THE CO-CULTIVATION OF RICE AND ALGAE TO IMPROVE PROCESS ECONOMICS FOR ALGAL BIOFUEL PRODUCTION

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Agricultural and Biological Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

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Abstract

Fossil fuels are becoming more and more scarce as the demand for them increases with an increasing population. One way to meet these demands is by increasing the production of fuels from algae. Algal biofuels are currently limited by high costs for land, cultivation ponds, nutrients, and labor resulting in a total cost of \$10.9 per gallon of gasoline equivalent (gge^{-1}) according to Lundquist et al., (2010). These can be mitigated by integrating the cultivation of algae with rice and/or by using wastewater as a source of nutrients for rice and algae production. Some algae growth already occurs naturally within a rice field, and it serves as a fertilizer for the rice. This study proposes a co-cultivation system where the growth of algae is actively encouraged and then harvested and processed into a biofuel. Improvements in cost were made by using a plastic HDPE tarp as a way to make harvesting of algae easier, decreasing the overall volume for tanks, not supplying aeration, processing wet biomass into fuel via hydrothermal liquefaction, and using the same land that rice is being grown on. These changes result in a feedstock cost of \$7.60 gge^{-1} before wastewater treatment credits. The amount of fuel potentially produced by this system was also investigated, experimentally. These experiments also quantified the simultaneous treatment of wastewater and the amount of rice grain produced in a co-cultivation system. The experiment was conducted with 4 treatments or approaches to rice cultivation and with 4 replicates of each treatment. The first, the baseline treatment: Water Plus No Plastic with a yield of 7.7 metric tons $hectare^{-1}$. Another treatment tested the effect of an HDPE tarp: Water Plus Plastic which had a yield of 8.7 metric tons $hectare^{-1}$. An additional treatment tested the effect of having wastewater and algae grow together: Swine Manure Lagoon Effluent Plus Plastic which had a yield of 7.9 metric ton $hectare^{-1}$. The final treatment demonstrated the E2 Energy Process by integrating a wastewater produced during the experiment: Swine Manure Lagoon Effluent/Post Hydrothermal Liquefaction Wastewater Plus Plastic which had a yield of 10.3 metric tons $hectare^{-1}$. All results were analyzed using ANOVA and post-hoc analysis was conducted using least significant difference (LSD) and Tukey's honest significant difference (HSD) tests. These experimental results were used to determine the effect of co-cultivation on the rice yield. LSD analysis showed that the Swine/PHWW Plus Plastic treatment was statistically different compared to the baseline treatment of Water Plus No Plastic while HSD analysis determined it was not statistically different. Overall, it was determined that yields were at worst the same while there is potential for an increase in yield by using a Swine/PHWW mix since the LSD test determined significance. A credit for wastewater treatment is included and divided between the amount of nitrogen and BOD removed. The experimental results showed that the algae could remove up to 99% of ammonia, 92% of nitrate, and 90% of COD. This results in an overall treatment credit of \$4.50 gge^{-1} which means the total cost for biofuel production decreases to \$3.10 gge^{-1}. The amount of land area devoted to rice cultivation is large, and if this co-cultivation system was implemented in every rice field around the world, 36% of the world's crude oil supply could come from algae grown in co-cultivation systems. This is a significant portion of the oil supply as biodiesel is currently providing less than 1% of the United States oil supply. A co-cultivation system like this could address multiple broad societal issues including the production of cost-effective biofuels on a large scale and improved water quality in agricultural areas.

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Chapter 1

Introduction

1.1 Motivation

1.1.1 Energy Security

As the world's population increases, so does the demand for transportation fuels. Today, transportation fuels come almost entirely from non-renewable petroleum sources. This means that the world will eventually run out of these fuels. Estimates range from 50-100 years left of oil (Nashawi et al., 2010). This fact poses a long-term supply problem with an increased demand for these same fuels.

