DEVELOPMENT AND OPTIMIZATION OF A PRODUCED WATER, BIOFILM-BASED MICROALGAE CULTIVATION SYSTEM FOR BIOCRUDE CONVERSION USING HYDROTHERMAL LIQUEFACTION

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biological Engineering

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2018
ABSTRACT

Development and Optimization of a Produced Water, Biofilm Based Microalgae Cultivation System for Biocrude Conversion Using Hydrothermal Liquefaction

By

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Utah State University, 2018

Major Professor: Dr. Ron Sims
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Utah’s hydraulic fracturing industry produces large quantities of wastewater, also known as produced water. This water contains high levels of contaminants such as salts and hydrocarbons, and current techniques for dealing with this produced water are costly resulting in subsurface injection and evaporation. Due to the high cost, most produced water is treated as purely waste and is reinjected back into the subsurface. This project approaches this water as not waste but as a source of nutrients used to grow and cultivate microalgae. These microalgae can then be converted into a product stream such as biocrude oil and valuable pharmaceutical products.

The objectives of the project were to (1) cultivate biomass on produced water, (2) alter the material of construction of the RABR, (3) decrease the rotations per minute of the disks within the operating design, and (4) optimize the conversion of the microalgae into biocrude oil using hydrothermal liquefaction (HTL). Microalgae were grown in mixed culture using a Rotating Algal Biofilm Reactor (RABR) that was rotated in produced water taken from the Uinta Basin in Eastern Utah. The RABR was built at pilot scale to increase yield, and two substrates were used in construction, polystyrene and
cotton rope. After studies were carried out on motor power consumption, polystyrene was chosen to lower cost of RABR construction and was oriented in a way to increase the ratio of growth surface area to produced water volume.

The microalgae strains that were cultivated were genetically identified as unique to the Logan Lagoons in Logan, Utah, and the Great Salt Lake in Salt Lake City, Utah, and once harvested were converted into biocrude oil using hydrothermal liquefaction (HTL). The conversion of the microalgae to biocrude gave a yield of 35% ash free dry weight being obtained in laboratory HTL tests and 58% of feedstock energy recovered in the biocrude.

(74 pages)
Development and Optimization of a Produced Water, Biofilm Based Microalgae Cultivation System for Biocrude Conversion Using Hydrothermal Liquefaction

Benjamin L. Peterson

Extraction of oil and gas in Utah’s Uintah Basin results in large quantities of wastewater, or produced water, with nutrients and residual organic chemical that represent a significant resource for producing energy-related and value-added products. Produced water was obtained as a biomass producing nutrient source from industries operating in Utah’s Uintah Basin. Within the Uintah Basin (defined as Uintah and Duchesne Counties within Utah) approximately 93 million barrels of water were produced in 2013 while only 11% of the water was disposed of through evaporation, with the national average at 2%. The rest is reinjected into the subsurface.

The goal of this project was to design a system that utilizes produced water as a nutrient source for growing microalgae biomass in a biofilm form using a Rotating Algal Biofilm Reactor (RABR). The biomass would then be harvested and converted into biocrude oil using hydrothermal liquefaction (HTL). The objectives were to (1) cultivate biomass on produced water, (2) optimize the reactor to reduce energy costs to operate while increasing biomass productivity, and (3) increase feedstock quality for HTL.

The RABR was constructed out of polystyrene disks, and experimentation was carried out to optimize rotational speed of the reactor. Two strains of algal biomass were identified as biofilm formers and grown using produced water as the nutrient source. The
biomass was then utilized as a HTL feedstock that gave an average yield of 34.5% ash free dry weight.
ACKNOWLEDGMENTS

I would like to acknowledge the Huntsman Environmental Research Center (HERC) and the Sustainable Waste to Bioproducts Engineering Center for financial support and all those who work within. I would like to thank my professors and colleagues for all their support and encouragement. Without you I would not have been able to accomplish this. Thank you.
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<th>Description</th>
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<td>ANOVA</td>
<td>Analysis of Variants</td>
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<tr>
<td>COD</td>
<td>Carbon Oxygen Demand</td>
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<tr>
<td>cRPB</td>
<td>clinoptilolite Rotating Photo Bioreactor</td>
</tr>
<tr>
<td>GSL</td>
<td>Great Salt Lake</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>HTL</td>
<td>Hydrothermal Liquefaction</td>
</tr>
<tr>
<td>LCA</td>
<td>Life Cycle Assessment</td>
</tr>
<tr>
<td>LLC2</td>
<td>Logan Lagoons Culture 2</td>
</tr>
<tr>
<td>MFSP</td>
<td>Means Fuel Selling Price</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically Active Radiation</td>
</tr>
<tr>
<td>PPFD</td>
<td>Photosynthetic Photon Flux Density</td>
</tr>
<tr>
<td>RAB</td>
<td>Rotating Algal Biofilm</td>
</tr>
<tr>
<td>RABR</td>
<td>Rotating Algal Biofilm Reactor</td>
</tr>
<tr>
<td>RBC</td>
<td>Rotating Biological Contactor</td>
</tr>
<tr>
<td>RPM</td>
<td>Rotations Per Minute</td>
</tr>
<tr>
<td>SWBEC</td>
<td>Sustainable Waste to Bioproducts Engineering Center</td>
</tr>
<tr>
<td>TDN</td>
<td>Total Dissolved Nitrogen</td>
</tr>
<tr>
<td>TDP</td>
<td>Total Dissolved Phosphorus</td>
</tr>
<tr>
<td>TEA</td>
<td>Techno-Economic Analysis</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Suspended Solids</td>
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</table>
CHAPTER 1

INTRODUCTION AND OBJECTIVES

Current technologies for treating wastewater from oil and gas operations, called produced water, are too expensive and lead to companies simply reinjecting the water back into the subsurface, which causes an increased risk in contamination of local drinking water supplies. This thesis describes a project that incorporates a biological component into the treatment process, microalgae, to provide an alternative revenue stream to help offset the cost of treatment. The project used a microalgae cultivation system developed by the Sustainable Waste to Bioproducts Engineering Center (SWBEC) at Utah State University called the Rotating Algal Biofilm Reactor (RABR) to grow microalgae as a biofilm. Additionally, reactor design changes were implemented to improve microalgae production, while decreasing energy consumption.

For this project, several key objectives were proposed: (1) cultivate biomass in produced water; (2) alter the material of construction of the RABR from a frame covered in a cotton cloth to a polystyrene disk configuration; (3) decreasing the rotations per minute of the disks within the operating design; (4) optimize the conversion of the microalgae into biocrude oil using hydrothermal liquefaction (HTL). The list of objectives addresses the optimization of the Rotating Algal Biofilm Reactor (RABR) with the focus for increasing the production value of the microalgae grown on produced water. This optimization stems from the techno-economic analysis conducted by Jay Barlow [21] regarding the integration of a RABR into a wastewater treatment system for production of biocrude oil. In the analysis, it is stated that to make the integration viable, an optimization of the reactor must take place to reduce the capital cost associated with microalgae production in regard to bio-crude oil production. In order to address this optimization challenge highlighted by Barlow et al. [21] I proposed various changes to the
design, with the intention of decreasing the energy consumption of the motors turning the reactor shafts while minimizing microalgae productivity losses or possibly increasing the microalgae productivity.

One of these changes included altering the material of construction from a cylinder frame covered in a growth material to a polystyrene disk configuration. This proposed change not only increased the growth surface area to water volume ratio, which increased the microalgae productivity, but also decreased the overall weight of the reactor. By decreasing the weight of the reactor, it was hypothesized that the motor will be required to use less electricity to rotate the reactor. The reduction in electricity required to operate the reactor results in a corresponding increase in the value of the microalgae produced by decreasing the cost to produce it.

In a further attempt to reduce the power required to rotate the reactor, I also proposed decreasing the rotations per minute of the disks within the design. It was hypothesized that, with a decreased rotation speed, the energy required to grow the microalgae will be much less while minimally affecting microalgae production. While conducting this analysis, I also collected environmental data during the microalgae growth phase. In doing this, I could attempt a correlation of microalgae production with specific growth factors. This correlation will hopefully help identify the optimal growth factors required for increased microalgae production.

After these changes to the microalgae production system were implemented, I additionally proposed looking at the conversion of the microalgae into biocrude oil using hydrothermal liquefaction (HTL). I took the microalgae grown during my RPM trials and converted it into biocrude oil using the process of HTL, which uses high temperature and pressure to convert wet microalgae biomass into biocrude oil. HTL conversion data previously
gathered in a different study shown in Table 1, was used for comparison. Additionally, I conducted an experiment to try to increase HTL productivity by manipulating the quality of the microalgae feedstock. This experiment involved adding a washing step within the procedure to decrease the ash content in the microalgae. It was hypothesized that with less ash content in the feedstock more of the biomass can be converted to produce biocrude. Increasing HTL productivity is important for improving the viability of the RABR as a microalgae productivity system.

