the macronutrients were leached at the previously enhanced conditions of pH 2.5 and 40 °C into cyanidium media (CM) prepared without phosphates and ammonia in it, to be used to grow *G. sulphuraria*. A lower pH media was conducive the growth of the microalgae. The leached phosphates and ammoniacal nitrogen were used to grow *G. sulphuraria*; first in a 250 µL microplate assay and followed by a volumetric scale up in a 6 ml tubular photo bioreactor at 40 °C and 2-3 vol. % CO₂, in an incubator. Growth was done up to ten replicates for each condition. The growth curves
were compared to standard cyanidium media. Comparable growth was observed in leached nutrients as against regular nutrients in the standard growth media.

These works in part or whole is planned to be the basis of all future works in the field of nutrient recycling from biochar for microalgae. It will set a foundation for all future endeavors into this innovative idea that can reduce the price of biofuels produced from microalgae.
1.6. References


Keat Teong Lee, Steven Lim, Yean Ling Pang, Hwai Chyuan Ong, Wen Tong Chong. 2014. Integration of reactive extraction with supercritical fluids for process intensification of biodiesel production: Prospects and recent advances. Progress in Energy and Combustion Science. 45, 54-78.


2. HYDROTHERMAL LIQUEFACTION OF TWO ALGAL SPECIES

2.1. Introduction

Meeting an increasing energy demand of an ever-growing population is a prominent obstacle in the way of modernization. The growth of the human population is directly proportional to the net demand for energy. The call for sustainable development has been the greatest proponent for the use of non-conventional energy resources. Non-conventional fuels ensure this growing demand is met with carbon-negative or neutral sources like wind, tidal, hydel, solar and bio-energy. Bio-energy is mainly considered as one of the most important components for eliminating greenhouse gas emissions and substitute of fossil fuels (T. Aysua et. al.; 2015). The energy in biomass is from the sun and as such solar energy is abundant. It cannot be called carbon-negative, but can be designed to be carbon-neutral or as close to it as possible.

Feedstock used for energy production from biomass is generally classified into three generations. The first generation includes edible starch rich feedstock like corn, sugarcane and vegetable oils; that may cause an ethical dilemma due to food shortages in many parts of the world. The ethical issue furthered the use of second generation lignocellulosic sources, mostly non-edible seeds, woody biomass and agricultural wastes. The inconsistency in availability of such feedstock led to great thought for a better source of biomass. The most recent feedstock generation includes algal biomass that has a higher growth rate, considerable potential for bio-fuel production and is non-edible (S.
Microalgae are proven to be grown in freshwater, seawater, wastewater and mine wastes (Tamilarasan et. al.; 2014 and K.T. Lee et. al.; 2014). Microalgae are proven to be grown in freshwater, seawater, wastewater and mine wastes (P.D. Álvarez-Díaz et. al.; 2017, A. Taleb et. al.; 2016, J.J. Schmidt et. al.; 2016 and S. Raikova et. al.; 2016).

**Figure 2.1:** United States Consumption of Energy for 2015. (Source: U.S. EIA Monthly Energy Review, April 2016)

The conversion of microalgae into useable fuels is an energy-intensive process. The high energy demand leaves a researcher wondering if one could reduce this demand.
per unit fuel produced. It would be profitable to convert more microalgae using the same heat, volume and operational time. This asks for the scaling of bench-based technologies that would be viable in biofuel production. Some other issues seen with the use of microalgae-based energy systems are the demand for water to grow biomass and dewatering harvested biomass to feed thermochemical processes.

Thermochemical processes for fuel generation employs elevated temperatures to convert biomass into useable energy intensive fuels (M. Mathew et. al.; 2017). Thermochemical generation of useable energy products from algae have been proven possible by: hydrothermal liquefaction (N. Sudasinghe et. al.; 2014), gasification (T. M. Aida et. al.; 2016) and combustion (C. Gai et. al.; 2015). Among this, hydrothermal liquefaction presents a unique advantage: the process does not require complete removal of initial moisture content to be used as a feedstock. Both gasification and combustion are optimized in terms of energy demand if the biomass is pre dried or dewatered to moisture content below 10 %. The process of dewatering is usually energy and/or time intensive and reduces the net viability of these processes. The process of liquefaction is energy intensive and optimization of this process is still a topic of interest. Hydrothermal liquefaction is a high pressure (5 - 20 MPa); medium temperature (300 - 350 °C) thermochemical conversion of biomass into useable energy products in the presence of moisture. The presence of water in the biomass is advantageous in this technology. When water is heated under pressure, its dielectric constant and density changes, resulting in change of its solvent and reactant properties (A. Kruse and E. Dinjus 2007). This property of water at elevated conditions ensures conversion of biomass into energy-rich bio-crude,
a nutrient rich aqueous phase, biochar and gases. Algal cell precipitates derived from centrifugation, which are of high moisture content, are thus good raw materials for liquefaction (S. Amin, 2009). The presence of catalysts has been shown to have synergic effects on the bio-crude yield and quality (P. Biller et. al.; 2011 and D. Zhou et. al.; 2010).

In liquefaction, wet biomass is decomposed into small molecules which further depolymerize into oily products with a wide range of molecular weight distribution and high energy density (V. Patil et. al.; 2008). Liquefaction can be direct or indirect. Direct liquefaction is the fast pyrolysis of biomass to produce biocrude, liquid tar and condensable organic vapor. In contrast, indirect liquefaction is performed in the presence of catalyst to convert non-condensable products and gases from pyrolysis or gasification into liquid products (R.H. Williams and E.D. Larson; 2003). The biocrude content and calorific value are normally in the range of 32 - 60 wt. % and 30 - 45 MJ kg\(^{-1}\). This calorific value is comparable to that of petroleum oil (41 MJ kg\(^{-1}\)). Hence, liquefied biocrude can be readily utilized as a combustion fuel.

The process of hydrothermal liquefaction can achieve 10 – 65 % of biocrude, gaseous mixture content of 10 - 20% and an ash content of 0.2 to 0.5% (Z. Shuping et. al.; 2010 and A.B. Ross et. al.; 2010). HTL products vary greatly with change in operational conditions like temperature (B.E. Eboibi et. al.; 2014 and P.J. Valdez et. al.; 2012), solid loading (P.J. Valdez et. al.; 2012), reaction time (B.E. Eboibi et. al.; 2014 and P.J. Valdez et. al.; 2012), catalysts (B. Patel et. al.; 2017) and strain of the microalgae (D.L. Barreiro et. al.; 2013). Liquefaction produces water insoluble oils of
high viscosity and usually requires solvents, reducing gases such as CO or H₂ and/or catalysts to be present in addition to biomass for optimum product recovery. Many works have been reported trying to optimize the process of hydrothermal liquefaction on the bench-scale (J.L. Faeth and P.E. Savage; 2016) and a few have attempted its optimization in larger systems (K. Prapaiwatcharapan et. al.; 2015). The reactors and fuel requirements for liquefaction are slightly complex and this makes the process more expensive (P. McKendry; 2002). Most of these works have come to the conclusion that the process may not be simplest technology to be scaled-up especially due to the problems in pumping the feedstock and loss in mass transfer and heat transfer efficiencies in larger systems.

This chapter seeks to study the possibility of scaling a bench-based hydrothermal liquefaction unit from Parr Industries (Parr Instrument Company, Illinois, USA). The study is done for two seasonal strains of microalgae: Galdieria sulphuraria and Kirchneriella cornutum; between 100 ml and 250 ml. This work will further analyze the product yields from each of the strains in each of the reactors to study if the volumetric increase changed the product yield. The novelty is due to the reported works in the past scaling the system directly from a bench-based technology to a continuous system. Scale-ups of that magnitude if not done with the safety factors in place, would pose to fail.
2.2. Materials and Methodology

2.2.1. Microalgae and Materials

The unicellular red algae G. sulphuraria CCMEE 5587.1 (J.A. Toplin et. al.; 2008) used in this study was obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon) now at Environmental Molecular Sciences Division at Pacific Northwest National Laboratory. The algal stock solution was grown in Standard Cyanidium medium (J.A. Toplin et. al.; 2008), which was modified to contain twice the standard ammonium sulfate concentration (T. Selvaratnam et. al.; 2014). The inoculum for test algal cultures used in this study was derived from single colonies scaled up autotrophically in the modified Cyanidium medium recipe. The freshwater microalgae Kirchneriella cornutum used in this study was obtained from the Arizona Center for Algae Technology and Innovation (Arizona State University). AzCATI (Arizona Center for Algae Technology and Innovation) grows both these strains in crop rotation throughout the year to maximize year-round biomass output. The incubator (Percival, IA, USA) employed was set at 40 °C with a 14-h light/10-h dark cycle. The CO₂ level inside the incubator was maintained at 2–3% (vol/vol). The G. sulphuraria and Kirchneriella cornutum biomass used to produce the HTL products at 300 °C was grown outdoors in a 4000 L photobioreactor similar to a previously reported work (T. Selvaratnam et. al.; 2014).

The chemicals, growth media feedstock and dichloromethane (DCM) and all other solvents used in analysis were purchased from Sigma Aldrich, Saint Louis, MO, USA.
2.2.2. Instruments

A Parr 4576A 250 ml accompanied by a 4843 controller unit and a Parr 4593 100 ml Micro reactor stainless steel bench top reactor accompanied by a 4843 controller unit manufactured by Parr Instrument Company (Moline, Illinois, USA) was used for conducting hydrothermal extraction and liquefaction experiments. Both reactors are equipped with pressure gauges to monitor pressure. Each of the equipment was sterilized prior to change in feedstock to remove any error due to the strain’s chemical compositions.