1.1.2 State of Biofuels

One way to mitigate the problem of finite and unsustainable petroleum based fuels is by using biofuels. The US government has mandated through the Renewable Fuels Standard 2 that 36 billion gallons per year of renewable fuels have to be produced in the US by the year 2022 (US Environmental Protection Agency, 2010). 21 of the 36 billion gallons is supposed to be produced from advanced biofuels defined as a biofuel that can reduce a minimum of 50% greenhouse gas emissions when compared to gasoline (US Environmental Protection Agency, 2010). Advanced biofuels include cellulosic, biodiesel, and other advanced biofuels including algae. 16 billion gallons of the 21 billion for advanced biofuels were supposed to be produced from cellulosic biofuels, but the cellulosic industry has failed to meet their quotas since the enactment of the standard such that the quotas have been adjusted to match the actual production. For example, as of 2013, the US was able to produce 0.8 million gallons of cellulosic fuels (US Environmental Protection Agency, 2014) which is only 0.005% of the goal of 16 billion gallons. This suggests that other ways of producing advanced biofuels should be looked at. One option is to produce various kinds of fuels from algae. There are multiple ways to produce liquid fuels from algae. One is via the process of hydrothermal liquefaction. The challenge with making biofuels from algae is the current cost of conventional approaches to producing algal biomass can be quite high (>$1000/ton) and this results in fuels that are not cost competitive with traditional petroleum fuels. Biodiesel made from algae can be as expensive as $405 barrel^{-1} (Chisti et al., 2007). Current crude oil costs $50 barrel^{-1}, but can range from $30-140 barrel^{-1} according to the US Energy Information Administration (Energy Information Association, 2016). Without a mandate, algal fuels will not be used in the near future since they cannot compete with non-renewable fuels like crude oil at current price points.

1.1.3 Need to Improve Algae Cultivation Economics and Scale

It is essential to consider ways to improve the economics of producing algal fuels. A comprehensive breakdown of the capital costs associated with contemporary algal biofuel production using high-rate raceway ponds was provided by Lundquist et al., (2010) as shown in the figure below.

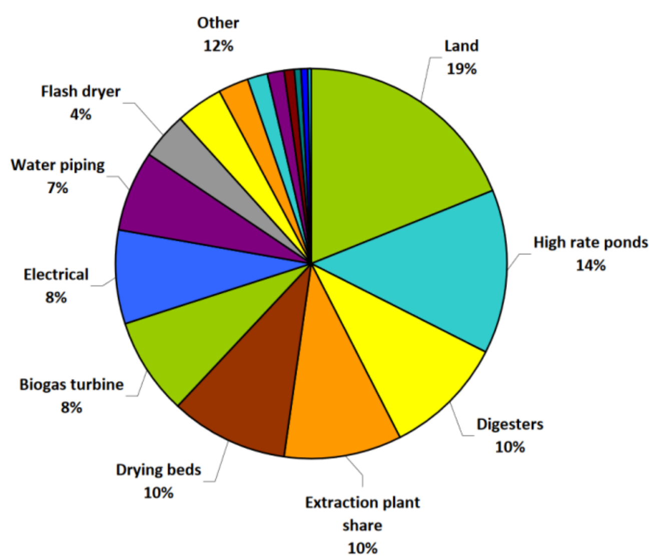

Figure 1-1: Capital Cost Components (from Lundquist et al., 2010)

This figure shows that the largest percentage of the capital cost in producing algal biofuel is for land followed by the construction of high rate ponds which consist of plastic-lined ponds with spinning wheels used to grow the algae/bacteria biomass mixture (ABM). Rice cultivation has a significant amount of land devoted to it, and much of this land has berms and soil types that facilitate shallow ponds similar in depth to high-rate algae ponds. Thus, co-cultivation of rice and algae offers significant potential for cutting the costs for land and ponds, which could have a large impact on the overall cost of algal biofuels.

Additionally, it is too costly to add nutrients to a system to grow algae. Addressing these two key factors could significantly decrease the cost of algal fuel production close to that of non-renewable petroleum. One option to solving the nutrient problem is to use wastewater. Wastewater has a high nitrogen and phosphorous content, two nutrients essential for algae growth. Wastewater treatment plants pay \$8,130 ton^{-1} of nitrogen removed and \$49,500 ton^{-1} of phosphorous removed (Hey et al., 2005). Fortunately, algae can use the nitrogen and phosphorous to grow and essentially treat the wastewater. The wastewater provides free nutrients for algae while also providing an additional revenue stream for algal fuel production due to the economic credit received from treating wastewater.

In addition to the higher cost for algal biofuels, it is also hard to produce the fuels on a large scale. According to the Lundquist et al., (2010) study, algal biofuels can only achieve up to 1% of total US liquid fuel consumption due to their constraints for land, light, temperature, and CO_2 source. Therefore, even if the cost was reduced to compete with conventional fuels, the amount of land viable for algal fuel production is limited and would not contribute significantly to US liquid fuel consumption.

1.1.4 Land Use

The largest cost factor is land use since a lot of land will be required to produce a lot of fuel. Therefore, it makes sense to look at things produced around the world at a large scale. Table 1-1 illustrates these commodities.