**Table 1**

Initial biocrude yield data obtained from HTL conversion (Barlow 21).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Biocrude yield (% dw)</th>
<th>Biocrude yield (% afdw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>16.4</td>
<td>33.0</td>
</tr>
<tr>
<td>Trial 2</td>
<td>19.1</td>
<td>38.4</td>
</tr>
<tr>
<td>Trial 3</td>
<td>16.6</td>
<td>33.3</td>
</tr>
<tr>
<td>Average</td>
<td>17.4</td>
<td>34.9</td>
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</table>

*dw = dry weight basis

*afdw = ash-free dry weight basis
CHAPTER 2

LITERATURE REVIEW

2.1 General Review

Microalgae are small aquatic organisms that can be grown in a wide range of environments and environmental conditions. More specifically, microalgae can adapt to harsh growth conditions, such as high salinity and large pH ranges, allowing for several different growth substrates for a variety of microalga species. It is this flexibility that has led to researchers combining microalgae cultivation with wastewater treatment. Due to the microalgae’s ability to adapt to harsh environments, systems are being developed that utilize wastewaters containing harsh contaminants for microalgae cultivation. Microalgae cultivation where the biomass utilizes nutrients in the wastewater contributes to water remediation and simultaneously serves as a feedstock for bioproducts such as renewable biofuels [1]. The cultivation provides improved environmental quality and the resulting fuel product may offset the high costs of wastewater treatment.

One objective for this project was to determine the ability of microalgae to grow in the high salinity environment of produced water. Produced water, shown in Figure 1, is a byproduct of the oil and gas industry when fluid is drawn from the subsurface during mining operations and can be either all-natural water or contain fracking fluid and other chemical contaminants added in the mining process. It is a highly saline waste that contains a large array of chemical contaminants [2]. Approximately 14 billion barrels of the water are produced annually [3,4], and a large
portion of the water goes untreated. On average seven barrels of produced water are generated for every 1 barrel of crude oil generated [5]. Currently the industry does not treat the water and instead reinjects it back into the land from which it was drawn. This leads to a risk of contamination of the local drinking water [6]. The problem however, is that current disposal and treatment methods are expensive and thus dissuades companies from allocating resources focused on water treatment. Depending on the composition of the water, the treatment methods focus on objectives such as de-oiling, disinfection, suspended solids removal, desalination, and dissolved gas removal. Each objective requires different treatment methods with one such example of centrifugation or the use of an API gravity separator when focusing on oil removal [5]. Depending on the location of the site and the contents of the water, disposal can range from $0.30 a barrel to $105 a barrel [7]. This high cost is due to the lack of cost effective methods of wastewater treatment. The high costs of disposal along with the large amount of produced water to dispose is what leads to the development of a microalgae cultivation system that integrated wastewater treatment with bioproduct production in this project.
Along with the produced water being abundantly available, the water also shows high potential for microalgae growth due to the supply of nutrients in the water. One such sample of produced water taken from Utah’s Uintah Basin (Table 2) shows that it contains various levels of organic carbon, nitrogen, and phosphorus that are all key elements required for microalgae cultivation. One parameter of note is the Total Kjeldahl Nitrogen, which is the sum of organic nitrogen, ammonia and ammonium when analyzing samples of wastewater. These elements have been shown to be limiting factors in various systems and usually require amending to maintain microalgae production. With such nutrient levels in the produced water available for the microalgae to utilize, a system utilizing produced water as a nutrient source would need to replace the water with water containing more nutrients much less frequently. This would greatly improve the viability of the wastewater as a nutrient source if microalgae could be cultivated to withstand the other contaminants such as the high salinity. The SWBEC group has previously

### Table 2

Wastewater characteristics of produced water sampled from Utah’s Uintah Basin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Result</th>
<th>Minimum Reporting Limit</th>
<th>Units</th>
<th>Analytical Method</th>
<th>Preparation Date/Time</th>
<th>Analysis Date/Time</th>
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<td>Total Inorganic Nitrogen</td>
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<td>0.2</td>
<td>mg/L</td>
<td>Calculation</td>
<td>10/13/2015 09:21</td>
<td>10/13/2015 9:21</td>
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<tr>
<td><strong>Inorganic</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia as N</td>
<td>7.7</td>
<td>0.2</td>
<td>mg/L</td>
<td>SM 4500 NH3-D</td>
<td>10/06/2015 13:30</td>
<td>10/6/2015 13:30</td>
</tr>
<tr>
<td>Biochemical Oxygen Demand</td>
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<td>5</td>
<td>mg/L</td>
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<td>5</td>
<td>mg/L</td>
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<tr>
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<td>1210</td>
<td>100</td>
<td>mg/L</td>
<td>Hach 8000</td>
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<td>Nitrate as N</td>
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<td>2.0</td>
<td>mg/L</td>
<td>EPA 300.0</td>
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<td>Nitrates as N</td>
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<tr>
<td>Oil &amp; Grease (HEM)</td>
<td>ND</td>
<td>5</td>
<td>mg/L</td>
<td>EPA 1664A</td>
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<td>10/5/2015 14:00</td>
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<td>pH</td>
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<td>0.1</td>
<td>pH Units</td>
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<td>Phosphate, ortho as P</td>
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<td>20</td>
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<td>0.01</td>
<td>mg/L</td>
<td>EPA 8260B</td>
<td>09/30/2015 13:06</td>
<td>9/30/2015 17:56</td>
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</table>
accomplished this and was able to maintain a culture of microalgae, identified as LLC2 [8], that not only could survive but produce large quantities of biomass when cultivated in produced water. The LLC2 was coupled with a microalgae production and harvesting unit to grow large quantities of microalgae to then use for bioproducts.

Traditional microalgae growth systems take advantage of raceways where water is mixed, and microalgae is cultivated in suspension [9]. This method is not optimal however, due to the high-energy costs related to removing the microalgae from suspension [10] and the low microalgae productivity due to the turbidity of most wastewaters that tends to prevent sunlight from penetrating far enough into the water to facilitate microalgae growth [11]. Additionally, the high-energy issue arises from the cultivation designs where the microalgae are suspended in solution because to separate the microalgae from the water, the solution is generally centrifuged, which is an energy intensive process [12]. The low microalgae production issue arises due to the turbidity of the water because microalgae are cultivated in more shallow raceways as the sunlight penetrates the water for an only short depth. These two major issues of high energy costs and low productivity cause microalgae productivity for wastewater treatment to be traditionally suboptimal. To address these issues, systems of microalgae cultivation are being developed that utilize biofilm microalgae.

2.2 Rotating Biological Contactors (RBCs)

2.2.1 Martin Gross:

In 2013, Gross et al. [13] developed a unique biomass cultivation system to grow microalgae as a biofilm in order to incorporate a scraping-style harvest system [13]. This approach was carried out to avoid traditionally energy intensive harvesting methods such as flocculation and centrifugation. With this goal in mind, he designed the Rotating Algal Biofilm
(RAB) system for use at both laboratory and pilot scales. The objectives of his study were (1) to prove the concept of the RAB system, (2) explore optimal operation conditions of the system, and (3) explore the possibilities of system scale up.

After defining his objectives, Gross began to develop the system by conducting material substrate evaluations. He evaluated a total of 16 different materials for use as an attached growth substrate. The selected materials were chosen because they are inexpensive, durable, and easy to obtain/produce. The materials chosen to evaluate were: muslin cheese cloth, armid fiberglass, PTE coated fiberglass, chamois leather cloth, vermiculite, microfiber, synthetic chamois cloth, fiberglass, burlap, cotton duct, velvet, Tyvek, poly-lactic acid, abraised poly-lactic acid, vinyl laminated nylon, and polyester. Microalgae attachment trials were conducted using a rocker shaker. The shaker was operated in a plexiglass-chamber, and the materials were rocked in and out of Bold’s Basal Medium.

After the material trials were conducted, a lab-scale RAB system was designed, shown in Figure 2. The growth material was stretched around 3 shafts in a form of a triangle with one of the points of the triangle flowing through a growth medium reservoir. The material is pulled through the reservoir, exposing the biomass to nutrients, then into the air exposing it to light and carbon dioxide. The laboratory-scale system was operated in another plexiglass chamber maintained at 25°C, with air being

![Fig. 2 A schematic of the RAB designed by Martin Gross. Biomass is allowed to grow on the surface of the attachment material which, is exposed to both light and nutrients. [13]](image-url)
continually pumped into the chamber. The liquid in the reservoir was replaced at a ratio that gave the system a 5-day hydraulic retention time (HRT). Additionally, a suspended microalgae culture was built and maintained under identical conditions for RAB system comparisons. Multiple experiments were conducted varying rotational speed, carbon dioxide concentrations within the inlet air, and harvesting frequency all designed to determine their influence on biomass production.

Along with laboratory-scale testing, a pilot scale RAB-enhanced raceway was constructed and installed inside a greenhouse. Four pilot scale RABs were built of cotton duct and placed in a 3 by 8-meter-long raceway. Additionally, two open ponds were placed in the same greenhouse for comparison. The reactors were allowed to operate during January-February and May-June 2013 while being exposed to natural light.