A separatory flask was employed to isolate the aqueous phase from the hydrocarbons. The biochar was removed from the product mixture using Whatman No. 1 filter paper. The solvent (Dichloromethane) was separated using a vacuum oven at 39 °C.

2.2.3. HTL experimental procedure

Both the above mentioned Parr bench-top reactors can be operated up to 350 °C and 200 bars pressure. Reactors were filled to 50 % volume with microalgae to improve heat and mass transfer in the system. This slurry was fed to the reactors and nitrogen was purged to sweep away the undesirable air in the reactor. The unreactive atmosphere reduced random oxidation in the unit and increased process safety. The reactors were heated after initial pressurizing with nitrogen at 350 psi. This helps control rapid boiling of water. The residence time is taken from the point when the reactor reaches a desired temperature. All the experiments were conducted with 10 % biomass loading, 300 °C reaction temperature and 30 min of reaction time in triplicates by varying the strains
between *G. sulphuraria* and *Kirchneriella cornutum*. The same procedure was repeated in the 250 ml reactor to study changes due to volumetric increase and design changes.

After liquefaction, the reactor was cooled down to room temperature and the gases produced during the reaction were vented off. 30 mL of dichloromethane (DCM) was added to the reactor and stirred for 10 min to extract the biocrude oil present in the reaction mixture and from the reactor walls. The product mixture was then transferred to the separation unit to separate the biochar using a filter paper and to separate the DCM using a separation funnel. The biochar was washed with 5 mL of DCM to collect the residual biocrude and then dried before storing. The DCM was evaporated using a vacuum oven and the thus separated biocrude oil along with the remaining aqueous phase was stored separately at −5 °C for further analysis. Yields of biocrude oil, biochar and water soluble compounds were calculated using equations presented below:

\[
\text{Biocrude Yield (\%)} = \frac{\text{Weight of biocrude}}{\text{Dry weight of biomass}} \times 100
\]

\[
\text{Biochar Yield (\%)} = \frac{\text{Weight of biochar}}{\text{Dry weight of biomass}} \times 100
\]

\[
\text{Aqueous phase Yield (\%)} = \frac{\text{Wt. of aq. phase} - \text{Intial moisture weight}}{\text{Dry weight of biomass}} \times 100
\]

Gaseous products are calculated as the difference in weight of biocrude, biochar and aqueous phase with that of the dried microalgae. Similarly, the equations for energy recovery studies in each case are given below:
Energy in Biocrude (MJ) = Weight of biocrude * HHV of biocrude (in $\frac{MJ}{kg}$)

Energy Recovery (%)

$$= \frac{(\text{Biocrude Weight} \times \text{HHV of biocrude}) + (\text{Biochar Weight} \times \text{HHV of biochar})}{(\text{Dry weight of biomass} \times \text{HHV of biomass})} \times 100$$

2.3. Analytical methods

The residue left after heating the algae sample at 600 °C for 24 h was considered as ash. The NREL protocol for the determination of carbohydrates, lipids and proteins was employed in this study to understand the composition of the two strains: *G. sulphuraria* and *Kirchneriella cornutum* (Laurens; 2013).

A 6725 Semi micro calorie meter manufactured by the Parr Instrument Company (Moline, Illinois, USA) was used to determine the high heating value of the microalgae, biochar and biocrude oil samples. Ultimate Analysis of the dry biochar and biocrude samples was performed using an Elemental Analyzer (Perkin Elmer 2400 Series II CHNS/O Analyzer). All the HTL experiments are done in 3 replicates and analysis in 5 replicates. The values presented are the average of the above mentioned number of replicates. All results related to biomass analysis is shown in Table 2.
2.4. Results and discussions

2.4.1. Analysis of the microalgae and HTL products

The heating value and elemental composition of the HTL products are summarized in Table 2.1. Higher heating value for all products and microalgae like *G. sulphuraria* and *K. cornutum* was done using a Parr 6725 Semi micro calorimeter. The higher heating values observed are comparable to other reported literature (*Y. Huang* et al.; 2016 and *T. Muppaneni* et al.; 2017). To correlate the quality of the bio-crude, the H/C ratio is reported. A higher H/C ratio ensures a successful burn when the product is ignited. Further, the lower O/C numbers ensure higher thermal efficiency during combustion. Carbohydrates present in the biomass have a tendency to be hydrolyzed faster than the proteins (*Z. Srokol* et al.; 2004). The carbohydrates present in the biomass could undergo many different reactions to form a wide range of products depending upon the reaction conditions (*Z. Srokol* et al.; 2004). From the current work it is evident that, the reported bio-crude yield as seen in Table 2.2; is found to be more than the initial lipid content, proving that there is conversion of other major components in the biomass like proteins and carbohydrates into bio-crude. The initial lipid content for *G. sulphuraria* (8.85 %) and *K. cornutum* (12.62 %) is about 3.25 to 3.55 times that of the highest observed percentage of biocrude produced through HTL.

From analysis, it can be concluded that *G. sulphuraria* is a low ash content, high protein strain that may be grown in Arizona during the summer months while; *K. cornutum* is a high ash content strain of microalgae that can be easily cultivated in cooler
months of the year. From the H/C and O/C ratios, we can conclude that the products from HTL of both strains offer a good fuel intermediate in the form of biocrude. Also, the biochar produced from HTL of *G. sulphuraria* at 300 °C and 30 minutes is a good combustion source with H/C at 1.31. The higher O/C ratio would signal a low calorific value burn, but the biochar combustion would be consistent.

**Table 2.1:** Chemical Analysis for two strains and HTL products: *G. sulphuraria* and *K. cornutum*.

<table>
<thead>
<tr>
<th></th>
<th><em>G. sulphuraria</em> Products (250 ml)</th>
<th><em>K. cornutum</em> Products (250 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV MJ/kg</td>
<td>Bio-crude 36.42</td>
<td>Biochar 24.51</td>
</tr>
<tr>
<td>Ash content wt %</td>
<td>2.41</td>
<td>6.22</td>
</tr>
<tr>
<td>Lipids wt %</td>
<td>8.85</td>
<td>12.62</td>
</tr>
<tr>
<td>Proteins wt %</td>
<td>53.25</td>
<td>28.11</td>
</tr>
<tr>
<td>Carbohydrates wt %</td>
<td>18.13</td>
<td>26.47</td>
</tr>
<tr>
<td>Carbon wt %</td>
<td>51.44</td>
<td>73.89</td>
</tr>
<tr>
<td>Hydrogen wt %</td>
<td>7.83</td>
<td>9.13</td>
</tr>
<tr>
<td>Nitrogen wt %</td>
<td>11.14</td>
<td>7.25</td>
</tr>
<tr>
<td>Sulphur wt %</td>
<td>1.83</td>
<td>2.31</td>
</tr>
<tr>
<td>Oxygen* wt %</td>
<td>27.76</td>
<td>7.42</td>
</tr>
<tr>
<td>O/C mol/mol</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>H/C mol/mol</td>
<td>1.83</td>
<td>1.48</td>
</tr>
</tbody>
</table>
2.4.2. Influence of volume increase on product distribution

The reactors were run using the same controller connected to similar geometry reactors from the same manufacturer (Parr Instruments Company, Illinois, USA). These similarities would ensure negligible heat and mass transfer restrictions in the system.

In Table 2, the products from hydrothermal liquefaction of microalgal strains like *G. sulphuraria* and *K. cornutum* is expressed in percentage yield with standard deviation. Both reactors were produced from the same manufacturer but, may have varied in design and the technological upgrades would have added effects on the product yields. All data in the Table 2.2 is averaged from five replicates. The standard deviation was calculated from all cases and a statistical analysis based on standard deviation was done to confirm significance of variation in each case. The variation in product composition for *G. sulphuraria* was less significant when liquefaction was done in the 100 ml and 250 ml reactors. The difference was more visible in the case of the high-lipid, low protein (see Table 2) *K. cornutum*. In the case of *G. sulphuraria*, the increase in volume only reduced the biochar production by a significant percent. This reduction may be due to the greater dissociation of the initial biomass into biocrude and aqueous phase. Further, the loss of biomass as gases is seen to decrease with increase in volume.
Table 2.2: Product ratio from hydrothermal liquefaction of biomass: *G. sulphuraria* and *K. cornutum* on increasing reactor volume from 100 to 250 ml.

<table>
<thead>
<tr>
<th>Microalgal Strain</th>
<th>Reactor Size ml</th>
<th>HTL Products (<em>calculated as difference</em>)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Biocrude</td>
<td>Biochar</td>
<td>Aqueous Phase</td>
<td>Gas Phase*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Galdieria sulphuraria</em></td>
<td>100</td>
<td>31.22</td>
<td>0.62</td>
<td>4.85</td>
<td>0.02</td>
<td>8.20</td>
<td>2.05</td>
<td>55.74</td>
<td>2.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>31.48</td>
<td>0.83</td>
<td>2.54</td>
<td>0.01</td>
<td>13.78</td>
<td>6.33</td>
<td>52.20</td>
<td>7.17</td>
<td></td>
</tr>
<tr>
<td><em>Kirchenella cornutum</em></td>
<td>100</td>
<td>41.00</td>
<td>0.41</td>
<td>14.23</td>
<td>0.01</td>
<td>14.73</td>
<td>2.21</td>
<td>30.05</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>38.05</td>
<td>0.50</td>
<td>12.34</td>
<td>0.04</td>
<td>11.09</td>
<td>2.72</td>
<td>38.51</td>
<td>3.25</td>
<td></td>
</tr>
</tbody>
</table>

The increase in volume showed significant variation in product composition for *K. cornutum*. In the case of this strain of microalgae, the increase in volume varied the composition of biocrude and biochar by a significant percent. The liquefaction process in the larger reactor reduced the biocrude and biochar production by 2.95 and 1.89 % respectively. Further, the loss as gaseous phase is seen to increase by 8.46 % to 38.51 % in a 250 ml reactor (see Fig. 2.2). This reduction may be due to the HTL temperature effect in such freshwater species. The reactor may have differential heat zones that lead to non-uniform heating and thereby, more flashing of the biomass to be lost as gases. To conclude, the increase in volume ensures that there is a greater conversion of biomass into biocrude, but losses increased with positive change in volume of the reactor. This observation may vary with the strain employed.
Figure 2.2: Variation in products from HTL as the reactor volume is increased from 100 ml to 250 ml for two strains of microalgae.