Commodity	**Production (in metric tons)**
Sugar Cane	1,899,991,846
Maize	1,021,616,583
Milk	758,222,163
Rice	740,955,973
Soybean	308,436,056

Table 1-1: Global Commodity Production (Food and Agriculture Organization of the United Nations Statistics Division, 2016)

From this table, it is evident that rice is produced on a large scale. According to the Food and Agriculture Organization of the United Nations, as of the year 2016, 740,955,973 metric tons of rice per year are grown making it the third most produced crop in the world next to sugar cane and maize. Since a large amount of land is needed to provide the world rice grain, the costs associated with production including that for land is justified as rice would not be produced in this amount if it was not profitable. Rice also needs a massive amount of standing water to grow. This standing water will naturally grow microorganisms including algae when in the field.

1.1.5 The Case for Co-Cultivation

There is extensive literature on microorganisms growing in rice fields naturally, mostly being used as a fertilizer (Alam et al., 2014; Roger, 1982). Since they grow in the rice fields naturally, it would be easy to harvest them and use them for fuel production. Land is already being set aside to produce the rice, so it makes sense to try to grow ABM in the water and harvest that ABM for fuel production. If the water used to water the rice is wastewater, the wastewater can provide an ample amount of nutrients for the ABM as well as the rice effectively bringing the cost of algal fuels down to a more comparable price to non-renewable fuels. With the decreased costs, rice farmers could grow ABM in addition, and since there is a lot of land for rice production, there will be a lot of land for ABM production contributing to the US liquid fuel consumption.

1.2 Objectives

As the demand for transportation fuel increases, a way to produce these fuels in a safe and renewable manner is essential. Currently cellulosic biofuels are not being produced enough to live up to the Renewable Fuel Standards set by the US EPA as stated previously. Therefore, advanced biofuels must be produced elsewhere. One way is with algae. Algae has its own set of unique problems that must be solved in order to make algal biofuels a reality. A way to bring the costs of biofuel production from algae down is by producing algae in conjunction with rice.

There are three objectives of this study:

1. <u>Define a system for co-cultivating rice and algae to produce cost-effective biofuels by conducting background research.</u> The proposed approach will reduce the cost of algal biomass feedstock production by sharing land and pond infrastructure with rice production. The proposed system will be designed to facilitate the growth and harvest of algae while maintaining the productivity of rice.

2. Develop an engineering-economic model for a system involving co-cultivation of algae and rice production. This will demonstrate the financial effects of implementing a co-cultivation system like this in many areas around the world.
3. Perform proof of concept experiments to verify key features of the proposed co-cultivation system. This study will confirm foundational elements of the proposed system and identify potential limitations that can be investigated in future work.

These objectives will show the impacts of coupling rice cultivation, algae cultivation, and wastewater treatment. Not only is the energy problem significantly lessened by producing a large quantity of cost-effective renewable fuel, but wastewater is also treated in a cost-effective manner which means more parts of the world can treat wastewater and re-use it if necessary. This can produce clean water to be provided to places that do not have clean water access. A system that helps solve two sustainable issues is definitely worth the investment.

Chapter 2

Literature Review for Development of a Co-Cultivation System for Rice and Algae

2.1 Rice Growth

To prove that a co-cultivation system can work, a system must be defined and developed through background research. Parameters must be selected such that good rice growth can be replicated as well as optimize for ABM growth. The goal is to develop a system that can produce the normal amount of rice that a rice paddy would produce as well as grow ABM in the standing water in the paddy. The parameters for this system will be selected to optimize both rice and algae growth.

2.1.1 Location

To implement a system that grows rice and ABM together, location must be considered first. This then suggests to look at the world's largest rice producers. The table below depicts that.

COUNTRY	METRIC TONS OF RICE PRODUCED	AREA HARVESTED (HECTARES)
CHINA, MAINLAND	182,278,000	29,009,000
INDIA	129,196,000	44,999,000
INDONESIA	44,072,000	12,171,000
BANGLADESH	33,956,000	12,086,000
VIETNAM	30,796,000	7,531,000
UNITED STATES	6,551,000	1,156,000

Table 2-1: Production of Top 5 Rice Producers in the World Plus United States Production for 2013-2014. (Adapted from Wailes, Eric J. and Chavez, Eddie C., 2012).

This shows that these are locations throughout the world that an ABM and rice co-cultivation system would most likely be implemented. It is evident that China is the world's largest rice producer, and the United States is the 12th largest. Therefore, a co-cultivation system should be investigated for implementation for both China and the United States. If the system was implemented in the United States, it would most likely be in the states listed in the table below as they are the top rice producers in the US according to the USDA's Crop Production 2015 Summary (USDA, 2016).