After the experimentation was completed, the data collected were analyzed to show proof of concept and system optimization conditions. Data from the laboratory scale tests showed that for the chosen criteria of rotational speed, harvesting frequency, and carbon dioxide levels, the optimal conditions were 4 rpm, harvesting every 7 days, and any carbon dioxide concentration since the concentration of carbon dioxide ranging from atmospheric concentration of .03% to an increased concentration of 3% carbon dioxide did not directly affect the biomass productivity. When the system was compared to the open raceway, the microalgae composition was essentially comparable to that of open raceway microalgae. It was also determined that the RAB produced this comparable alga at a much higher rate of 3.51 ± 0.48 g m⁻² day⁻¹ than the open raceway at 0.26 g L⁻¹ day⁻¹ and energy cost to harvest the biomass was much less. These findings were confirmed within the pilot scale tests. [13]
2.2.2 Ashton Young:

For Ashton Young’s Utah State University MS research, he chose to address the issue that, due to ammonia gas volatilization, the optimal molar ratio of 16:1 N:P was not being met with traditional microalgae based wastewater treatment systems [14]. For his research, Young developed a zeolite-based photobioreactor that utilized clinoptilolite, an ammonium selective zeolite, to sequester nitrogen from ammonium ions to be in a form that is bioavailable for biomass growth.

Young defines zeolites as “…a group of naturally occurring framework of hydrated alumino-silicate of alkali and alkaline earth cations… with high cation exchange capacities… without change of crystal structure.” [14]. It was these properties that led to the zeolite, clinoptilolite, to be chosen for the design based on its affinity for ammonium. This choice was made after bench scale tests were carried out over 21 days. These tests showed that, when clinoptilolite was utilized to sequester nitrogen from ammonium ions, biomass could be produced.

After demonstrating the zeolite’s capability for growing biomass, Young designed a bioreactor, which he referred to as the cRPB (clinoptilolite Rotating Photo Bioreactor), shown in Figure 3. The design incorporated 4 key criteria: (1) reactor rotation speed to be held between speed...
of 1-5 rpm, (2) 40% of the growth substrate would be submerged in the aqueous phase while the remaining 60% of the growth substrate would be exposed to the gaseous phase, (3) a centrally driven shaft through the cRPB drum would be utilized for reactor rotation, and (4) growth substratum would be available for facilitated microalgae biofilm harvesting. In constructing a reactor to meet those criteria, Young developed a RPB form out of plastic piping, which was sealed to create a vacuum. Using this form, he attached the zeolite to a 3-inch standard pipe using a mixture of epoxy resin and a hardener. The mixture was evenly applied using the applied vacuum where the vacuum pulled the resin mixture through the zeolite void space and, using qualitative analysis, it was shown that the process produced a uniform matrix around the granules. After the mixture was allowed to dry at room temperature for 24 hours, the composite was machined on a lathe and sanded with sand paper to produce a biofilm conducive growth surface. Finally, a threaded rod was inserted through the middle of the pipe to provide rotation to the reactor.

After fabricating the reactor, analysis showed that 64% of the total surface area of the cRPB was exposed zeolite. Experiments were then carried out to establish the reactor’s capability to exchange ammonium. In these experiments, the reactors were rotated in synthetic wastewater at 4.6 rpm. The water was kept at 21°C with a pH of 7.7, which allowed for 2% of the $NH_4^+ - N$ to be available in the gaseous ammonia form. Ammonia levels were measured periodically within a 24-hour experimentation period. This experimental period allowed Young to demonstrate the validity of his ammonium exchange reactor.
After the proof of concept experiments were carried out, Young designed an experiment to determine the effectiveness of the zeolite-based reactor compared to inert reactors of the same design with regards to biomass production. A statistical design, shown in Figure 4, was implemented using five identically sized reactors differing in surface composition. The experiment was carried out at the same environmental conditions as the previous proof of concept experiment, while in semi-batch mode. Additionally, the reactors were rotated at the same speed of 4.6 rpm. Biomass productivity was measured over a 35-day experimental period. Although zeolite-based reactors produced more biomass than the controls through 21 days, there was no differences in the reactors after 35 days. Additional analysis showed that there was no

**Fig. 4** The statistical design of the cRPB experiment where C = cation exchange surface, S = Sand as an inert surface, E = Epoxy as an inert surface, W = week, and R = replicate. [14]
statistically significant difference between the reactors when comparing biomass productivity based on grams per day. [14]

2.3 The Rotating Algal Biofilm Reactor

2.3.1 Logan Christenson:

In 2010 the Logan Lagoon Waste Water Treatment Plant was notified of the requirement to lower the effluent emissions of total nitrogen and phosphorus concentrations within the treated wastewater from 8.3 mg L\(^{-1}\) to 3.0 mg L\(^{-1}\) for nitrogen and 4.1 mg L\(^{-1}\) to 1.0 mg L\(^{-1}\) for phosphorus. To accomplish this, the city was interested in implementing an alga based nutrient removal system. That removal system was designed by Christenson as his MS research, when he designed the Rotating Algal Biofilm Reactor (RABR) [15,16].

The RABR was designed based on existing principles utilized by rotating biological contactors (RBCs), which capitalize on the ability to efficiently grow bacterial biofilms for secondary wastewater treatment. The RBC design was followed by Christenson due to the compact design nature, along with its good gas exchange and high shock load tolerance. The RABR was designed to maintain the good RBC properties while focusing on tertiary wastewater treatment with inorganic carbon-based microalgae growth. This tertiary treatment focus is because the RABR produces a mixture of microalgae and bacteria biofilms instead of the heterotrophic biofilms utilized for secondary treatment in traditional RBC operations.

Once the RABR was designed, it was then tested and optimized to meet the treatment requirements set by Logan City at the Logan Lagoon Treatment Plant. To meet these requirements, both bench scale and pilot scale RABRs were built and experiments were conducted to evaluate the effectiveness based on pre-determined parameters. The major parameters chosen to be monitored for evaluation were biomass production and nutrient removal.
Both parameters were analyzed and compared with a traditional suspended growth microalgae treatment system. This comparison was chosen to show advantages that biofilm-based treatment systems have when compared with suspended growth treatment systems.

Christenson set goals to effectively design a system that was better suited for nutrient removal and biomass production compared to traditional biological based treatment systems. Those goals were: (1) design a reactor capable of reducing the nitrogen concentration levels in the Logan Lagoon wastewater from $8.3 \, mg \, l^{-1}$ to $3.0 \, mg \, l^{-1}$ and reducing the phosphorus concentration levels from $4.1 \, mg \, l^{-1}$ to $1.0 \, mg \, l^{-1}$; (2) test and evaluate multiple growth substrata for optimum biomass growth; (3) design and operate a biomass harvesting system to remove the produced biomass after nutrient removal; and (4) compare the developed treatment system to that of standard suspended culture treatment systems while evaluating the ability of the new system to effectively remove nutrients and produce biomass at a higher capacity than traditional suspended growth systems.

Eight bench scale reactors were used in the substratum test and used when compared to bench scale suspended growth. The reactors were built from 3-inch diameter PVC pipes cut 40 inches long. The pipes were submerged within the wastewater 40% deep by cylindrical diameter and rotated at a speed of 4.8 rpm using 110V AC motors. Two styles, cord and sheet, of growth substratum configurations were chosen to cover the PVC with varying substrata compositions for each style. Nylon, polypropylene, cotton, acrylic, and jute were the substrata chosen to be tested in the cord construction with each cord measured at $\frac{1}{4}$ inches in diameter while polyester, high thread count cotton, and low thread count cotton were chosen to be tested in the sheet configuration.
Once the reactors were built, they were submerged in 8 L of wastewater taken from the Logan Lagoons. Total dissolved nitrogen and total dissolved phosphorus were measured within the water sample, and additional N&P were added in the form of NaNO$_3$ and KH$_2$PO$_4$ to bring the nutrient levels to the Redfield ratio of 16:1 N:P molar ratio. The reactors were operated in a fed-batch mode with N&P added every 48hrs. Plant growth fluorescent lights were used to give an average photosynthetic photon flux density (PPFD) of 170 $\mu$mol m$^{-2}$ s$^{-1}$, which is defined as the measurement of the photons that are exposed to the reactors when, in this case, utilizing a 14 hr. on and 10 hr. off lighting cycle. The water temperature ranged from a daily low of approximately 14°C and a high of 24°C, with an average of 19°C. The evaporated water was replaced daily with deionized water. During the experiment, biomass was only taken from the biofilm and the data collected were normalized using the plan view surface area of the system which was at 0.1858 m$^2$.

In addition to the RABRs, suspended growth cultures were operated in identical tanks under identical growth conditions. The suspended cultures were mixed using dual-blade paddle impellers, which draw 4.4 W of power, identical to the power demands the RABRs place on their motors. Both the paddles and RABRs were rotated without stopping and the overall experiment was conducted for 26 days before harvesting biomass. Water samples were collected every 48 hours for comparisons.
Along with small scale bench testing, Christenson carried out multiple larger scale experiments including the implementation of a pilot scale RABR treatment system on site at the Logan Lagoons. Before pilot scale test were conducted however, experimentation was carried out using medium sized RABRs. For this experiment, two treatment systems were set up in tanks that were 8 ft. long and 4 ft. wide while being 1.3 ft. deep. The first of the two systems was designed to be a traditional suspended biomass treatment system. The tank was filled with 535 gallons of wastewater taken from the Logan Lagoons and circulated using a paddle wheel. The second of the two systems was designed to be a RABR raceway hybrid. The system was assembled the same as the first; however, in addition to the paddle wheel, five RABRs were added to the tank. The RABRs were constructed out of plastic 15-gallon drums, 16 inches in diameter, which were then wrapped with a 350 ft. long 0.25-inch diameter solid braid cotton cord. The reactors were submerged 40%, similar to the bench scale reactors while being rotated at 5.4 rpm. An identical volume of water was added to the hybrid system, and the paddle wheels in both systems circulated the water while spinning at 5.4 rpm.