2.4.3. Influence of volume increase on energy recovery

Energy recovery (%) was calculated based on the higher heating values (HHVs) of biocrude, biochar and the feedstock. The equations for this calculation and any further graphs and tables in this section are based on previously stated equation (see Section 2.2.3). The measure of HHV was calculated using above-mentioned Parr bomb calorimeter. Further, energy content was based on the HTL product ratios reported in Table 2.2.
Table 2.3: Energy recovery for two strains: *G. sulphuraria* and *K. cornutum* on increasing reactor volume from 100 to 250 ml.

<table>
<thead>
<tr>
<th>HHV (MJ/kg)</th>
<th><em>Kircheneriella cornutum</em> 100 ml</th>
<th><em>Kircheneriella cornutum</em> 250 ml</th>
<th><em>Galdieria sulphuraria</em> 100 ml</th>
<th><em>Galdieria sulphuraria</em> 250 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>19.73</td>
<td>19.73</td>
<td>15.80</td>
<td>15.80</td>
</tr>
<tr>
<td>Biocrude</td>
<td>37.52</td>
<td>39.40</td>
<td>36.42</td>
<td>36.78</td>
</tr>
<tr>
<td>Biochar</td>
<td>23.48</td>
<td>23.95</td>
<td>24.51</td>
<td>26.72</td>
</tr>
<tr>
<td>Energy in Biomass (MJ/kg)</td>
<td>1973.00</td>
<td>1973.00</td>
<td>1580.00</td>
<td>1580.00</td>
</tr>
<tr>
<td>Energy in Biocrude or Biochar (MJ/kg)</td>
<td>1872.44</td>
<td>1794.70</td>
<td>1255.91</td>
<td>1225.68</td>
</tr>
<tr>
<td>Percent Recovery (%)</td>
<td>94.90</td>
<td>90.96</td>
<td>79.49</td>
<td>77.57</td>
</tr>
</tbody>
</table>

The highest energy recovery of 94.9% was achieved with *Kircheneriella cornutum* at 300 °C. Above 70% of energy recovery was attained at all conditions as biocrude oil and bio-char. Energy recovery reduced with increasing volumes and as the strain was changed from *K. cornutum* to *G. sulphuraria*. The increase in volume may have caused increased gasification of the biomass. The increased volume of nitrogen gas used for pressurizing the reactor prior to operation and moisture at higher pressure and temperatures may have aided in the expedited gasification of initial dry *K. cornutum* biomass into gases vented - out after cooling the reactor back to room temperature (gas phase increased from 30.05 to 38.51%). In the case of *G. sulphuraria*, reduction of energy recovery was combined with an increase in the aqueous phase recovery. The 5.58% increase in aqueous phase would have been the major cause for the 1.92% drop in energy recovery for *G. sulphuraria* as the volume of the reactor was increased 40%.
The higher lipid containing (see Table 2.3) *K. cornutum* showed greater energy recovery than the *G. sulphuraria* biomass. The recovery was above 90% for both conditions of *K. cornutum* as compared to above 70% recovery in every case including *G. sulphuraria*. In conclusion, change in reactor design and volume definitely affects the energy recovery from biomass at the same operational conditions. It was more significant in some species compared to the others. The choice of strain for HTL too plays an important role in any future works.

2.4.4. Effect of strain on product yields

The HTL reaction operating conditions including temperature, pressure and time were chosen based on our previous studies (*S. S. Toor et. al.; 2013, T. Muppaneni et. al.; 2017* and *T. Selvaratnam et. al.; 2015*) in order to achieve maximum conversion of algal biomass into products. Hydrothermal liquefaction of 20% loading of *G. sulphuraria* and *K. cornutum* at 300 °C for 30 minutes was done in triplicates. The reaction time was achieved in 15 - 20 minute with constant agitation. The process products were collected after cooling the reactor to ambient temperature to reduce loss of products. The gaseous loss from the process was not collected due to limitation of the experimental unit used in this work. The gas product yield was measured as the difference of the other major products from the dry microalgae weight used in each run.

The optimum temperature for liquefaction of *G. sulphuraria* to produce maximum bio-crude is reported to be between 300 and 350 °C (*S. S. Toor et. al.; 2013 and T. Muppaneni et. al.; 2017*). No hydrothermal liquefaction data for *Kirchneriella cornutum*
has been reported. Based on initial HTL studies (not included in this work) an optimum temperature of 300 °C can be expected for *K. cornutum*. The average biocrude yield varied with the strain as seen in many other works of the past (*H. Li et. al.; 2014, D.L. Barreiro et. al.; 2015 and X. Tang et. al.; 2016*). The production of biocrude from *K. cornutum* varied between 38.05 and 41.00 % as compared to an almost constant rate of 31.35 ± 1 % in *G. sulphuraria* (see Fig. 2.2). Percentage of solid residues produced from the process is reported to decrease with temperature and lowest at operational conditions above 300 °C (*T. Muppaneni et. al.; 2017*). From Figure 2.2, we observe the biochar production in any of the conditions was below 14.23 wt. % of the initial biomass. The biochar recovered amounted to 2.54 and 12.34 wt. % for *G. sulphuraria* and *K. cornutum* in a 250 ml reactor, respectively; as reported in Figure 2.2. It can be observed that the biochar produced is larger than the respective initial ash contents of 2.41 and 6.22 wt. %; warranting the assumption that at higher temperatures, oxidations of major components in the biomass like proteins and carbohydrates leads to increased biochar. This controlled oxidation; may in turn help reduce the ‘O’ content. The results presented in Table 2.2, show a reduced bio-crude ‘O’ content in biocrude of 7.42 and 7.94 wt. % in *G. sulphuraria* and *K. cornutum*, respectively.
2.5. References


Keat Teong Lee, Steven Lim, Yean Ling Pang, Hwai Chyuan Ong, Wen Tong Chong. 2014. Integration of reactive extraction with supercritical fluids for process intensification of biodiesel production: Prospects and recent advances. Progress in Energy and Combustion Science. 45, 54-78.


Yanqin Huang, Yupeng Chen, Jianjun Xie, Huacai Liu, Xiuli Yin, Chuangzhi Wu. Bio-oil production from hydrothermal liquefaction of high-protein high-ash microalgae including wild Cyanobacteria sp. and cultivated Bacillariophyta sp. Fuel 183 (2016) 9–19.


3. PHOSPHOROUS AND NITROGEN RECOVERY FROM BIOCHAR

3.1. Introduction

Hydrothermal liquefaction is an energy intensive thermochemical process that could be used to convert microalgal biomass into energy-rich products. The process produces three major products: biocrude, biochar, aqueous phase and a gaseous phase. Further, HTL of microalgae produces an energy intensive biocrude, energy and metal-rich solid residue called biochar and a carbohydrates and nitrogen-rich aqueous phase. Many works have been reported in the past explaining the use of nitrogen and carbohydrates present in the aqueous phase to supplement growth of microalgae cultures mixotrophically (U. Jena et. al.; 2011, P. Biller et. al.; 2012 and D.L. Barreiro et. al.; 2015).

All living organisms are carbon based. Plants even though carbon-based require nitrogen, phosphorous and potassium to grow. These elements are called macronutrients due to being required in large quantities for an ideal flora growth. Microalgae too require these nutrients in considerable ratios for their growth. The requirement of each strain of microalgae is varied based on biochemical composition (A. Bhatnagar et. al.; 2011 and P. Biller et. al.; 2012). The requirements for growth of Galdieria sulphurarria would be different from that of Kirchneriella cornutum. Biochar contains nutrients like nitrogen and phosphorous that could be used to grow microalgae. Analysis of biochar from HTL of microalgae has shown 1.7 - 17.1 wt. % phosphorous and 1.1 - 4.0 wt. % of nitrogen
The scale-up of hydrothermal liquefaction and commercialization of this technology would mean greater production of byproducts like biochar. The byproducts like biochar and aqueous phase if utilized optimally would increase the revenue generated from HTL, balancing the energy demand and operational costs incurred.