	AREA PLANTED			AREA HARVESTED		
	2013 (1,000 acres)	2014 (1,000 acres)	2015 (1,000 acres)	2013 (1,000 acres)	2014 (1,000 acres)	2015 (1,000 acres)
All rice						
Arkansas	1,076	1,486	1,306	1,070	1,480	1,286
California	567	445	423	562	442	421
Louisiana	418	466	420	413	462	415
Mississippi	125	191	150	124	190	149
Missouri	159	216	182	156	213	174
Texas	145	150	133	144	146	130
United States	2,490	2,954	2,614	2,469	2,933	2,575

Table 2-2: US Rice Producers (USDA, 2016)

The reasons for the location is due to various aspects regarding light availability, temperature, water availability, and soil types all of which will be discussed in the following sections. Therefore, this system should be implemented in one of the states listed above.

2.1.2 Light Conditions

As discussed before, light sensitivity plays a key role in how to design a co-cultivation system. Rice is generally grown outdoors for mass cultivation, where full sunlight provides up to 2000 µmol photons m^{-2} s^{-1} (Sharp et al., 1986), while the minimum amount of sunlight required for rice is 300 µmol photons m^{-2} s^{-1} but prefer 500-1000 µmol photons m^{-2} s^{-1} (Harrington, Sandra, 2010). Growing rice in places with little sunlight over the course of the year like Minnesota or Canada would not make sense as these get less sun (and have lower annual temperatures). For algae growth, the minimum amount of light needed is about 50-300 µmol photons m^{-2} s^{-1} (Zimmerman et al., 1997; Wang et al., 2007). Since the minimum required amount of light for algae is below the recommended amount for rice. Therefore, natural sunlight will be able to provide enough light for good algae and rice growth.

2.1.3 Temperature Range

The next parameter to select is the temperatures at which rice and ABM can grow well. Rice will need certain temperatures to establish various stages of growth. The optimum range for good rice growth during the day is 26-28°C while at night the range is 20-22°C (Moulton et al., 2012). Algae will grow best in the 25-35°C range (Dauta et al., 1990). When considering these temperature ranges, it is evident that this system again should not be established in places with cold temperatures for most of the year like in Minnesota or Canada. Places with warm year-long temperatures are ideal like in Arkansas and California. This again correlates to the places that already grow rice in the US.

2.1.4 Soil Selection and Range

In order to grow rice, it must have a certain type of soil for various reasons. First, since rice paddies are normally flooded with water, the soil should have the ability to retain the water without much seepage. Typically, rice will be grown in sandy loam, silt loam, or clay loam soils (Hardke et al., 2014). These soils will be present in varying amounts depending on the location. For example, sandy loams are very prominent in South Korea (Kang et al., 2007). The density of soil also depends on how far down the soil goes as clay soils will be more present at deeper depths (Cabangon et al., 2000). All of these factors must be considered when deciding where to implement this system as having to import soils would cost money. Therefore, the cultivation system should be implemented in a location that has enough of sandy loam or clay loam or a combination of the two.

2.1.5 Plant Spacing

To provide ample space for plant growth, the spacing between plants must be considered. The table below shows the effects of spacing on rice yield between various rice species.

Varieties/mutant strains	Spacing (cm)	No. of panicles per hill	Grain yield per hill (g)	Filled grains per panicle	1000 grain weight (g)	Panicle density per m^2	Grain yield per plot (kg)
Basmati 370	20 x 20	7.25	10.08	3.25	21.13	176.00	1.62
	22.5 x 22.5	10.50	11.26	37.50	21.58	312.75	2.27
	25 x 25	12.25	12.16	41.25	21.75	267.75	1.52
Basmati 370-32	20 x 20	11.50	14.76	51.75	21.85	288.75	2.58
	22.5 x 22.5	15.50	15.99	60.50	21.65	437.75	3.23
	25 x 25	17.75	15.39	64.50	22.38	374.25	2.45
Jajai 77	20 x 20	8.25	8.25	37.75	21.08	196.25	1.33
	22.5 x 22.5	9.75	9.75	41.50	21.20	313.75	1.98
	25 x 25	10.40	10.40	44.75	21.45	241.25	1.22
Jajai 77-30	20 x 20	20.72	20.72	61.00	23.15	367.75	3.27
	22.5 x 22.5	21.95	21.95	64.50	23.28	483.50	3.92
	25 x 25	22.60	22.60	68.25	23.50	430.50	3.16
Sonahri Sugdasi	20 x 20	11.49	11.49	34.75	23.88	190.75	1.90
	22.5 x 22.5	12.72	12.72	38.50	24.20	297.00	2.55
	25 x 25	13.37	13.37	40.75	24.35	249.00	1.79
Sonahri Sugdasi-6	20 x 20	15.52	15.52	51.50	24.13	297.25	2.77
	22.5 x 22.5	16.67	16.67	55.25	24.43	379.00	3.42
	25 x 25	17.35	17.35	57.25	24.60	345.50	2.66
Basmati 385	20 x 20	14.41	14.41	44.00	22.03	273.50	2.56
	22.5 x 22.5	15.87	15.87	47.75	23.00	362.25	2.84
	25 x 25	16.28	16.28	50.75	22.48	306.00	2.55

Table 2-3: Effect of Spacing on Yield and Yield Parameters Within Varieties and Mutant Strains of Rice. (Baloch et al., 2002).