After the two systems were constructed, a biofilm base was established for 10 days on the hybrid system to simulate operation at the treatment facility. The experiment was conducted for 20 days in August of 2010. During the experiment, 85-90% of the water was removed when the

![Fig. 5 A harvesting mechanism designed to remove biomass while re-spooling the growth substratum onto the reactor. [15]](image)
nutrient levels reached the benchmarks set and centrifuged to harvest suspended biomass in the suspended treatment system the water removed replaced with fresh wastewater at the same volume used at the beginning of the experiment. For the biofilm system, only the biofilm was harvested with the same amount of water being replaced as in the suspended raceway. The biomass was harvested using a harvester, shown in Figure 5, designed where the cord is passed through an adjustable diameter scraper, then through a pulley system, before being rewound onto the reactor. As the cord is being pulled through the scraper, the biomass falls into a collection tank underneath the scraper. The same harvesting mechanism was used when harvesting from the pilot scale reactor.

Along with bench and small-scale testing, a pilot scale RABR was designed and constructed out of two, 6\(\frac{1}{3}\) ft. diameter aluminum wheels. The wheels were placed on a 4-inch aluminum shaft 5 ft. from each other and connected using 10 aluminum strips. The growth substrate chosen to wrap around the strips was cotton cord \(\frac{1}{4}\) inch in diameter. The cotton cord was chosen after showing a greater potential for biomass production and because it was the most cost-effective material available at the large amount needed to completely cover the pilot scale reactor. Once constructed, the RABR was placed in a continuous flow channel and rotated at 1.2 rpm. This channel was 3 ft. deep and 6 ft. wide. Water flowed through the channel at a flow rate of 3 \(\text{gal min}^{-1}\) while water samples were taken 8 ft. apart. The hydraulic retention time (HRT) was 6.0 h.

Following completion of all experiments, a statistical analysis was performed on the results. For the substratum biomass growth studies, polypropylene rope and nylon rope both showed no biomass production for the entire duration of the experiment. Cotton cord was capable of developing a dry weight biomass density of 56 \(g \text{ m}^{-2}\) with an average dry weight
biomass productivity of 2.5 $g \, m^{-2} \, day^{-1}$ which was statistically more than any of the other materials tested in the experiment. It is this statistically better result, which led to the choice of cotton cord as the growth substratum for future research. In the suspension growth comparisons, a growth curve was developed comparing initial biofilms, regrowth biofilms, and suspended cultures (Figure 6). These growth curves showed that the RABR produced biomass at statistically higher yields than the suspended cultures typically used for wastewater treatment. It was also found that the biofilm grew at a much higher rate after the initial biofilm was harvested from the reactor. After harvesting in the RABR raceway hybrid system, the biofilm increased its average density from 58 $g \, m^{-2}$ to 99 $g \, m^{-2}$ after 18 days of operation, which resulted in a productivity of 5.5 $g \, m^{-2} \, day^{-1}$.

When the RABRs in the hybrid system were harvested after 20 days of operation, 390 $g \, m^{-2}$ of biomass was harvested, which gave an average productivity of 20 $g \, m^{-2} \, day^{-1}$. When compared to the system only containing suspended microalgae, with a harvest of 174 $g \, m^{-2}$ and a productivity of 8.7 $g \, m^{-2} \, day^{-1}$, the RABR enhanced system outperformed the suspended growth system. The improved performance included biomass production as well, which was shown in the reduced HRTs of the hybrid system when compared to the suspended system. The shorter HRT of 4.8 days for the hybrid system when compared to 6.3 days of the
suspended system was determined based on when the systems achieved the nutrient removal criteria set at the beginning of the experiments.

For the RABR-raceway hybrid experiment, comparisons were conducted on nutrient removal capabilities that showed much higher removal with the biofilm system when compared to the suspended system. Regarding the pilot scale reactor, 337 g m⁻² of biomass was collected after 12 days of operation giving a productivity level of 31 g m⁻² day⁻¹ for the experiment. With similar nutrient loading as the smaller scale experiments, the higher biomass production level was likely due to other environmental factors such as the seasonal variation. During the experiment, the pilot scale reactor met the total dissolved nitrogen (TDN) requirement of < 3.0 mg l⁻¹ with an average nitrogen level reduction to 1.1 mg l⁻¹. However, the pilot scale reactor failed to meet the TDP requirement of < 1.0 mg l⁻¹ by averaging a TDP of only 1.6 mg l⁻¹. A longer hydraulic retention time was suggested to meet the set TDP criteria. Additional units may have also been utilized to meet the lower TDP criteria but would have been more costly.

An energy balance was carried out for the pilot scale reactor that illustrated a larger energy requirement to rotate the reactor at the set rpm than would be required to rotate a typical paddle wheel in a suspended system (6.3 W m⁻² compared to 0.2 W m⁻²). However, when harvesting and processing energy is considered, the RABR is slightly more efficient when compared to a suspended treatment system (1.4 W m⁻² compared to 1.7 W m⁻²). Additional analysis of the biomass harvested from the RABR showed a solids content averaging 12-16%. This solids content is comparable to centrifuged suspended biomass.

In conclusion, Christenson et al. [15,16] set out to meet four key objectives with his project. He was able to design a reactor capable of reducing the nitrogen concentration levels in
the Logan Lagoon wastewater from 8.3 \(g \text{l}^{-1}\) to 3.0 \(mg \text{l}^{-1}\) and reducing the phosphorus concentration levels from 4.1 \(mg \text{l}^{-1}\) to 1.0 \(mg \text{l}^{-1}\) for the bench scale reactor. He tested and evaluated multiple growth substrata for optimum biomass growth while concluding that cotton rope was the best growth substratum. He also designed and operated a biomass harvesting system to remove the produced biomass, which allowed for biomass harvesting at solid content levels of 12-16%, which is comparable to suspended, centrifuged biomass as well as improving on the energy demand of the RABR at 1.4 W m\(^{-2}\) when compared to a suspended system at 1.7 W m\(^{-2}\). Finally, Christenson compared the RABR treatment system to that of standard suspended culture treatment systems while evaluating the ability of the RABR system to effectively remove nutrients and produce biomass at a higher capacity than traditional suspended growth systems, which he demonstrated utilizing growth curves and energy balances. [15,16]

2.3.2 Terence Smith:

Smith et al. [17] utilized a pilot scale RABR, shown in Figure 7, that was 74 inches in diameter and 60 inches long, with cotton rope as the growth substrate. The reactor was operated outdoors in an approximately 10,700-liter tank containing wastewater taken from the Logan Lagoon treatment facility. For his project, he wanted to accomplish two objectives: (1) develop a predictive model for the growth of algal biofilm biomass on the RABR and (2) develop a predictive model.
of nutrient removal by the RABR for wastewater remediation. The reactors were operated in a continuous flow mode while varying the HRTs at 11 hours and 6 days. The growth was monitored in one-month periods from Aug/Sept 2012 and Oct/Nov 2012 [17].

During the experiment, it was observed that in the warmer time period, the shorter HRT of 11 hours produced more biomass at 700 grams per square meter than the longer HRT of 6 days at 570 grams per square meter in terms of biomass productivity. This is in contrast to the results obtained in the colder time period when there were almost no differences between the two HRTs. After collecting data from the RABR operation, a predictability model of specific microalgae growth rate was developed. This predictability model of $u = u_{max} \times I \times T \times N \times A$ was established using the EPA’s Benthic Algae model as an example. The model incorporated the Steele equation (I), Arrhenius equation (T), Monod equation (N), and logistical area equation (A). Along with the growth model, Smith et al. [17] developed a nutrient uptake model for nitrogen and phosphorus shown in Figure 8. This model not only showed agreement between the predictive removal and observed removal, but also showed a distinct environmental influence on nutrient removal.

**Nutrient removal due to biomass:**

- $\frac{dN}{dt} = -unN \left(\frac{sa}{v}\right) + NFin - NFout$
- $\frac{dP}{dt} = -upP \left(\frac{sa}{v}\right) + PFin - PFout$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Identity</th>
<th>Units</th>
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<tbody>
<tr>
<td>N</td>
<td>Bioavailable nitrogen</td>
<td>mg/L</td>
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<tr>
<td>n</td>
<td>N content of biofilm biomass</td>
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<tr>
<td>P</td>
<td>Bioavailable phosphorus</td>
<td>mg/L</td>
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<tr>
<td>p</td>
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<tr>
<td>F</td>
<td>Flow rate</td>
<td>L/day</td>
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<tr>
<td>Sa</td>
<td>Surface area</td>
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<tr>
<td>V</td>
<td>Volume of tank</td>
<td>L</td>
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</table>

**Fig. 8** Nutrient removal models for the removal of Nitrogen and Phosphorous [17]
2.3.3 Maureen Kesaano:

It has been determined that biofilms are an effective way to treat wastewater while also producing biomass for bioproduct conversion [18,19]. This determination led Kesaano et al. [18] to conduct a study focused on enhancing algal biofilm growth and nutrient uptake during nutrient deplete culturing in which biomass was cultured in a media low in key nutrients such as nitrogen and phosphorus in an effort to increase biomass productivity while also increasing lipid production for biodiesel conversion. This goal was addressed through monitoring the effects of adding dissolved inorganic carbon in the form of 2mM $\text{HCO}_3^-$ to synthetic wastewater for algal biofilm growth [18,19].