The recycle of nutrients through HTL of microalgae has been a topic of great interest in the recent past. Recycle of HTL aqueous phase in growth of algal species was discussed to great extent for G. sulphuraria (T. Selvaratnam et. al.; 2015a and T. Selvaratnam et. al.; 2015b). Further, study of polycultures for HTL aqueous phase recycle was reported by C. M. Godwin et al.; this work used a polyculture consisting of Ankistrodesmus falcatus, Chlorella sorokiniana, Pediastrum duplex, Scenedesmus acuminatus, Scenedesmus ecorinis, and Selenastrum capricornutum. One of the first-ever reported works on aqueous phase recycle from liquefaction was in 2013 by L. G. Alba et al.; this work was reported using the microalgae Desmodesmus sp. Another work of interest is reported by T. Lane and his team; they have reported a method for nitrogen and phosphorous recovery as struvite from microalgae after initial transesterification (J.C. Hewson et. al.). Struvite (NH₄MgPO₄·6H₂O) is an ammoniacal phosphate that was expected to carry 100% of the P and a portion of the N solubilized from biomass. The viability for this chemical is that it is a common growth additive that is soluble in acidic. In higher pH, struvite precipitates. This restricts its usability for strains that grow in alkaline media.
Leaching is one of the most primordial methods used in mining and mineralogy. The process has been in use especially for the purification of metals like nickel, uranium, copper and gold from ores (B.K. Loveday; 2008, B. Avvaru et. al.; 2008, K.A. Lewandowski and S.K. Kawatra; 2009 and A.R. Alonso-Gómez and G.T. Lapidus; 2009). Here, the process is optimized based on temperature (V.H. Ha et. al.; 2014), reactant concentration (V.H. Ha et. al.; 2014, E.A. Oraby and J.J. Eksteen; 2016 and Divyamaan Wadnerkar; 2015) and pH (E.A. Oraby and J.J. Eksteen; 2016 and E. Yanuar; 2015). Similar uses have been found for leaching in bio-system to selectively remove a component or element of interest from a cluster of molecules like ores (W.A. Stubbings et. al.; 2016 and Stefanie Hopfe et. al.; 2017). Previous works on leaching of phosphorous from rock phosphates in China have reported the optimum pH to be between 1.5 and 2.5. Another work of interest by R. Huang and Y. Tang proved that HTL biochar from anaerobically digested and activated sewage sludge can be used to leach phosphorous as orthophosphates at an operating temperature of 225 °C that is in the hydrothermal liquefaction range. Leaching as orthophosphates ensures that this phosphorous can be in turn used for various applications assimilated by microalgae. Leaching of phosphorous and nitrogen as ammonia from defatted microalgae using water has shown the optimum conditions to be at 250 °C and 350 °C, respectively (T.M. Aida et. al.; 2017). Further, the past works on recycle of HTL aqueous phase cited above give a better view into the recycle of nitrogen-rich components from the aqueous phase and using them to grow microalgae.
This chapter aims at improving leaching of phosphorous from hydrothermal liquefaction biochar as orthophosphates and nitrogen as ammoniacal nitrogen. The initial nitrogen and phosphorous content in biochar may be leached out in other forms like meta- and inorganic phosphates in the case of phosphorous. These other possible leached products are not considered in this study as previous studies have shown the predominant leaching of P to be as orthophosphates (R. Huang and Y. Tang; 2016) and nitrogen as ammoniacal nitrogen (T. Selvaratnam et. al.; 2015b). This chapter will serve as the basis for future works with leaching of macronutrients like nitrogen and phosphorous from biochar produced through HTL of similar strains.

3.2. Materials and methodology

3.2.1. ICP-OES protocol for analysis of algal biomass

Two digestion protocols for followed to prepare the biomass prior to spectroscopic quantification using a Thermo Fischer iCAP 6300 ICP-OES unit. The digestions of samples were done in a CEM Mars 6 microwave digester (CEM Corporation, Matthews, North Carolina, USA).

About fifty milligram of lyophilized G. sulphuraria biomass, dried biocrude and desiccated biochar were weighed out using a Mettler Toledo ME103TE New Classic ME-TE Toploading Balance (Cole-Parmer Company, Vernon Hills, Illinois, USA) weighing balance into digestion tubes in duplicates. All weighed replicates of the samples used for ICP-OES varied within 5 % of the set weight. This was followed by addition of 5 ml metal grade nitric acid into the mixture and leaving the sample-acid mixture in a chemical
hood for off-gassing. After about 30 minutes of off-gassing, 1 ml of hydrofluoric acid is added to the mixture and left for a period of 30 minutes prior to sealing the digestion tubes. The use of HF ensured complete digestion. The tubes are loaded into the digester and run for a period of 80 minutes in ramp-hold operation mentioned in Table 3.1. The samples are collected after a period of 80 minutes and let to off-gas in a chemical hood to vent-out gases like nitrous oxides for a period of 30 minute. This is followed by complexation using boric acid. Six times the volume of boric acid (49 % concentrated) is added to the tube to deactivate the hydrofluoric acid present after digestion.

Similarly, 3 ml of the hydrothermal liquefaction aqueous phase was measured out into digestion tubes in replicates. This was followed by addition of 10 ml metal grade nitric acid into the mixture and leaving the sample-acid mixture in a chemical hood for off-gassing. After about 30 minutes of off-gassing the digestion tubes were sealed. The tubes are loaded into the digester and run for a period of 50 minutes in ramp-hold operation mentioned in Table 3.1. The samples are collected after a period of 50 minutes and let to off-gas in a chemical hood to vent-out gases produced from the addition of strong acids for a period of 30 minutes. Samples from both protocols were completely digested and stored in poly tetrafluoroethylene (PTFE) tubes at 4 °C prior to analysis using ICP-OES. Samples were diluted to within the operational range of the unit at the time of analysis.
Table 3.1: Microwave digestion protocol for biomass, biocrude, biochar and aqueous phase.

<table>
<thead>
<tr>
<th>Step</th>
<th>Target Temp °C</th>
<th>Press Max bar</th>
<th>Ramp Time min</th>
<th>Hold Time min</th>
<th>Power %</th>
<th>Protocol for digestion of aqueous phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>1 165 30 5 10 70</td>
</tr>
<tr>
<td>2</td>
<td>190</td>
<td>80</td>
<td>5</td>
<td>10</td>
<td>90</td>
<td>2 190 30 5 20 90</td>
</tr>
<tr>
<td>3</td>
<td>240</td>
<td>80</td>
<td>5</td>
<td>30</td>
<td>90</td>
<td>3 50 30 1 10 0</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>80</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>Complexation of HF using boric acid oxidant (Repeat step #3)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The choices of operational wavelengths to detect presence of the digested metallic ions were set according to the vendor’s operational manual. The spectroscopic analysis was done in axial mode to improve detectability. The wavelengths employed are mentioned in Table 3.2. In the case of multiple wavelengths, the average concentration was used to ascertain the amount of the metal present.
Table 3.2: Wavelengths used to ascertain concentration of elements through inductively coupled plasma optical emission spectrophotometer (ICP-OES).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Element</th>
<th>Name</th>
<th>Sign</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silver</td>
<td>Ag</td>
<td></td>
<td>328.068</td>
</tr>
<tr>
<td>2</td>
<td>Aluminium</td>
<td>Al</td>
<td></td>
<td>167.079 308.215</td>
</tr>
<tr>
<td>3</td>
<td>Arsenic</td>
<td>As</td>
<td></td>
<td>189.042 193.759</td>
</tr>
<tr>
<td>4</td>
<td>Barium</td>
<td>Ba</td>
<td></td>
<td>455.403</td>
</tr>
<tr>
<td>5</td>
<td>Berrylium</td>
<td>Be</td>
<td></td>
<td>311.107</td>
</tr>
<tr>
<td>6</td>
<td>Calcium</td>
<td>Ca</td>
<td></td>
<td>393.366</td>
</tr>
<tr>
<td>7</td>
<td>Cadmium</td>
<td>Cd</td>
<td></td>
<td>214.438</td>
</tr>
<tr>
<td>8</td>
<td>Cobalt</td>
<td>Co</td>
<td></td>
<td>228.616</td>
</tr>
<tr>
<td>9</td>
<td>Chromium</td>
<td>Cr</td>
<td></td>
<td>205.560</td>
</tr>
<tr>
<td>10</td>
<td>Copper</td>
<td>Cu</td>
<td></td>
<td>324.754</td>
</tr>
<tr>
<td>11</td>
<td>Iron</td>
<td>Fe</td>
<td></td>
<td>259.940</td>
</tr>
<tr>
<td>12</td>
<td>Mercury</td>
<td>Hg</td>
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<td>184.950 194.227</td>
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<tr>
<td>13</td>
<td>Pottassium</td>
<td>K</td>
<td></td>
<td>766.490</td>
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<tr>
<td>14</td>
<td>Lithium</td>
<td>Li</td>
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<td>670.784</td>
</tr>
<tr>
<td>15</td>
<td>Magnesium</td>
<td>Mg</td>
<td></td>
<td>279.553</td>
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<tr>
<td>16</td>
<td>Manganese</td>
<td>Mn</td>
<td></td>
<td>257.610</td>
</tr>
<tr>
<td>17</td>
<td>Molybdenum</td>
<td>Mo</td>
<td></td>
<td>202.030</td>
</tr>
<tr>
<td>18</td>
<td>Sodium</td>
<td>Na</td>
<td></td>
<td>589.592</td>
</tr>
<tr>
<td>19</td>
<td>Nickel</td>
<td>Ni</td>
<td></td>
<td>231.604</td>
</tr>
<tr>
<td>20</td>
<td>Phosphorous</td>
<td>P</td>
<td></td>
<td>177.495 213.618</td>
</tr>
<tr>
<td>21</td>
<td>Lead</td>
<td>Pb</td>
<td></td>
<td>220.353</td>
</tr>
<tr>
<td>22</td>
<td>Sulphur</td>
<td>S</td>
<td></td>
<td>180.731</td>
</tr>
<tr>
<td>23</td>
<td>Antimony</td>
<td>Sb</td>
<td></td>
<td>206.833</td>
</tr>
<tr>
<td>24</td>
<td>Selenium</td>
<td>Se</td>
<td></td>
<td>196.090</td>
</tr>
<tr>
<td>25</td>
<td>Tin</td>
<td>Sn</td>
<td></td>
<td>189.989 283.999</td>
</tr>
<tr>
<td>26</td>
<td>Strontium</td>
<td>Sr</td>
<td></td>
<td>407.771</td>
</tr>
<tr>
<td>27</td>
<td>Titanium</td>
<td>Ti</td>
<td></td>
<td>336.121</td>
</tr>
<tr>
<td>28</td>
<td>Telurium</td>
<td>Tl</td>
<td></td>
<td>190.856 276.787</td>
</tr>
<tr>
<td>29</td>
<td>Vanadium</td>
<td>V</td>
<td></td>
<td>309.311</td>
</tr>
<tr>
<td>30</td>
<td>Zinc</td>
<td>Zn</td>
<td></td>
<td>213.856</td>
</tr>
</tbody>
</table>
3.2.2. Preparation of leaching media and materials

The leaching studies were done varying the pH (between 0.5 and 7.0) and temperature (between 23 °C and 40 °C) of experimentation to optimize the conditions. Primary tests were done using DI water brought to required pH by addition of sulfuric acid or potassium hydroxide based on initial pH of the medium. The addition of pH variation agents were done drop-wise using a pipette and mixed and tested for pH variation, after addition of every drop. When cyanidium medium was used to leach the nitrogen and phosphorous, care was taken to follow the procedure mentioned in T. Selvaratnam et. al.; 2015b. All cyanidium media samples were made with no initial N or P compounds. It was expected to replace ammonium sulphate and potassium phosphate in normal cyanidium media (T. Selvaratnam et. al.; 2015b) with ammoniacal nitrogen and orthophosphates, respectively from the biochar. All media made from stock chemicals were autoclaved to sterilize the experimental conditions.