The rice grain yield per plot varies per rice cultivar, but as shown in Table 2-3, the 22.5 cm x 22.5 cm spacing yielded the highest amount of rice grain. The spacing can affect the compaction of the roots and overall profit margin. If the plants are too close together, then the roots cannot spread out as much and grab more nutrients, so the rice grain yield will go down. On the other hand, if the plants are spread too far apart, the roots will have plenty of room to grow, but since there are less plants per unit land, the productivity and profit decreases drastically. Therefore, a balance must be achieved between enough room for good growth and as many plants planted as possible to maximize revenue. This balance is what the 22.5 cm x 22.5 cm spacing represents. Therefore, 22.5 cm x 22.5 cm should be the spacing used in the proposed cultivation system.

2.1.6 Nutrient Supply

The next step in designing this system is to decide how the nutrients will be supplied. The most essential nutrients for growth of ABM and rice are nitrogen, phosphorous, and potassium. These can be supplied artificially via chemicals and fertilizers. For example, nitrogen, in the form of anhydrous ammonia, will cost \$480-890 ton^{-1} (Schnitkey, G., 2015). Urea can be purchased for \$370-470 ton^{-1} (Quinn, Russ, 2016). The cost of nutrients can be significant especially in large scale systems. One way to avoid the significant cost of nutrients is to use waste nutrients. These can come from agricultural waste, municipal waste, or animal waste. These also have varying amounts of nutrients. Agricultural waste mostly consists of nitrogen and phosphorous from farm runoff. Municipal and animal waste will be similar to each other and will have more solids and organics than agricultural waste. Treating waste also gives a treatment credit to the facility treating it. Municipal wastewater treatment plants typically pay \$8,130 - \$49,500 ton^{-1} of nitrogen or phosphorous removed (Hey et al., 2005). If the ABM can remove nutrients, then the system would provide significant value for it. Not only can wastewater be used to grow ABM, it can be used to supply nutrients for the rice as well. For this reason, municipal wastewater makes the most sense with respect to how to supply soluble nutrients to the system for ABM and rice growth. The only factor remaining is if the municipal wastewater can provide enough nutrients for ABM growth. ABM can use a variety of sources of nitrogen like ammonia and nitrate. ABM can grow on as little as 10 mg L^{-1} and can have a saturation effect past 20 mg L^{-1} of NH_3-N, as shown in the figure below, while other studies have shown that 350 mg L^{-1} of nitrogen are recommended for superior ABM growth (Mostert et al., 1987). The graphs below depict the algae biomass productivity relative to amounts of ammonia and nitrate.