Multiple laboratory-scale RABRs were constructed from 10-cm. in diameter plastic wheels onto which cotton rope was attached. The reactors were then rotated at 12 rpm in synthetic, medium strength domestic wastewater at 25°C. Groups of four reactors were placed under growth lights with a photosynthetically active radiation (PAR) of $227 \pm 65 \mu \text{mol m}^{-2} \text{s}^{-1}$ on a 14:10 light:dark cycle. One group of reactors was rotated in wastewater amended with 2mM $\text{HCO}_3^-$ in the form of $\text{NaHCO}_3$ and the other group was rotated in water without the amendment. The reactors were operated on a 5-day HRT for 18 days; then nitrogen stress was induced for another 5 days through nitrogen starvation.
After experimentation was complete, biomass production, along with nutrient uptake and biomass composition, was analyzed. For the bicarbonate-amended reactors, the biomass production mean was 0.18 grams of dry weight biomass per day and the non-amended mean production was 0.20 grams of biomass per day. Additionally, the production rates for the amended and non-amended were 1.45 $g \ m^{-2} \ day^{-1}$ and 1.79 $g \ m^{-2} \ day^{-1}$ respectively. Growth curves were developed, and statistical analysis was conducted, which showed that there were no statistically significant differences in the productivity between the amended and non-amended reactors shown in Figure 9. Finally, analysis of nutrient uptake in comparison to microalgae growth showed a link between the rate of production and nutrient removal. However, there were no significant differences between the reactor groups. [18-20]
2.3.4 Alan Hodges:

Hodges et al. [21,22] utilized bench scale RABRs operating at 20°C in continuous flow with HRTs of 24 and 48 hours while comparing them to an open lagoon system operated at a 36-hour HRT [19]. The reactors were operated in petrochemical wastewater that was collected from an API (American Petroleum Institute) separator. Wastewater characteristics of interest were chemical oxygen demand (COD), total suspended solids (TSS), nitrogen concentration (N), and phosphorus concentration (P). Water samples were taken weekly from the effluent streams for 12 continuous weeks to determine the nutrient removal capabilities.

Data analysis indicated the reactors reduced N, P, and TSS by 72.4%, 50%, and 53.6% for the 24-hour HRT reactors and by 70.8%, 55.6% and 61.3% for the 48-hour HRT reactors. Statistically, the RABRs removed more nutrients than the open lagoons, shown in Figure 10; however, there were no significant differences between the two HRT groups. Additionally, the RABRs did not outperform the open lagoons in COD reduction due to the availability of $CO_2$ in the gaseous phase as a carbon source for the biofilms. [21,22]
2.3.5 Jonathan Wood:

Wood et al [8] utilized bench-scale RABRs operating at 20±2°C with cotton rope as the growth substrate to produce cyanobacteria for phycocyanin extraction. Phycocyanin is a phycobiliprotein pigment found in cyanobacteria that has shown many uses in foods, cosmetics, medicines, and biotechnology.

**Fig. 10** Effluent results for COD, TSS, N, and P for the RABRs vs. the open lagoons. [21]
RABRs were constructed for phycocyanin production. The RABRs were operated in produced water and phycocyanin was extracted from the biomass harvested from the reactor. Phycocyanin content increased as the reactor operation time increased (Figure 11). [8]

2.3.6 Zachary Fica:

Fica et al. [23,24] utilized bench-scale RABRs operating at 7, 17, and 24°C with a 7 day HRT in varying total organic carbon (TOC) concentrations of 300, 600, and 1200 mg l\(^{-1}\) to determine the effects of temperature and TOC on biomass production. The reactors were operated in diary wastewater collected from Utah State’s Caine Dairy Farm evaporation pond. Biomass was harvested weekly to determine productivity. A statistical analysis was used to determine significance of the variables and if the variables interacted in a significant way to influence biomass productivity.

Through ANOVA testing it was shown that the variable interactions did not show any influence on productivity; however, the influence of temperature and TOC concentrations, shown in Figure 12, did show a statistically significant impact on biomass productivity when analyzed individually. In addition to this statistical analysis, Fica [23] was also able to derive a predictability equation to predict biomass production based on water
2.3.7 Jay Barlow:

After recognizing that there is a benefit from growing biomass as a biofilm versus as a suspended culture and recognizing that systems that implement this culturing technique while also integrating wastewater treatment have not been characterized in terms of economic viability and environmental impact, Barlow et al. [25] attempted to accomplish this regarding the RABR. Specifically, they characterized the economic viability and environmental impact of the RABR when coupling an open-lagoon wastewater treatment plant with a RABR facility co-located with a hydrothermal liquefaction (HTL) conversion plant. To obtain data for his models, Barlow conducted tests on HTL conversion of biomass harvested from a RABR.

Barlow et al. [25] conducted a techno-economic analysis. This analysis considered factors including biomass cultivation, biomass harvesting, HTL conversion, and wastewater treatment. In addition to the techno-economic analysis, Barlow et al. [25] also carried out a life-cycle assessment. In this assessment, he used factors such as the net energy ratio and the global warming potential of the designed system.

To make final comparisons, Barlow et al. [25] used a criterion called the minimum fuel selling price (MFSP) to account for all cash flows over the lifetime of the treatment and biocrude oil production facility. This criterion, is the basis of the recommendations for system optimization. These optimizations derive from factors within the system that affect the MFSP.
Barlow et al. [25] were able to identify three major optimization targets that were also identified in the life-cycle assessment, investigated in this project. The three main optimization targets are: (1) RABR operational costs, namely the amount of energy required to operate the reactor, (2) biomass productivity, and (3) HTL feedstock improvement, especially minimization of ash content. [25]

2.4 Summary

In summary, there are many different technologies to cultivate algal biomass with many different reasons to do so. These technologies range from zeolite-based RBC’s to cotton rope RABRs. The cultivation platforms are designed to both remove nutrients and produce biomass while also looking at potential products such as biocrude oil or phycocyanin. A summary of the projects discussed in this review is shown in Table 3.

**Table 3**

Literature summary table

<table>
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<th>Project</th>
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<th>Substratum material</th>
<th>Scale</th>
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<td>Varied (cotton)</td>
<td>Lab &amp; Pilot</td>
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CHAPTER 3

ROTATING ALGAL BIOFILM REACTOR OPTIMIZATION

3.1 Introduction

The Rotating Algal Biofilm Reactor (RABR) is a rotating biological contactor designed to produce algal biofilm while removing nutrients from wastewater. The original design was developed by Christensen et al. [15,16] whose project was discussed in detail in Chapter 2.3 and was built to remove N&P from municipal wastewater taken from the Logan Lagoons [15]. The reactor was built out of plastic barrels (lab scale) and aluminum wheels (pilot scale). Cotton rope was used as the growth substratum after initial growth substratum testing showed that the rope was the best substrate for the design.

The initial RABR design was used primarily as a wastewater treatment platform and not as much for biomass production. This focus was altered by Smith et al. [17] Kesaano and [18] and Kesaano et al. [19] with the focus of increasing biomass production. Along with Smith and Kesaano, Wood et al. [8] used the reactor as a platform to produce biomass for conversion into a high value pharmaceutical. Fica [23,24] used the reactor to produce biomass utilizing dairy wastewater with the focus of biomass production and wastewater treatment. All these projects used the RABR based on the original design of Christenson [15] to achieve their goals, but this design has not been optimized for efficient use. This was pointed out by Barlow et al [25] in a lifecycle assessment (LCA) and techno economic analysis (TEA) for a system that incorporates the RABR with wastewater treatment with the goal of conversion into biocrude oil using a co-located HTL plant [25].
The LCA and TEA indicate inefficiencies within the system and it is these inefficiencies that this project addresses. This project focused on the objective of optimizing the RABR when it comes to the energy required to operate the reactor. This was a key issue identified in the LCA and TEA because the reactor originally was designed using a heavy skeleton and an easily biodegradable growth substrate (cotton rope). This combination proved to be costly. The Reactor was redesigned into a disk configuration utilizing a lightweight polystyrene composite. The disk design is energetically favorable to rotate and increases the growth surface area to wastewater volume ratio volume for the disk RABR when compared with the barrel RABR.

3.2 Materials and Methods

To optimize the RABR, two different reactors were built for comparison. One reactor was built in accordance with Christenson’s original design [15]. This reactor was built of two PVC barrels approximately 19 cm in diameter, 70 cm in length, and covered in cotton pads. The reactor was attached through the middle by a stainless-steel rod to a motor that continually rotated the barrel in the wastewater. The reactor was operated in a raceway that held approximately 175 liters of produced water that was taken from a produced water pond in the Uintah Basin in Eastern Utah. The raceway was divided into two lanes of produced water. The produced water was analyzed by Chemtech Ford, a wastewater analytical laboratory in Salt Lake City, Utah.