3.2.3. Design of experiments and preliminary optimization

The enhancement of phosphates leaching from biochar was studied based on the following parameters: temperature of leaching and pH of the leach media. Preliminary studies mentioned below were completed to study the effect of above mentioned parameters on efficiency of leaching. Final leaching studies were done at improved conditions.
3.2.3.1. Study of effect of temperature in leaching

Biochar produced from hydrothermal liquefaction was collected by methods mentioned in Chapter 2 and stored prior to leaching. The biochar from liquefaction of *G. sulphuraria* was chosen to study the influence of temperature on leaching of phosphates. Thirty milligram of biochar was weighed out in triplicates for each condition into 50 ml polypropylene (PP) centrifugal tubes. The conditions investigated was: (i) Biochar in water at 23 °C, (ii) Biochar in water at 39 °C, (iii) Biochar in cyanidium media (CM) at 23 °C and (iv) Biochar in CM at 39 °C. Into each of the 50 ml tubes, 40 ml of the medium was added, capped and finally sealed to restrict evaporation of the medium. Water medium was maintained at neutral pH. The pH in CM was adjusted to lower pH by adding sulfuric acid. To increase pH, potassium hydroxide was employed.

Leaching was done in PP tubes stored at set temperatures in an incubator under sterile conditions. The experiment was done for 4 days with phosphate readings taken using a HACH test kits every second day in triplicates. The final pH after a period of 4 days was tested to confirm no significant change in pH or other operational conditions. The efficiency of leaching of phosphates from biochar was calculated based on the following equation:

\[
\text{Leaching Efficiency} \, (\%) = \frac{\text{Phosphorous leached as phosphates into the medium}}{\text{Total Phosphorous in 30 mg } G.\, sulphuraria \, \text{HTL biochar}}
\]
3.2.3.2. Study of effect of pH in leaching

Biochar produced from hydrothermal liquefaction was collected by methods mentioned in Chapter 2 and stored prior to leaching. The biochar from liquefaction of *Micractinium sp.* was chosen to study the influence of pH on leaching of phosphates. Thirty milligrams of biochar was weighed out in triplicates for each condition into 50 ml polypropylene (PP) centrifugal tubes. All leaching was done at 40 °C. The conditions investigated were: (i) Biochar in water at pH 2.5, (ii) Biochar in water at pH 4.0, (iii) Biochar in water at pH 7.0, (iv) Biochar in water at pH 10.0, (v) Biochar in water at pH 11.5 and (vi) Biochar in CM at pH 2.5. Into each of the 50 ml tubes, 40 ml of the medium was added, capped and finally sealed to restrict evaporation of the medium. The pH in CM and water was adjusted to prechosen by adding sulfuric acid or potassium hydroxide.

Leaching was done in PP tubes stored at set temperatures in an incubator under sterile conditions. The experiment was done for 7 days with phosphate readings taken using a HACH test kits on the second, third, fifth and seventh day in triplicates. The final pH after a period of 7 days was tested to confirm no significant change in pH or other operational conditions.

3.2.4. Enhanced leaching of phosphates and ammonia from biochar

For leaching, 30 mg of biochar obtained from HTL at above improved conditions of pH 2.5 and temperature 40 °C were weighed out and added to four different 50 ml polypropylene (PP) centrifugal tubular reactors. Lower pH was tested to test theoretical optimums based on pKₐ values. Each 50 ml tubular reactor was added with 40 ml of: (i)
CM with no initial phosphates at pH 2.5 corrected using sulfuric acid, (ii) CM with no initial phosphates at pH 7.0 corrected using potassium hydoxide, (iii) CM with no initial phosphates at pH 1.0 corrected using sulfuric acid, (iv) CM with no initial phosphates at pH 0.5 corrected using sulfuric acid (v) Deionized water at pH 2.5 corrected using sulfuric acid and (vi) Deionized water at pH 7.0. Leaching experiments were done for a period of 7 days; in 5 replicates. Phosphate and ammoniacal nitrogen levels in the media were measured periodically day in triplicates. The change in the fraction of phosphate and ammonia leached from the biochar over time was calculated. The pH levels in all reactors remained the same during the experimental period.

3.2.5. N and P measurements

The nitrogen and phosphorous content was measured using a HACH DR5000 spectrometer (HACH Company, Colorado, USA) during leaching studies. The media removed were replenished with deionized (DI) water before resuming the growth studies. The amount of N and P were found in terms of ammoniacal nitrogen (NH$_3$-N) and phosphates (PO$_4^{3-}$) using the Salicylate TNT Method 10031 and Phosver 3 Method 8048, respectively.

3.3. Results and discussions

3.3.1. Effect of temperature on leaching

The operational conditions of leaching are still a topic of interest in many works (B. Behnajady and J. Moghaddam; 2017 and M. Kavousi et. al.; 2017). In this
experiment, the temperature and pH of the leaching media was seen to have a significant effect on the leaching of phosphorous and nitrogen from HTL biochar. The effect of temperature study mentioned in Section 3.2.3.1 on leaching of phosphates from HTL biochar was shown in Table 3.3. Initial studies omitted leaching of nitrogen as ammoniacal nitrogen as, this project is majorly aimed at phosphorous recovery through HTL.

**Table 3.3:** Effect of leaching temperature on phosphorous recovery from HTL biochar, as phosphates.

<table>
<thead>
<tr>
<th>Run</th>
<th>Description</th>
<th>Temp °C</th>
<th>pH</th>
<th>t = 0 day mg/L</th>
<th>t = 2 day mg/L</th>
<th>t = 4 day mg/L</th>
<th>Efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water + Char</td>
<td>23</td>
<td>7.0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>4.54</td>
</tr>
<tr>
<td>2</td>
<td>Water + Char</td>
<td>39</td>
<td>7.0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>10.91</td>
</tr>
<tr>
<td>1</td>
<td>CM + Char</td>
<td>23</td>
<td>2.5</td>
<td>0</td>
<td>30</td>
<td>54</td>
<td>49.09</td>
</tr>
<tr>
<td>2</td>
<td>CM + Char</td>
<td>39</td>
<td>2.5</td>
<td>0</td>
<td>30</td>
<td>68</td>
<td>61.81</td>
</tr>
</tbody>
</table>

In the case of both media seen above, the elevated temperatures guaranteed greater leaching of phosphorous as phosphates. In the case of water at neutral pH, this increase is by about 58.38 % with increasing temperature while, the increase is much lesser in the case of an acidic cyanidium media (20.58 %). This warrants the assumption that pH is a major operational condition in the optimization of leaching from biochar. The assumption is furthered by the pKa value of phosphoric acid being 2.15 for the first dissociation. The increase in temperature showed differing increase in leaching efficiencies in two different media varying in pH and composition. The difference in
leaching may be an effect of the pH alone or a synergistic effect of both the pH and ionic strength. A time based increase in phosphates leached from biochar is shown in Figure 3.1.

![Phosphates leached over time](image)

**Figure 3.1:** Increase in phosphates leached over a period of four days in a 50 ml PP centrifugal tube.

### 3.3.2. Effect of pH on leaching

In leaching of elements from impure sources, pH is a major point of process optimization and improvement. The pH test was done using a similar strain of microalgae *Micractinium sp.* It was liquefied in the same protocol as mentioned in Chapter 2 in a 250
ml reactor. The biochar was stored prior to leaching studies. The pH ranges employed in this experiment were chosen based on growth conditions of common strains of microalgae (M. Wan et. al.; 2016, Q. Gong et. al.; 2014 and W. Shi et. al.; 2016). This was done as to reduce the number of steps necessary to leach out nutrients and utilize them in a growth reactor. If the pH for leaching was too basic or acidic compared to the growth conditions of the strain to be supplemented with the nutrients, a secondary pH adjustment step would become necessary, this increases operational and capital costs and even decrease process efficiency. The effect of pH mentioned in Section 3.2.3.1 on leaching of phosphates from HTL biochar was shown in Table 3.4. The control for this study is leaching of HTL biochar using CM at pH 2.5. Again, leaching of nitrogen as ammoniacal nitrogen is not considered during initial stages of optimization as recovery and recycle of phosphorous is the major target in this project. The table below shows averaged data from three replicates.

Table 3.4: Effect of pH on recovering phosphorous from HTL biochar, as phosphates.