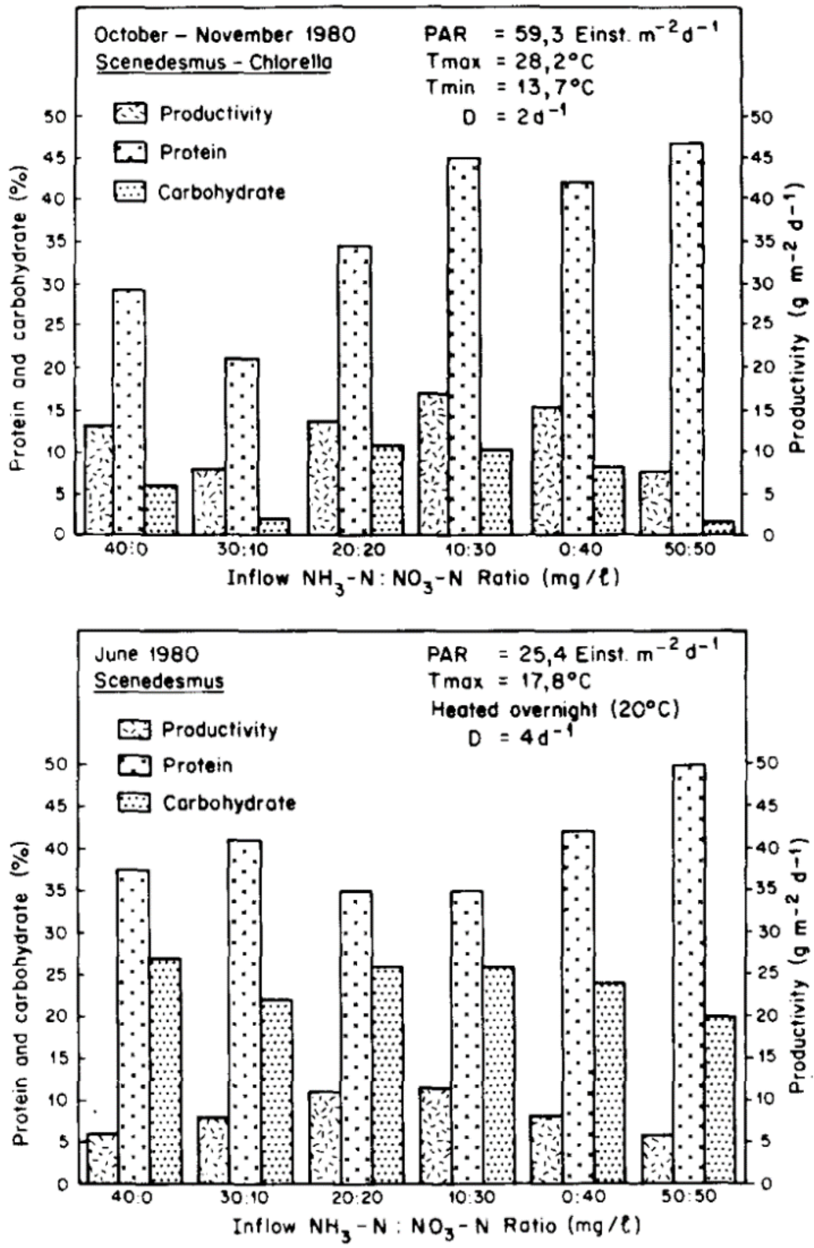

Figure 2-1: The Influence of Ratios of NH_3-N and NO_3-N on Productivity, Crude Protein Content and Carbohydrate Content of the Algae (from Mostert et al., 1987)

Another aspect to be considered is the effects using wastewater has on the various crops involved in this system. With all different kinds of crops, different types of wastewater have been used as a free source of nutrients throughout history (Hussain et al., 2002). It has also been reported that although wastewater can provide free nutrients to the system, nutrients in excess can cause an array of effects ranging from delayed maturity to crop yield reduction (Hussain et al., 2002). Yield reduction generally occurs when wastewaters that are unfiltered and therefore provide even more nutrients (Hussain et al., 2002). A table below shows some of the effects wastewater has on rice yield.

Effluent concentration (% V/V)	**Plant height (cm)**	**Leaf area (cm^2)**	**Seed dry weight (g plant $^{-1}$)**	**Root dry weight (g plant $^{-1}$)**	**Number of seed (plant $^{-1}$)**	**Seed weight (g plant $^{-1}$)**
0	48 ± 4	275 ± 17	10.8 ± 0.9	8.4 ± 0.3	758 ± 31	15.0 ± 0.9
2.5	49 ± 5 (2%)	300 ± 24 (9%)	12.0 ± 1.2 (11%)	9.5 ± 0.8 (13%)	790 ± 39 (4%)	18.3 ± 1.9 (22%)
5	56 ± 6 (16%)	325 ± 31 (18%)	12.9 ± 1.5 (19%)	10.5 ± 1.3 (25%)	825 ± 7 (8%)	20.0 ± 2.3 (33%)
10	43 ± 4	240 ± 15	9.5 ± 0.8	7.0 ± 0.5	710 ± 30	14.2 ± 1.5
25	35 ± 3	200 ± 11	8.0 ± 0.6	5.6 ± 0.3	485 ± 24	11.0 ± 1.3
50	28 ± 2	150 ± 9	6.1 ± 0.4	4.5 ± 0.2	350 ± 19	9.8 ± 0.9

Table 2-4: Plant Height, Leaf Area and Mean Dry Mass or Quantity of Various Plant Parts of Rice Grown on Soil Irrigated with Various Effluent Concentrations. Values in Parenthesis Indicate % Increase with Reference to Control (±SD) (adapted from Singh, K.K. and Mishra, L.C., 1987)

This table shows the potential negative effects of adding a concentration of nutrients that is too high. It shows that the seed dry weight and number of seeds per plant decrease past a concentration of 5 mg L^{-1}. The potential effects of nutrient poisoning will have to be investigated with a future experiment.

Since wastewater is being used, the depth at which the water is being kept at will have an effect on the yield of the rice, so careful selection is required. The figure below shows the effect of different water levels on the 1000 grain weight (a common way to measure grain yield).