In addition to the barrel reactor, a re-worked design was built to compare to the old design where growth substrate was wrapped around a cylinder. A polystyrene slab was obtained from the local Home Depot and cut into disks approximately the same diameter as the barrel reactor, which was 19cm in diameter. From the slab, 23 disks were cut and, using washers on each side secured approximately 11.5cm apart from each other by nuts, and were skewered by an
identical rod as the one used in the barrel reactor. The resulting disk configuration matched the length of the barrel reactor almost identically. Voltage meters were connected to the two reactors to monitor the power consumption over the course of the experiment. The comparison study was conducted for approximately 13 days in August 2016.

Along with the reactor design comparisons, energy consumption experiments were carried out on the polystyrene reactor to determine if the rotational speed of the reactor affected the biomass production and, if so, would lowering the speed of the reactor in an effort to lower energy cost of the system negatively affect the biomass production. To accomplish this experiment, an additional 23 disks were cut and assembled in an identical fashion as the previous reactor. The new set of disks were placed in the same raceway as the older disks in order to operate them in identical environments. The reactors were then rotated at 0.5, 1, 2, and 5 rpm and the rpm were assigned at random. Energy required to operate the reactors was monitored using the voltage meters and the reactors, which operated for approximately 29 days from mid-September 2016 to mid-October 2016 and mid-October 2016 to mid-November 2016. The biomass was collected and weighed at the end of the experiment and statistical analysis was conducted on the results to determine the effect of rotational speed on biomass productivity. Reactors used in experiments are shown in Figure 13. The reactors were housed in a greenhouse, which was temperature controlled with a heater and the growth substrate for both reactors were rotated perpendicular to the path of the sun.
3.3 Results and Discussion

After both the barrel and disk reactors were operated at 1 rpm for approximately 350 hours each, the barrel reactor consumed 7.78 MJ of energy while the disk reactor only consumed 4.54 MJ of energy. This is a large difference between the two reactors and is expected to increase as the reactors collect more biomass. The difference is considered to be caused by the additional energy that the larger and heavier barrel reactor requires to rotate within the water.

After the comparison tests were carried out, the additional lane of disks was installed, and the RPM trials were conducted. In the first set of trials the reactors rotated at 1 and 2 rpm for a total of 677 hours. The reactor rotating at 2 rpm consumed 9.14 $Wm^{-2}$ of energy, while the reactor rotating at 1 rpm was consumed 9.51 $Wm^{-2}$ of energy. This result seems counter intuitive because the slower rotating reactor consumed more energy, however, this extra energy

**Fig. 13** From left to right – PVC barrel reactor using cloth pad as the growth substrate; Polystyrene disk reactor consisting of 23 disks for comparison to barrel reactor; side by side polystyrene reactors used to determine effect of reactor rotation speed on biomass productivity.
requirement can be accounted for when considering the difference in biomass productivity. For the 2-rpm reactor, 357.7 g of wet biomass was collected with an average solids content of 13.4% was produced resulting in approximately 47.9 g of dry biomass. The 1-rpm reactor produced 449.52 g of wet biomass with an average solids content of 13.1% was produced resulting in approximately 58.9 g of dry biomass. The extra approximately 100 g of wet biomass distributed over the 1-rpm reactor is increasing the weight of the reactor enough to require more energy used by the reactor. When comparing the productivity of the two rotational speeds regarding dry biomass yield per watt consumed by the reactor, while the 1-rpm reactor does consume more energy with 6.78 W when compared to the 6.52 W consumed by the 2-rpm reactor, the 1-rpm reactor produces at a higher yield of 8.69 grams of dry biomass per watt when compared to the 7.35 grams of dry biomass per watt produced by the 2-rpm reactor.

Statistical analysis was carried out on the RPM trials to determine which factors affecting the biomass productivity of the reactor were significant. The factors considered were the combination of the side of disk harvested (east facing side vs. west facing side) and the disk position within the reactor, the combination of the rpm and the disk position, the combination of the rpm and the side harvested, and the individual factors of side, position and rpm of the reactor. These factors were analyzed (Table 4) using the statistical program SAS and it was shown in the rows containing multiple factors that none of the established combinations were significant because the p-values in the far-right column for those rows are greater than 0.05. This insignificant result for the combination of the factors allows for the analysis of the individual factors. For the effects of the individual factors on biomass productivity, side of the disks harvested for both rows was not significant whereas both the rpm and the disk location within the produced water reservoir were significant at p-values of 0.0087 and <0.0001 respectively, since
those values are less than the significance criteria of 0.05. With a statistically significant result for the rpm factor it is then determined that the productivity of the reactor is most effected by the rotational speed of the disks and that the productivity is statistically significant between all the rotational speeds. In addition, the disk position is most likely explained by inadequately mixed nutrients within the wastewater and can be better handled with a small pump to slightly agitate the system. The significance of the rpm is rather encouraging and requires more study.

Table 4
SAS analysis of RPM trials conducted for the polystyrene disk RABRs. Factors tested were the speed the disks were facing (rpm), the side of the disk harvested (direction) the combination of the rotation speed and side harvested (rpm*direction), the location of the disk in the water (disk), the combination of location the disk and how fast it was rotated (rpm*disk), and the combination of the location of the disk and what side was harvested (direction*disk). Significant factors were rpm and disk.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpm</td>
<td>1</td>
<td>0.47967953</td>
<td>0.47967953</td>
<td>23.54</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>direction</td>
<td>1</td>
<td>0.00435543</td>
<td>0.00435543</td>
<td>0.21</td>
<td>0.6484</td>
</tr>
<tr>
<td>rpm*direction</td>
<td>1</td>
<td>0.02577001</td>
<td>0.02577001</td>
<td>1.26</td>
<td>0.2728</td>
</tr>
<tr>
<td>disk</td>
<td>22</td>
<td>1.27699140</td>
<td>0.05804506</td>
<td>2.85</td>
<td>0.0087</td>
</tr>
<tr>
<td>rpm*disk</td>
<td>22</td>
<td>0.88377633</td>
<td>0.04017165</td>
<td>1.97</td>
<td>0.0594</td>
</tr>
<tr>
<td>direction*disk</td>
<td>22</td>
<td>0.22563888</td>
<td>0.01025631</td>
<td>0.50</td>
<td>0.9425</td>
</tr>
</tbody>
</table>

To further analyze the effect of rpm on biomass productivity, another trial was conducted at 0.5 rpm and 5 rpm. The reactors were operated for 686 hours in identical environments. The 5-rpm reactor was observed to consume 32.11 MJ (13.0 Wm^{-2}) of energy, while the 0.5 rpm reactor was observed to consume 7.63 MJ (4.33 Wm^{-2}) of energy. When comparing biomass production, it was found that the 0.5 rpm reactor produced approximately 130.65 g of wet biomass with an average solids content of 13.5% resulting in approximately 17.6 g of dry biomass.
biomass, while the 5-rpm reactor produced 505.78 g of wet biomass with an average solids content of 13.2% resulting in approximately 66.8 g of dry biomass being produced. These production values show a significant difference between the two reactors. However, when productivity is compared to the 1 rpm reactor, a difference of 56.26 g of extra wet biomass while consuming an extra 15.59 MJ of energy does not appear to be worth the potentially slightly higher productivity. The productivity isn’t higher however when the two reactors are compared regarding grams of dry biomass produced per watt used by the reactors. For the 5-rpm reactor, 5.14 grams of dry biomass was produced for every watt used by the reactor, which is higher than the 4.07 grams of dry biomass produced per watt by the 0.5-rpm reactor, but not higher than the 8.69 grams of dry biomass produced per watt by the 1-rpm reactor. This result suggests that there is a potentially optimal operating speed when considering biomass produced versus energy consumed by the reactor.

3.4 Conclusions

The focus of the experimentation was to address the issue of energy demand associated with the operation of the RABR when growing biomass for biocrude production. The difference in energy requirements between the original RABR design and the improved disk design was shown to be substantial due to the heavier materials of PVC and cotton rope of the original design compared with the lighter polystyrene material. Additionally, the improved design utilizes a higher growth substratum surface area to water ratio that improves reactor efficiency over the original design. Once it was shown to be a more efficient design when compared to the original design, additional tests were carried out to determine if the speed of rotation for the reactor could be reduced without losing biomass productivity.
The rotational trials were carried out using two lanes of identical polystyrene disks in identical growth environments. The first trial conducted compared 1 and 2 rpm rotational speeds and found that there was a statistically significant impact on productivity, with the 1-rpm disks producing more biomass than the 2-rpm disks. To further analyze the effects of rotation, a second trial was conducted at 0.5 and 5 rpm. This second trial found that there was a statistically significant difference in biomass productivity for the two reactors rotating at different rotational speeds; however, when the higher productivity of the 5 rpm in trial 2 was compared to the slightly lower production of the 1 rpm in trial 1, the slight increase of 11% in total biomass production using a faster rotation was not favorable when considering the much larger increase in energy demand. This is made apparent in the dry biomass per watt data for the reactors (Table 5). We can then conclude that, with the optimization goal of decreasing operation energy cost proposed by Barlow et. al. [21], the RABR can be operated at a reduced rotational rate in order to lower energy demands while maintaining comparable biomass productivity with the most optimal rotational speed set at 1 rpm.