<table>
<thead>
<tr>
<th>Run</th>
<th>Description</th>
<th>pH</th>
<th>t = 0 day mg/L</th>
<th>t = 2 day mg/L</th>
<th>t = 3 day mg/L</th>
<th>t = 5 day mg/L</th>
<th>t = 7 day mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avg</td>
<td>Std Dev</td>
<td>Avg</td>
<td>Std Dev</td>
<td>Avg</td>
</tr>
<tr>
<td>1</td>
<td>Water + Char</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>Water + Char</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>9.0</td>
<td>1.0</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>Water + Char</td>
<td>7.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.0</td>
<td>0.0</td>
<td>6.5</td>
</tr>
<tr>
<td>4</td>
<td>Water + Char</td>
<td>10.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>Water + Char</td>
<td>11.5</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>CM + Char</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>14.5</td>
<td>2.5</td>
<td>14.5</td>
</tr>
</tbody>
</table>
Lower pH definitely increased the leaching potential for phosphorous from 100 mg of biochar (2.86 wt. %) calculated from ICP-OES. The leaching was at 8 mg/L of phosphates after 7 days at a pH of 11.5 while, this increased up to 20.5 mg/L at pH 4.0. A pH of 2.5 showed slightly lower leaching potentials with a 10.0 mg/L leach of phosphates over a period of 7 days. This decrease in potential may be due to the extreme acidity of the medium dissociating the measure orthophosphates as inorganic phosphates or other non-measured forms of phosphorous. The theoretical optimum leaching of P is expected at pKa of 2.5 or a pH of 1.12 but, the study with *Micractinium sp.* did not agree with this assumption. An interesting result is the higher potential of leaching for CM at pH 2.5 when compared to water at the same conditions. This warrants the possibility that the presence of strong ions in CM aid in the leaching of phosphorous. This effect of ionic strength has net been investigated in this optimization study. A time based increase in phosphates leached from biochar is shown in Figure 3.2.
Figure 3.2: Increase in phosphates leached over a period of seven days in a 50 ml PP centrifugal tube.

3.3.3. Improved leaching of phosphates from HTL biochar

From ICP-OES, it was observed that 15.98% of phosphorous is present in biochar (Fig. 4.4). The CHNS studies reported 5.27% of nitrogen (Table 2.1). Acidity or basicity of a media aids the leaching of nutrients from materials like biochar. The increased presence of H⁺ or OH⁻ in the media is assumed to have a denaturing effect on the biochar surface much like the activation of carbonaceous compounds (D. Saha et. al.; 2014 and P.D. Mines et. al.; 2017). Similar effects are assumed to be the cause for leaching in this study.

The choice of a lower pH range for this study stems from the optimum growth conditions for G. sulphuraria (S.M. Henkanatte-Gedera et. al.; 2016 and T. Selvaratnam
This range would ensure that the leaching can be done both before and in the growth reactor. A higher pH would call for an extra step before growth aimed at reducing the medium pH closer to 2.5. From Figure 3.3.a, it is seen that leaching of phosphates from biochar varied greatly with pH. At lower pH values of 2.5, 1.0 and 0.5, the amount of leached phosphates into the media was seen to be 154.61, 159.50 and 129.00 mg/L as compared to 40 mg/L at a pH of 7.0. This increase in leaching capacity can be attributed to the difference in acidity. Similar results were observed in the control condition of leaching into water at the two pH values (see Figure 3.3.a). Past reported works have proved that phosphorus leaching using phosphate rocks is greater by an order of magnitude in lower pH (between 1.0 and 2.5) compared to neutral pH (L. Jiang et. al.; 2016). Further, the pKₐ value for 2.15 therefore, theoretically the greatest leaching of phosphorous as phosphates can be expected at a pH of 1.12. Since leaching is observed to be better at lower pH, the leached nutrients in the media can be used to grow *G. sulphuraria*; as the optimum growth pH for the strain of microalgae is acidic.

Further, from leaching of ammoniacal nitrogen from biochar (see Figure 3.3.b.) it was observed that the effect of pH was negligible. At a lower pH of 0.5, 1.0 or 2.5, ammoniacal nitrogen leached into the CM amounted to 128.41, 131.41 and 124.03 mg/L respectively compared to 105.19 mg/L at neutral pH. The swing in pH did very little to improve the leaching of nitrogen into the growth media as usable ammoniacal nitrogen, similar trends can be observed in the control conditions using water at similar pH. In the case of the controls (see Fig. 3.3.b.) leaching was observed to be about 80 mg/L lower.
than that in CM. This difference may be due to possible interactions of the media with the bio-char surface and possible effect of dissociated ions in the media.
**Figure 3.3:** Leaching study results for: a) Phosphates over a period of 7 days and b) Ammoniacal nitrogen over a period of 7 days
The initial amount of phosphorous and nitrogen in the biochar was reported to be 15.98 wt. % (Fig. 4.4) and 5.27 wt. % (Table 2.1), respectively. The process of acidic leaching improves recovery of phosphorous and nitrogen. The optimum pH condition was observed to be 1.0 with 159.50 mg/L of phosphates from phosphorous and 131.24 m/L of ammoniacal nitrogen. Leaching at a slightly higher pH of 2.5 compared to optimum pH of 1.0, ensured 92 % and 96 % recovery of compounds with lesser inputs to increase the acidity of the leaching media. By the use of the above-mentioned acidic leaching process at pH of 2.5, leaching of phosphorous as phosphates was 39 % (i.e. 4.95 wt. % leached) efficient and 61 % (i.e. 3.21 wt. % leached) for leaching of nitrogen as ammoniacal nitrogen.
Figure 3.4: Study of effect on leaching of nitrogen and phosphorous from G. sulphuraria HTL biochar at varying pH at the end of 7 days.
3.3.4. Mass balance of products from HTL and leaching studies

The products from HTL were analyzed for carbon, hydrogen, nitrogen and sulfur by CHNS (see Table 2.1) and phosphorous using ICP-OES. The results are tabulated in Table 3. The table shows a simple mass balance and concludes with recovery % based on the product composition expressed in Table 1. It can be observed that the recovery of carbon was at 52.27 %. It can be assumed that the remaining 47.73 % carbon was a part of the gaseous products of liquefaction which were not studied as a part of this work. The recovery percentage for oxygen is 24.67 %. This means about 75 % of the initial oxygen was lost in the gas phase. Some past works have reported the major composition of HTL gas phase to be predominantly oxides of carbon, nitrogen and sulfur with traces of hydrocarbons (B. E. Eboibi et al.; 2015 and G. L. Alba et al.; 2012).

Table 3.5: Mass Balance from hydrothermal liquefaction (* recovered as bio-crude, bio-char or aqueous phase, **calculated as difference).

<table>
<thead>
<tr>
<th></th>
<th>Dry Algae</th>
<th>Bio-crude</th>
<th>Bio-char</th>
<th>Aqueous Phase</th>
<th>Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Carbon</td>
<td>51.44</td>
<td>73.89</td>
<td>42.90</td>
<td>29.82</td>
<td>52.27</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>7.83</td>
<td>9.13</td>
<td>4.67</td>
<td>6.80</td>
<td>44.95</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>11.14</td>
<td>7.25</td>
<td>5.27</td>
<td>14.88</td>
<td>31.33</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.83</td>
<td>2.31</td>
<td>0.74</td>
<td>0.90</td>
<td>44.91</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>1.45</td>
<td>0.44</td>
<td>15.98</td>
<td>11.74</td>
<td>88.29</td>
</tr>
<tr>
<td>Oxygen**</td>
<td>26.31</td>
<td>7.41</td>
<td>30.44</td>
<td>35.86</td>
<td>24.67</td>
</tr>
</tbody>
</table>

In the case of phosphorous, stellar recovery of 88.29 % was reported. This is the highest reported through the process of HTL and for the strain employed. The major recovery of phosphorous is in the aqueous phase (54.54 %) and biochar (29.54 %). Also, about 9.19 % and 1.19 % of the initial nitrogen is recovered in the aqueous phase and
biochar. These nutrients in the biochar were leached using the above mentioned acidic leaching at pH 2.5. From this process of acidic leaching, about 49.49 % of the phosphorous was recovered as orthophosphates. The author believes this leaching to be greater should other forms of phosphates be included in the calculation. Similarly, about 95.21 % of the initial nitrogen is recovered as ammoniacal nitrogen. There were no tests done to study the leaching of nitrogen as nitrates, nitrites or any other derivatives.

Based on the data accumulated from CHNS elemental analysis and ICP-OES of the *G. sulphuraria*, the Redfield ratio / Redfield stoichiometry can be calculated. It is the ratio of carbon, nitrogen and phosphorous required for a biomass to grow and thrive in the natural habitat. For *G. sulphuraria*, the C:N:P ratio is calculated to be 91:17:1. It was based on the following equation:

\[
\text{Moles of Carbon} : \text{Moles of Nitrogen} : \text{Moles of Phosphorous}
\]
3.4. References


Paul D. Mines, Damien Thirion, Basil Uthuppu, Yuhoon Hwang, Mogens H. Jakobsen, Henrik R. Andersen, Cafer T. Yavuz. Covalent organic polymer functionalization of


4. GROWTH OF *GALDIERIA SULPHURARIA* IN LEACHED NUTRIENTS RECOVERED FROM BIOCHAR

4.1. Introduction

Macronutrients like nitrogen, phosphorous and potassium are necessary for the ideal growth of any plant. Microalgae being of the plantae kingdom are not any different in their requirements. The ideal nutrient ratio for each of the strains of microalgae may vary, but they still require each of the macronutrients in significant amounts.