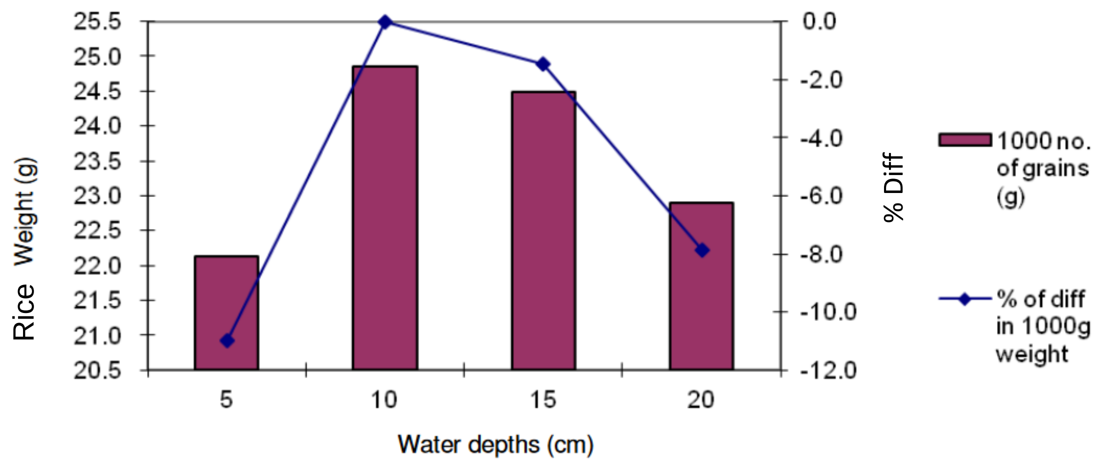

Figure 2-2: Weight of 1000 grains and their Difference in Percentage (from Talpur et al., 2013)

This figure shows that clearly having too much and too little water depth, the 1000 grain weight (and therefore the yield) decreases. Therefore, the proposed system should have 10 cm of water because this depth provides the greatest 1000 grain weight, and the ABM can grow in 10 cm of water.

2.2 Algae Cultivation

After the parameters for rice cultivation have been selected, the next step in system development is to decide how the ABM will be cultivated. The parameters decided here are essential to how economical the system will be.

2.2.1 Harvesting Method

2.2.1.1 Plastic Sheeting

The method used for harvesting the ABM must be carefully selected. To optimize the harvesting yield of the ABM, a thin sheet of plastic to be placed over the part of the field that is to be harvested is proposed. The plastic sheet will serve as a way for ABM to grow a fixed film on the bottom. The sheet also provides a way to prevent weeds from competing for nutrients with the rice plants. This would decrease costs required for weed control as less weed control would be required. The main purpose of the sheet is that it will also make it easier to collect more biomass as whatever ABM settles to the bottom could be easily recaptured. This would increase the harvesting yield of the ABM so as to maximize the cost effectiveness. Whether High Density Polyethylene (HDPE) plastic or Low Density Polyethylene (LDPE) plastic should be used is based on the cost and durability of the plastic. Since LDPE plastic is by definition less dense, this plastic would not be able to last many rice growing cycles and would have to be replaced every crop cycle given the amount of abuse the plastic will take as people are walking across the field. The other factor involved in deciding which plastic to use is the cost of the materials. After some conversion, it is reported that LDPE film grade plastic costs between \$1860 and \$2004 ton^{-1} assuming LDPE densities between 0.917 and 0.93 g cm$^{-3,}$ (Probst, Thomas, 2016; British Plastics Federation, 2016). HDPE injection molding- grade plastic costs between \$1860 and \$1925 ton^{-1} assuming densities between 0.935 and 0.96 g cm$^{-3,}$ (Underwriters Labs Prospector, 2016). The thickness of these plastic sheets range from 0.003 cm for LDPE plastic tarp to 0.014 cm for HDPE plastic tarp. The thicker the plastic, the more durable it will be. Since the costs are relatively the same per ton of plastic and the thicker plastic will be more durable allowing for multiple uses, HDPE plastic is the kind of plastic that will work best in this system. This plastic sheet should have 5 cm diameter holes cut out of the plastic spaced just as far as the plant spacing (discussed in section 2.1.5) is on center. 5 cm holes were chosen as a rough estimate of the width of the plant stems, but ultimately the rice plants will be able to expand out of the 5 cm depth if necessary.