Table 5
Reactor rotational speed trials for rotation speeds of 0.5 rpm, 1 rpm, 2 rpm, and 5 rpm

<table>
<thead>
<tr>
<th>Speed</th>
<th>Operational Time (h)</th>
<th>Watts (W)</th>
<th>W/m²</th>
<th>Dry Yield (g)</th>
<th>g (dry)/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 RPM</td>
<td>686</td>
<td>3.09</td>
<td>4.33</td>
<td>17.6</td>
<td>4.07 ± 0.87</td>
</tr>
<tr>
<td>1.0 RPM</td>
<td>677</td>
<td>6.78</td>
<td>9.51</td>
<td>58.9</td>
<td>8.69 ± 0.42</td>
</tr>
<tr>
<td>2.0 RPM</td>
<td>677</td>
<td>6.52</td>
<td>9.14</td>
<td>47.9</td>
<td>7.35 ± 0.31</td>
</tr>
<tr>
<td>5.0 RPM</td>
<td>686</td>
<td>13.0</td>
<td>18.2</td>
<td>66.8</td>
<td>5.14 ± 1.2</td>
</tr>
</tbody>
</table>
CHAPTER 4

BIOMASS CHARACTERIZATION AND ENVIRONMENTAL CORRELATIONS

4.1 Introduction

The RABR is a versatile reactor capable of removing nutrients from wastewater, while utilizing those nutrients for biomass production. The biomass produced can be converted into valuable products such as pharmaceuticals, biocrude, biogas, and animal feed. Ideally, the reactor would be operated in the best possible conditions to produce the most biomass regarding RABR biomass production, it is important to analyze the environmental factors associated with the production system. These factors may include water nutrient levels, water pH, water temperature, ambient air temperature, and photosynthetically active radiation (PAR). The goal of biomass production is to operate the production system in the most optimal environment available for the highest productivity.

Barlow et al. [25] suggested in their TAE and LCA that the biomass productivity of the RABR should be increased for a more effective system. Other researchers have attempted to increase the productivity including Smith et al. [17] who modeled biomass productivity of the RABR using municipal wastewater and Kesaano et al. [18] who attempted to correlate biomass productivity with wastewater nutrient levels. Fica [23] focused on optimizing the RABR biomass productivity when coupled with dairy wastewater. All these projects achieved interesting and promising results that establish the need for experimentation to analyze environmental correlations with biomass productivity.

This study involved correlating environmental factors that may impact biomass productivity of a mixed culture of novel strains of biomass being grown on RABRs utilizing
produced water as the nutrient source. The strains were isolated from the Logan Lagoons
Wastewater Treatment Facility in Logan, Utah and the Great Salt Lake in Salt Lake City, Utah.
The factors of interest were wastewater pH, waste water temperature, ambient air temperature,
and PAR. This analysis was conducted to investigate optimizing the production of this mixed
culture when grown in produced water.

4.2 Materials and Methods

During experimentation, two strains of biomass that were previously identified as capable
of withstanding the highly saline growth environment of produced water were combined into one
mixed culture. One strain was isolated from cultures taken from the Lagoons Wastewater
Treatment Facility in Logan, Utah and the other strain was taken from Great Salt Lake in Salt
Lake City, Utah. They were then sampled and isolated to extract DNA for DNA analysis using
Polymerase Chain Reaction (PCR). Samples of the biomass were frozen at -80 °C for several
hours, thawed at room temperature, and vortexed to disrupt cell walls to release the DNA. This
process was repeated three times to ensure adequate DNA was available for PCR amplification.
The PCR reaction mixture consisted of 29.5 µL H₂O, 5.0 µL 10X buffer, 8.0 µL 25 mM MgCl₂,
2.0 µL dNTPs, 1.0 µL DMSO, 1.0 µL each of 50 mM forward and reverse 23S primers, 0.5 µL
TAQ polymerase, and 2.0 µL DNA template taken from the supernatant of the centrifuged DNA
samples. A 1% agarose gel was mixed for gel electrophoresis to confirm algal DNA. DNA
samples were then concentrated and sent to the Genomics Laboratory in the Center for Integrated
BioSystems at Utah State University for DNA sequencing. The resulting sequences were then
imputed into the NCBI blast database to check against previously sequenced DNA.
In addition to genetic characterization, the biomass was grown in flasks for use in RABRs as seed inoculum for biomass production experimentation. Four sets of 500mL flasks were filled with either 200mL each of BG11 growth media, BG11+1% NaCl, produced water, or produced water that had been aerated for five days (Figure 14), with 0.6g of nitrogen (\(\text{NaNO}_3\)) and 0.103g of phosphorus (\(\text{K}_2\text{HPO}_4\)). The flasks were fitted with hanging cotton rope and inoculated using biomass samples taken from the Logan, Utah Wastewater Treatment Facility and the Great Salt Lake in Salt Lake City, Utah. The biomass matured over several weeks within the flasks and then were inoculated in the larger RABRs such as the ones used in the experiments described in Chapter 3. Once inoculated in the reactors, environmental factors were monitored during two different growth trial periods taking place during August 2016 and during late September through early October 2016 using temperature, PAR, and pH probes. The probes were attached to a Campbell Scientific data logger that recorded values regularly during experimentation. These values were then used for correlation to biomass productivity.
4.3 Results and Discussion

After isolating DNA of the biomass samples through PCR amplification, gel electrophoresis was conducted to confirm the DNA length at the expected 400bp shown in Figure 15. Samples of DNA with an average concentration of 16.6 nanograms of DNA per microliter were sent to the Genomics Laboratory in the Center for Integrated BioSystems at Utah State University. The results of the sequences were then BLASTed against NCBI databases, which showed that the samples were novel sequences not previously identified for both alga types. These sequencing results confirmed the sequencing results of LLC2 carried out previously by Wood et al. [8] and additionally led to identifying a new strain isolated from the Great Salt Lake. Further analysis under the microscope showed shared similarities between the two species. These similarities,

**Fig. 15** Gel electrophoresis results showing the sample DNA having a base pair length of 400 bp.

**Fig. 16** Microscopic images at 40X magnification of LLC2 on the left and GSL on the right. The images show the similarities of the two species.
shown in Figure 16, are filamentous cells and the blue green nature of a possible cyanobacterium with GSL showing a very close sequencing match to a cyanobacterium isolated out of saline waterbodies in Australia [26]. This is promising because like the LLC2 analyzed by Wood et al. [8], the GSL may contain phycocyanin, which is a valuable product that can be used such as cosmetics, food, medicine, and biotechnology [27-29].

After the biomass was inoculated on the RABRs, the reactors were operated for several months and environmental data collected using the Campbell Scientific data logger. These values, shown in Appendix 1, resulted in an average water temperature of 20.5 °C with a range from 15.5 – 28.5 °C, an average pH of 9.3 with a range from 7.5 – 9.9, and an average PAR density of 112.4 μmol m⁻² s⁻¹ with a range from 0 – 900 μmol m⁻² s⁻¹ for the first trial which had a total operation time of 311 hours. An average water temperature of 19.6 °C with a range from 14.9 – 26.7 °C, an average pH of 8.3 with a range from 5.8 – 9.6, and an average PAR density of 84.8 μmol m⁻² s⁻¹ with a range from 0 – 715 μmol m⁻² s⁻¹ was observed for the second trial which had a total operation time of 686 hours. When compared to the environmental conditions of two trials, the trials are similar when it comes to pH and water temperature as expected since the reactors were in a temperature controlled green house.

Additionally, when looking at the average PAR experienced within the two trials, it is found that the larger levels of PAR in the first trial is to be expected since the trial occurred during a growth period during the late summer month of August that was higher in solar availability when compared to the second trial which had a growth period during the early fall months of late September and early October. This increase in PAR may also have a positive impact on the biomass productivity since the biomass uses photons as a key factor in growth. This was evident in the results of the two trials when 807 g of wet biomass was harvested from
the first and 636.43 g of wet biomass harvested from the second trial. This 22% higher production in the first trial can be contributed to the higher levels of PAR observed during that growth period. The difference in PAR between the two trials is not significant enough for concern, however, when conclusions are drawn for comparing the trials that took place at different growth periods in Chapter 3. To further optimize the reactor, operate the reactor in the summer months while the PAR is at its peak and at 1 rpm to reduce the energy demand.

4.4 Conclusions

To develop a system that can produce biomass on produced water for biocrude conversion, it was required that robust strains of biomass are needed to grow and thrive in the harsh environment of produced water (Table 2, Chapter 2). Two strains of biomass were identified, are from the Logan Lagoons (LLC2) and one from the Great Salt Lake (GSL) that could be grown in biofilm form on produced water. The DNA of these strains was amplified using PCR and the resulting sequences were analyzed and compared to the database of sequenced microalgae cultures in NCBI databases. The strains were unique using DNA identification and they were mixed together in order to increase the productivity when utilized on the RABR in produced water. This was done in hopes to maximize the culture’s ability to grow in produced water, which contains high levels of salinity and other contaminants.