The energy consumption in United States of America from petroleum, natural gas, coal and nuclear sources amounts to 89.09 quadrillion Btu in 2016 (*World Energy Outlook, 2015* and *Annual Energy Outlook, 2016*). A recent projection of energy consumption in the United States has been shown in Figure 4.1. Biofuels only supply 2.2 % of this demand (Fig. 2.1). Among the sources providing energy to United States, about 89.09 quadrillion Btu is produced from non-conventional sources like fossil fuels and nuclear power. Based on experimental HHV values for *G. sulphuraria*, If 10 % of this demand (i.e. 8.9 quadrillion Btu) was to be met by microalgae based power plants, we would annually require 656.27 million US tons of *G. sulphuraria*. The ICP-OES data (Table 3.4) gives a brief picture of the amount of phosphorous required to meet this demand. The demand for *G. sulphuraria* to meet 10 % US energy consumption would require 9.52 million US tons of phosphorous. The production of phosphorous in the United States in the year 2015 was 30.42 million US tons of phosphorous (*USGS MCS, 2015*).
The demand for microalgae-based biofuels to meet as low as 10% of the national energy requirement would mean a 31% increase in phosphorous production and mining. Phosphorous being a macronutrient that is mined out of the ground, this demand alone would spike the prices of the mineral and thereby biofuels in the market. This would seriously impair the usability and viability of this fuel.

Another possible application for biofuels is in aviation. The annual demand for aviation fuel in the United States is 20,272 million gallons ([FAA Aerospace Forecast Fiscal Years 2015-2035](#)). Based on preliminary calculations as shown above, the requirement for phosphorous to meet 100% of this demand would be equal to the annual consumption of phosphorous towards production of fertilizers. Presently microalgae are fed using mined nutrients like inorganic phosphates and nitrates or other forms of nitrogen. This increase in demand leads to escalated leaching on fertilizers. Mined phosphorous is presently the major contributor to meeting the demand for the agricultural industry. About 3.5 million US tons of phosphorous was required in 2010 to meet the demand in fertilizer production ([USDA ERS Website](#)). Annual demand of phosphorous to grow algae and meet 10% of the national energy demand is about 2.7 times that required to grow plants. Fertilizers that should be used to grow more crops would then be used to grow microalgae and fuel the country. It leads to a food-energy-water nexus like the ones being discussed extensively in the field of biofuels ([A. Endo et. al.; 2015 and D. Wichelns; 2017](#)). The possibility of an alternate source for these nutrients would greatly help both the biofuel and food industry. It would reduce the operational costs incurred due to growth nutrients for microalgae. Phosphates are acidic radical groups with the
potential to be leached out efficiently at a lower pH range between 1.5 and 2.5 (L. Jiang et al.; 2016) much similar to conditions at which acidophilic *G. sulphuraria* grows (pH 2.5). This in turn improves the efficiency of phosphorous recovery from HTL biochar as phosphates; that can be used directly to grow algae. The direct utilization reduces number of steps within the plant and overhead costs for incorporating such a recycle step in the process. Further, a low energy recycling step would reduce the energy demand for mining and processing of these nutrients. The reduced cost of operation at no significant increase in capital cost or energy investment would potentially better the Return on Investment (ROI) for such a step.

**U.S. primary energy production by major source, 2015**

![Bar chart showing energy production by major source](chart)

*Source: U.S. Energy Information Administration, Monthly Energy Review (April 2016), preliminary data*

**Figure 4.1:** Production of energy in the United States for 2015.
HTL biochar is rich in phosphorous and nitrogen (*P. Biller and A.B. Ross; 2011* and *Julia L. Faeth et. al.; 2016*). This biochar if utilized as a source for phosphorous and nitrogen in growth media would essentially reduce the demand for mined nutrients for algal growth. The present work studies the nutrient content and viability of its recovery in a byproduct from HTL, biochar. Among other uses, biochar has been used as a source for water treatment (*H. Jin et. al.; 2016*) and soil amendment (*R. Li et. al; 2016*), but the possibility of nutrient recovery prior to many of these other proven usages increases the revenue generated from the process of HTL. This in turn is expected to reduce the cost of biofuels per gallon. A similar work done in biochar produced from hydrothermal carbonization of sewage sludge (*R. Huang, Y. Tang; 2016*) reports an increased possibility of inorganic phosphorous recovery at elevated HTL temperatures. This would ensure uptake kinetics of nutrients from the medium by the microalgae is similar to that observed in most reported works. This present work utilizes phosphorous and nitrogen from HTL biochar of *G. sulphuraria*, to regrow the same strain of algae using nutrient leaching. It further, investigates the toxicity of leached phosphates and ammoniacal nitrogen to the biomass. Finally, the work done compares growth of *G. sulphuraria* in Cyanidium media (CM); prepared with leached macronutrients and inorganic stock solutions.
4.2. Materials and Methodology

4.2.1. Algae Strain collection and maintenance

Experiments in this study were performed with the red algae *Galdieria sulphuraria* CCMEE 5587.1 *(Toplin et al., 2008)* obtained from the Culture Collection of Microorganisms from Extreme Environments (Pacific Northwest National Laboratory; Richland, Washington, USA). Cultures were scaled up from single colonies to tissue culture flasks and axenic *G. sulphuraria* (5587.1) cultures were verified by sequencing regions of the 18S rRNA and RuBisCo LSU genes *(Toplin et al., 2008)*. A cleaved amplified polymorphic sequence (CAPS) method *(S.P. Fulbright et. al.; 2015)* was developed for *G. sulphuraria* (5587.1) using these markers as a diagnostic tool to ensure cultures were not compromised throughout the scale up process and during experiments (data not shown). Axenic stock cultures were maintained in tissue culture flasks with gyration at 40 °C in a lighted incubator (Percival Scientific, IA, USA) supplemented with 2% CO$_2$ under continuous light (100 μmol photons m$^{-2}$ s$^{-1}$). A modified Cyanidium medium at pH 2.5 *(Toplin et al., 2008)*, was used for verification, scale up, and experiments. The composition of the medium, per liter, was as follows: (NH$_4$)$_2$SO$_4$, 2.64 g; KH$_2$PO$_4$, 0.20 g; NaCl, 0.12 g; MgSO$_4$·7H$_2$O, 0.25 g; CaCl$_2$·2H$_2$O, 0.07 g; Nich's Trace Element Solution, 0.5 mL; FeCl$_3$ (solution = 0.29 g L$^{-1}$), 1.0 mL.

4.2.2. Growth study with leached nutrients

The standard CM was prepared using the following recipe: (NH$_4$)$_2$SO$_4$, 3.60 g L$^{-1}$; KH$_2$PO$_4$, 1.80 g L$^{-1}$; NaCl, 0.12 g L$^{-1}$; MgSO$_4$·7H$_2$O, 0.25 g L$^{-1}$; CaCl$_2$·2H$_2$O, 0.07 g L$^{-1}$;
Nitch's Trace Element Solution, 0.5 mL; FeCl₃ (solution 0.29 g L⁻¹), 1.0 mL, and the pH adjusted to 2.5 with 10 N H₂SO₄. Includes the vitamin component of f/2 algal medium (like; vitamins B1, B12 and biotin).

The leached nutrient rich medium was prepared from methods mentioned earlier in Chapter 3. Upon collection of this sample, the medium was autoclaved (at 121 °C); and stored at 4 °C. At the beginning of each test, the inoculum was centrifuged using a Beckman Coulter Allegra X-15R Centrifuge (Beckman Coulter Inc., California USA) and the algae pellets were suspended in the control set medium. Biomass growth was quantified periodically, in terms of the optical density (OD) measured with a HACH DR5000 UV-Vis spectrophotometer at a wavelength of 750 nm.

4.2.3. Experimental conditions for growth studies

4.2.3.1. Growth in 250 µL microplate assay

Initial growth studies were conducted in a 250 µL microplate assay to study the influence of leached nutrients in comparison to the standard growth media. Initial results from the growth experiments are summarized in Table 4.1. 10 reactors of each condition were used in this study. The optical density over the period of the growth experiments were tested using a HACH DR5000 Spectrometer. The microplate was housed inside an incubator (Percival Company, IA, USA) where the CO₂ level was maintained at 2-3% (vol./vol.) throughout experiments.
4.2.3.2. Growth in 6 ml tubular photo bioreactor

A volumetric scale-up was done to study possible effects on the growth that could change the growth rate. The cultures were grown in 16 mL borosilicate glass tubes, capped with plastic caps and sealed with parafilm to reduce evaporative losses. Each tube was inoculated with 6 mL of culture and placed in the outer rim of a roller drum (New Brunswick Scientific Company, Eppendorf, CT, USA) rotating at 16 rpm. The roller drum was housed inside the same incubator (where the CO₂ level was maintained at 2-3%) mentioned above; throughout experiments.

4.2.4. Growth and optical density measurements

The growth of *G. sulphuraria* was quantified periodically in terms of optical density (OD) at 750 nm using an UV-Vis spectrometer. The biomass density was found in terms of ash-free dry weight (AFDW) in g/L that was related to the OD at 750 nm using the relation *(T. Selvaratnam et. al.; 2014)*:

\[
AFDW \ (g \ L^{-1}) = 0.54 \times (OD@750 \ nm) + 0.023
\]

4.2.5. N and P measurements

The nitrogen and phosphorous content was measured using a HACH DR5000 spectrometer (HACH Company, Colorado, USA) during leaching studies; and at the initiation and completion of the growth studies. The media removed were replenished with DI water before resuming the growth studies. The amount of N and P were found in
terms of ammoniacal nitrogen (NH$_3$-N) and phosphates (PO$_4^{3-}$) using the Salicylate TNT Method 10031 and Phosver 3 Method 8048, respectively.