In addition to the biomass characterization, environmental factors were observed and compared during the RPM trials. PAR, average water temperature, and average water pH levels were compared between the two trials to determine if they had an impact on biomass production. Of the three factors, only the PAR values differed enough from each other to merit concern and this was due to it being the least controlled factor. The higher PAR level in Trial 1 led to a 22% higher overall biomass productivity, which was shown when the first trial produced an overall
higher amount of 807 g of wet biomass than the second at 636.43 g of wet biomass. This leads to the conclusion that in order to address the optimization criteria of increasing biomass productivity, operate the reactor during the summer months when PAR is most intense.
5.1 Introduction

Hydrothermal Liquefaction (HTL) is the process that uses high pressure and temperature to convert wet biomass into biocrude oil which then can be further refined into various types of fuels. This process is unique because of the wet nature of the feedstock. Traditional biocrude conversion techniques such as pyrolysis require a dried biomass for biocrude conversion. Drying the feedstock is a time and energy intensive process requiring additional feedstock processing steps to separate the water from the biomass, which is traditionally grown using a paddlewheel driven raceway growing the biomass in suspension in the water. To do this separation, the biomass is either centrifuged or processed through a flocculation system, which are both energy intensive processes. Additionally, when biomass is grown in suspension, the biomass is produced at a low solids concentration, which means not enough biomass is being produced to meet the demands for biocrude production.

One method for solving this issue is growing the biomass in a biofilm form using the RABR. Once the biomass is ready for harvesting, the biomass is manually scraped off the reactor, and no separation step is necessary. Coupling this biofilm reactor with a conversion system capable of handling wet feedstock, such as HTL, removes the need to further process the biomass after harvesting. This system allows for more efficient production of biocrude oil shown in Figure 17.

This method of biomass cultivation for biocrude conversion was analyzed by Barlow et al. [25] in their TEA and LCA analyses to determine the validity of such a system. It was shown
that though it is possible, it is not economically feasible with the current operating parameters. One suggestion taken from his analysis is to improve the feedstock quality taken from the RABR to increase the biocrude productivity of the HTL. More specifically it was recommended to reduce the ash content of the feedstock. That is, reduce the amount of salt and other inorganics that do not convert into biocrude oil. It is this suggestion that is addressed in the following section.

The experiments were conducted to improve the feedstock quality by decreasing the salinity of the biomass. This decrease in salinity is important because by decreasing the salinity of the feedstock there is an increase in organic content available within the feedstock to convert into biocrude oil. This decrease was achieved through manual washing to remove the salts and inorganic compounds that may have accumulated in the high salinity environment that is produced water. The feedstock was then converted into biocrude oil for comparison against biocrude oil produced using feedstock biomass that was not washed.
5.2 Materials and Methods

Mixed culture biomass was produced using a RABR operating in produced water. The biomass was analyzed for solid and ash content using ovens at 105 °C and 550 °C respectively. The biomass was then converted into biocrude oil using a 500-ml HTL pressure reactor, shown in Figure 18, utilizing a Parr 4520 controller. The operating parameters were determined by Barlow et al. [21] to be within the range of identified optimum values.

To reduce the ash content within the biomass feedstock with the goal of increasing biocrude production, a wash procedure was carried out. Biomass was harvested from the RABR that was grown in produced water. The biomass was filter washed 2 times through a Buchner funnel using deionized water and the filter cake was re-suspended in deionized water in equal parts to maintain solids content between the washed and unwashed samples. Samples of the biomass were taken before and after washing and baked at 103 °C to determine solids content and 550 °C to determine ash content. Additionally, using a hand-held salinity probe, salinity measurements were taken of the feedstock before and after washing to determine effectiveness of the wash.

The wet biomass feedstock was loaded into the vessel with the headspace vented and pressurized to 2MPa with nitrogen. For the reaction, the reactor was heated to 325 °C ± 6 °C at an average rate of 7.6 °C per minute for a 60-min retention time once the reaction temperature
was reached. During the reaction, the pressure of the vessel ranged from 14.5-16.2 MPa. After the reaction was complete, the reactor was cooled to 40 °C using an internal water loop and then vented.

Following completion of the reaction, the contents of the vessel were extracted using an equal volume of dichloromethane. The mixture was manually agitated for 3 minutes and then centrifuged for 10 minutes at 3700 x g. After decanting, the aqueous and nonpolar phases were filtered separately. The solid phase was resuspended in dichloromethane, centrifuged, decanted, and filtered twice more to ensure full biocrude recovery.

5.3 Results and Discussion

After the first conversions were carried out using feedstock that had not been manually washed, the biocrude yield was obtained. It was shown that with no alteration to the feedstock, an average biocrude yield of 34.9 % afdw of the total yield was possible. This yield is significant considering the biomass feedstock used had an average ash content of 50 %, which is important because ash is the component of the feedstock that is unavailable for biocrude conversion. To address this high average ash content the biomass feedstock was subjected to a manual washing attempting to remove and reduce the built-up of ash on the surface of the cells.

The feedstock was hand washed to limit energy cost within the system to maintain the advantage the biofilm-based reactor has over suspended growth. This hand washing technique was used to limit energy demand within a washing system while also being an experimental proof of concept. Following washing, the feedstock was analyzed for ash composition and compared to samples that had not been washed. The washing technique was not effective however, with the washed samples having almost identical ash compositions when compared to
the unwashed samples at an average of 53%, even though when measuring the salinity levels of the feedstock, the salinity concentration decreased, shown in Table 6, from 68.5 mS to 47.9 mS. This difference in salinity is a 30% decrease in the salinity level of the washed feedstock when compared to the original feedstock. This result leads to a conclusion that a majority of the ash is most likely located inside the microbial cells of the feedstock. Without reducing the feedstock ash quantity, the wash step would be unsuccessful at increasing the HTL biocrude production.

### 5.4 Conclusions

After biomass was cultured and harvested using the RABR in produced water, the biomass was used as feedstock for HTL conversion into biocrude oil. Using biomass feedstock with 50% ash content, an average biocrude yield percent in ash free dry weight was 34.9%. To address the optimization criteria stated by Barlow et al. [25] of increasing the biocrude yield through feedstock improvement, feedstock biomass was subjected to a washing step. This washing step was focused on reducing the sorbed salinity of the feedstock without adding more energy to the system. The wash was unsuccessful, leading to the conclusion that the majority of the ash content of the feedstock is found inside the cell, despite a wet feedstock salinity reduction of 30%. To address this, it could be suggested to alter the wash by first soaking the feedstock in

### Table 6

Feedstock salinity levels during manual washing.

<table>
<thead>
<tr>
<th>Number of washes</th>
<th>Feedstock Salinity of washwater (mS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>68.5</td>
</tr>
<tr>
<td>1</td>
<td>53.6</td>
</tr>
<tr>
<td>2</td>
<td>47.9</td>
</tr>
</tbody>
</table>
deionized water overnight which would potentially allow the cells to flush out the ash within the cells as well as remove the ash on the surface.
CHAPTER 6

SUGGESTED FUTURE WORK

To develop a system capable of microalgae-based biomass growth utilizing a RABR rotating in produced water with the goal of HTL biocrude production, it was important to address key issues such as operational costs, environmental conditions, and feedstock composition to reduce the overall production costs of the biocrude and increase the value of the final product. The experiments carried out addressed these issues with various levels of success, but additional suggestions can be made to optimize this system.

Addressing the new design, further experiments should be conducted to determine if there are inexpensive materials that are durable at larger scale than the reactor substrate used in these experiments and to test more RPM trials. The polystyrene performed adequately at this scale but as its size is increased, it will become less stable as a growth platform by becoming less rigid and could pose a problem. Further experimentation needs to be conducted to determine if there are comparable materials that produce biomass at relatively the same rate while keeping the energy demands low. Additionally, for RPM trials, experimentation could include adding short periods at decreased reactor speed or even stopping rotation to further reduce energy requirements.

For addressing the environmental impact on the biomass productivity, the effects of PAR and water temperature characteristics during non-optimal growing periods such as winter should be investigated. Further research would incorporate a statistically rigorous experimental design for determining how significant each of the factors are at influencing biomass productivity. This
experimental design can also focus on the individual biomass strains to determine the most optimal growth conditions.

Finally addressing the feedstock quality for HTL conversion, additional experimentation should be conducted to explore options to better analyze the feedstock to determine the amount of ash on the surface vs. inside the cells. Additionally, experimentation on growth conditions that reduce the dust in the air that would settle on the biomass would be advisable because dust settling on the biomass would increase the ash content of the biomass feedstock. Along with feedstock quality it would be beneficial to investigate optional avenues to improve the value of the biocrude such as recycling the aqueous phase taken from the conversion, which is high in nutrients needed, to produce more biomass. This added recycle stream could have the possibility of increasing the productivity level of the biomass within the RABR.
REFERENCES


Appendix A – Environmental Data for Growth Trials

Appendix A-1 Average water temperature (Celsius) of growth trial 1 (Sept. 29, 2016 through Oct. 09, 2016)
Appendix A-2 Average water pH of growth trial 1 (Sept. 29, 2016 through Oct. 09, 2016)
Appendix A-3 Average photosynthetically active radiation (PAR) density of growth trial 1 (Sept. 29, 2016 through Oct. 09, 2016)
Appendix A-4 Average water temperature (Celsius) of growth trial 2 (Oct. 15, 2016 through Nov. 05, 2016)
Appendix A-5 Average water pH of growth trial 2 (Oct. 15, 2016 through Nov. 05, 2016)
Appendix A-6 Average photosynthetically active radiation (PAR) density of growth trial 2 (Oct. 15, 2016 through Nov. 05, 2016)