4.3. Results and discussion

4.3.1. Toxicity study of biomass in leached media

Presence of dichloromethane (DCM) in the growth media in concentrations above 78 mM has been observed to be toxic to biomass growth (H.K. Byers and L.I. Sly; 1993). In the same work, concentrations between 0.156 and 78 mM were observed to be growth inhibitory. Since DCM is used in HTL prior to leaching and may remain on the surface of the biochar when leaching occurs, the leached media has a higher incidence of DCM. Toxicity was tested over a period of 15 days in an incubator at 40 °C maintained at 2-3 vol. % CO$_2$ gas. At the beginning of each test, an inoculum from a positive-growth photobioreactor was centrifuged using a Beckman Coulter Allegra X-15R Centrifuge (Beckman Coulter Inc., California USA) and the algae recovered from centrifugation of Galdieria sulphuraria CCME 5587.1 were suspended in the leached cyanidium media. This was done in duplicates to confirm observations made. This test served as a preliminary study and hence no statistical study was employed. Biomass growth was quantified periodically, in terms of the optical density (OD) measured with a HACH DR5000 UV-Vis spectrophotometer at a wavelength of 750 nm. To reduce effect due to media evaporation, the reactors were sealed. Also, the amount of sample taken for OD studies was replaced with equal amount of distilled water.
The biomass was observed to grow positively over the period of 15 days. There seems no significant drop in growth rates compared to past literature in CM media (T. Selvaratnam et. al.; 2014, T. Selvaratnam et. al. 2015 and S.M. Henkanatte-Gedera et. al.; 2015). The growth curve observed is plotted in Figure 4.2. Over a period of 7 days, the observed increase in biomass of 1.759 mg/L was comparable to past literature (T. Selvaratnam et. al. 2015) with an increase of 1.25 mg/L in the same period of time. This similarity in growth rates over the same period of time warrants the conclusion that leached media can be used as a valid growth nutrient media.

![Growth of G. sulphuraria with Time](image)

**Figure 4.2:** Inhibition study of *G. sulphuraria* over a period of 17 days in a 50 ml photobioreactor.
4.3.2. Comparative growth of *G. sulphuraria* in standard and leached media

The growth is shown in terms of ash-free dry weight (g L\(^{-1}\)) in Figure 3. The initial study was completed in a 96 well plate microplate assay (see Fig. 4.3 (A)) and then scaled up to a 6 ml tubular reactor placed in a roller drum reactor (see Fig. 4.3 (B)). This study attempted to prove that leached nutrients from biochar produced after HTL could be successfully employed to grow *G. sulphuraria* at rates comparable with the standard CM. This would serve as a basis for future studies to study viability and economics of such a recycle step in a microalgae-based bio-refinery. The composition of leached media was normalized to match ammoniacal nitrogen levels to 360 mg/L and phosphates at 170 mg/L. This eliminated nitrogen availability as a possible limiting variable in the growth experiments intended to evaluate *G. sulphuraria* growth in CM media both leached and standard.

**Table 4.1:** Growth data in term of ash free dry weight for *Galdieria sulphuraria* grown at 40 °C and 2-3 vol. % CO\(_2\) in: a) Inorganically mixed cyanidium media and b) Cyanidium media with ammonia and phosphates leached from HTL biochar.

<table>
<thead>
<tr>
<th>Day</th>
<th>CM_Leached</th>
<th>SD</th>
<th>Phosphate</th>
<th>Ammoniacal Nitrogen</th>
<th>CM_ASU</th>
<th>SD</th>
<th>Phosphate</th>
<th>Ammoniacal Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.100</td>
<td>0.000</td>
<td>180.0 ± 3.0</td>
<td>448.5 ± 27.5</td>
<td>0.100</td>
<td>0.000</td>
<td>180.0 ± 3.0</td>
<td>448.5 ± 27.5</td>
</tr>
<tr>
<td>1</td>
<td>0.142</td>
<td>0.009</td>
<td>0.106</td>
<td>0.014</td>
<td>0.152</td>
<td>0.015</td>
<td>0.219</td>
<td>0.017</td>
</tr>
<tr>
<td>2</td>
<td>0.212</td>
<td>0.014</td>
<td>0.219</td>
<td>0.017</td>
<td>0.314</td>
<td>0.010</td>
<td>0.442</td>
<td>0.039</td>
</tr>
<tr>
<td>3</td>
<td>0.297</td>
<td>0.010</td>
<td>0.442</td>
<td>0.039</td>
<td>1.341</td>
<td>0.296</td>
<td>78.1 ± 14.4</td>
<td>90.5 ± 54.1</td>
</tr>
<tr>
<td>5</td>
<td>0.693</td>
<td>0.018</td>
<td>0.503</td>
<td>0.062</td>
<td>0.503</td>
<td>0.062</td>
<td>101.5 ± 2.7</td>
<td>46.0 ± 6.4</td>
</tr>
<tr>
<td>6</td>
<td>0.942</td>
<td>0.187</td>
<td>101.5 ± 2.7</td>
<td>46.0 ± 6.4</td>
<td>0.942</td>
<td>0.187</td>
<td>101.5 ± 2.7</td>
<td>46.0 ± 6.4</td>
</tr>
</tbody>
</table>
Growth curves of *G. sulphuraria* cultivated in a microplate assay were summarized in Figure 4.3.A. This initial study warranted that growth of *G. sulphuraria* in leached CM was comparable to that in standard media. It can be ascertained that there was no toxic effect on the biomass growth by the leached nutrients as the slopes are similar. The almost similar growth rates prove that the use of leached media to grow microalgae is possible. Also, it may be ascertained that there is no visible growth inhibition due to leaching.
Figure 4.3: Growth of *Galdieria sulphuraria* in: a) 96 well microplate assay and b) 16 mm tubular reactor
A secondary confirmation using a tubular photo bioreactor showed similar results (Fig. 4.3 (B)) as in the microplate assay. This study was a volumetric scale-up from the 250 μL reactor size to 6 ml. This scale-up (~24 fold) did not show significant difference in the results. The ash-free dry weight (AFDW) increased from 0.128 g/L to 1.341 g/L in the leached media as compared to 0.539 g/L in standard media. Further, the increase in biomass in the 6 ml reactors were comparable with those observed in the microplate assay studies (Fig. 4.3 (A)) and other reported works on the same strain (T. Selvaratnam et. al.; 2014 and S.M. Henkanatte-Gedera et. al. 2016). These results confirm that *G. sulphuraria* can be successfully grown using recycled biochar from hydrothermal liquefaction.

A pie chart depiction of composition of biochar can be seen in Figure 4.4. The increase in growth of *G. sulphuraria* in a leached media when compared to a standard cyanidium media can be attributed to the presence of other leached elements like magnesium, calcium, iron and sodium. These elements together amounts to about 7 wt. % of the biochar. If we assume about 90 % efficient leaching of these nutrients, they will aid in better growth of the microalgae compared to the standard media. The slower uptake of these elements may have been the cause for similar growth observed in both cases till the third day and henceforth, an increase in growth rate till the eight day of experimentation.
Figure 4.4: Pie chart depiction of biochar composition through ICP-OES.
4.4. References


Dennis Wichelns. The water-energy-food nexus: Is the increasing attention warranted, from either a research or policy perspective? Environmental Science & Policy 69 (2017) 113–123.

Federal Aviation Administration Aerospace Forecast Fiscal Years 2015-2035


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5. SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1. Summary

Chapter 1 gives introduction about sources of renewable energy, current energy consumption and possible energy sources. A view into different microalgal species and possible recovery routes are discussed. The viability of hydrothermal liquefaction in conversion of microalgae is debated and the various uses for each of the HTL products are discussed. Also, this section concludes with a brief outlook into the following chapters and topics of interest.

Chapter 2 speaks about hydrothermal liquefaction in depth for two strains grown at Arizona Center for Algae Technology (AzCATI), Arizona State University throughout the year 2016-2017. These strains are grown in crop rotation and the production of biocrude from them is expressed in percentage yields. Further, the reactor was varied and HTL was done to investigate possible changes in energy recovery and biocrude yields. Significant drops in product recovery were seen in almost all cases.

Chapter 3 explains the leaching procedure employed to recovery nitrogen and phosphorous compounds from HTL biochar produced from microalgae. Preliminary studies showed the most favorable conditions for leaching N and P to be: low pH and high temperatures. These favorable conditions were employed to leach nutrients from G. sulphuraria HTL biochar. Cumulative mass balances were done in each step to study recovery efficiencies in HTL and leaching.
Chapter 4 is a proof of concept for the proposed recovery of macronutrients into the growth media and used to successfully grow *G. sulphurar*ia. Initially a study to test toxicity of leached macronutrients on the microalgae was done successfully over a period of 15 days. After initial studies, a final growth test was done on two scales. A consistent growth was observed both in a microplate assay (250 µL) and in a tubular reactor (6 ml).
5.2. Recommendations for future research

This thesis depicts the possibility of recovery of nutrients from hydrothermal liquefaction biochar through leaching into growth media used. It was done on the scale from 50 µL to 6 ml. This lab scale experiment would in the future need to be tested in a scale of consideration. The author believes a reactor size of about 1,000 L would be ideal to study the viability of such a leaching step in a large algal bio-refinery.

Further, this work only serves as a proof of concept. No study has been done to understand the economic implications of such a nutrient leaching step in an algal bio-refinery. A techno-economic analysis (TEA) on the proposed recycling with detailed investigation of the increase in capital investments and reduction in operational costs have to be studied. Based on these projections, a new ROI on biofuel projects may be generated. This future work will serve as the building block of a new era of bio-refineries that produce biofuels at reduced overhead costs. Also, a Life Cycle Analysis (LCA) on this recycle step would help increase the affability of such a technology.

Hydrothermal liquefaction has been optimized over the past decade for various strains of microalgae, but the compendium of this data is still not complete. Hydrothermal liquefaction technique of various algae can be performed and process parameters are to be optimized. The scale-up study done in this thesis can be used to predict a model that will better help understand the possible efficiency drops in energy recovery and change in product distribution.


Dennis Wicheln. The water-energy-food nexus: Is the increasing attention warranted, from either a research or policy perspective? Environmental Science & Policy 69 (2017) 113–123.


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