2.2.1.2 Collection

Since there is the potential for multiple kinds of biomass to grow in the field, each kind of biomass must be able to be collected in the system. In order to collect the various kinds of biomass, the water/ABM would be drained biweekly by lifting the levee gate at the edge of the rice field. The water would drain towards the edge of the perimeter of the rice field where the levee gate is. This accomplishes three things. First, the water that is drained out of the field could contain suspended microalgae. This water containing suspended microalgae will be sent to a clarifier where the suspended microalgae could settle and be further processed and dried. Second, any settled ABM that had collected in the rice field over time would be carried by the draining water and collecting in the clarifier that the drained water has been sent to. This previously settled biomass would then settle again in the clarifier to be processed and dried. Third, the action of draining the field using gravity would in effect cause the floating ABM at the top of the water to collect at the bottom of the field on the plastic sheet. A trench is located at the edge of the field where the ABM would collect when the field is drained. This would concentrate the ABM solids. Depending on where the co-cultivation system is located would dictate the method in which the remaining ABM would be collected. For example, in the Philippines and China where labor costs are relatively low, this system proposes having workers with brooms going down the rice field and sweeping the leftover biomass into the trenches. This type of labor costs between \$9.56 to \$10.38 day^{-1} in the Philippines and \$4.49 to \$9.78 day^{-1} in China (Business World Research, 2014). Compare this to US wages of \$58 day^{-1} assuming the national minimum wage of \$7.25 hr^{-1} for the average number of working hours per day being eight hours.

So in East and Southeast Asia, labor is the more viable option for finding a way to collect the biomass. The size of the rice field will determine how many people are needed to complete this operation. As a baseline, an assumption will be made that it will take 8 man hours to sweep 1.5 acres. Overall, it is clear that having people sweep with a broom in East and Southeast Asia can be very cost advantageous. In these locations, after biomass has been swept, the workers would then use a net, screen or filter to collect the biomass in the trenches since most of the biomass will be macro species and are easy to collect in a net. In the United States, labor is more expensive, so a different method should be used. This system proposes using a pressurized water approach where a worker would shoot pressurized water down the rows to push the ABM into the trench. This should take the same amount of time that it takes to broom up the biomass per acre, but will require less people. To collect that biomass that is in the trench, nets will be used to lift the biomass out of the trench. This biomass would then be directly transported to the dewatering process. This would be the same process of using a net regardless of where the co-cultivation system is implemented. After the ABM is collected, the field would then be refilled with fresh wastewater. A diagram of the rice field is shown below. The yellow circles represent the location of the rice stalks, and the green circles represent ABM which would then be swept into the trench at the end of the field.

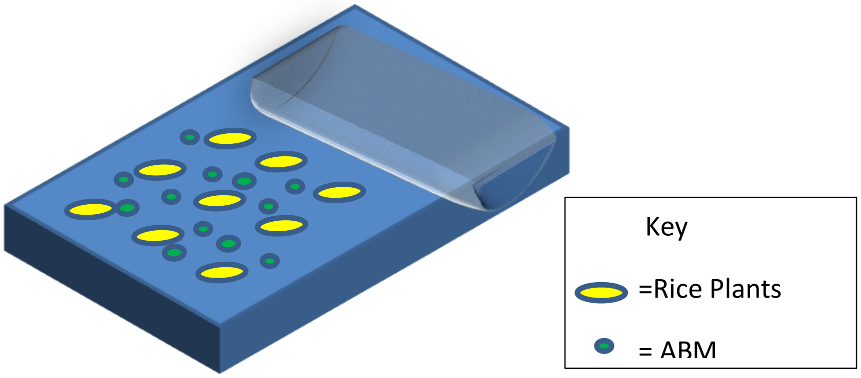

Figure 2-3: Schematic diagram of co-cultivation field

2.2.2 Harvesting Frequency

The frequency of the harvest will depend on several factors. First, the ABM productivity will be the main factor in determining when to harvest the biomass. The average ABM productivity for the whole growing season in a rice field was found to be approximately 7.54 g m^{-2} day^{-1} as discussed in section 3.1.2. To determine the harvesting interval, one must consider the optimal density that the ABM should be at in order to begin harvesting. The density at which ABM is harvested ranges from .02% to 0.06% total suspended solids (TSS) (Shelef et al., 1984). In order to achieve a minimum of .04% TSS or a density of 400 mg L^{-1}, it would take 6 days to achieve this density when the ABM grows at 7.54 g m^{-2} day^{-1}, but a more efficient density would be about 1000 mg L^{-1}. This would take 20 days to achieve this density with the same productivity mentioned above. Therefore, the ABM should be harvested every 20 days. This means that over the course of a year where 10 months (300 days) out of the year ABM is growing, harvesting will occur 15 times or 15 days since a harvesting event will occur in one day. The other factor is the availability of untreated or partially treated wastewater. The rice field will be assumed to be located next to a source of wastewater so that the field could be replenished after a harvesting event. This is to supply fresh nutrients to the field so more ABM can grow. This would allow for the harvesting of ABM to occur as frequently as it can grow. An inoculation tank will be located in the same facility to establish a dominant culture in the rice field.

The figure below depicts what the proposed system would look like.

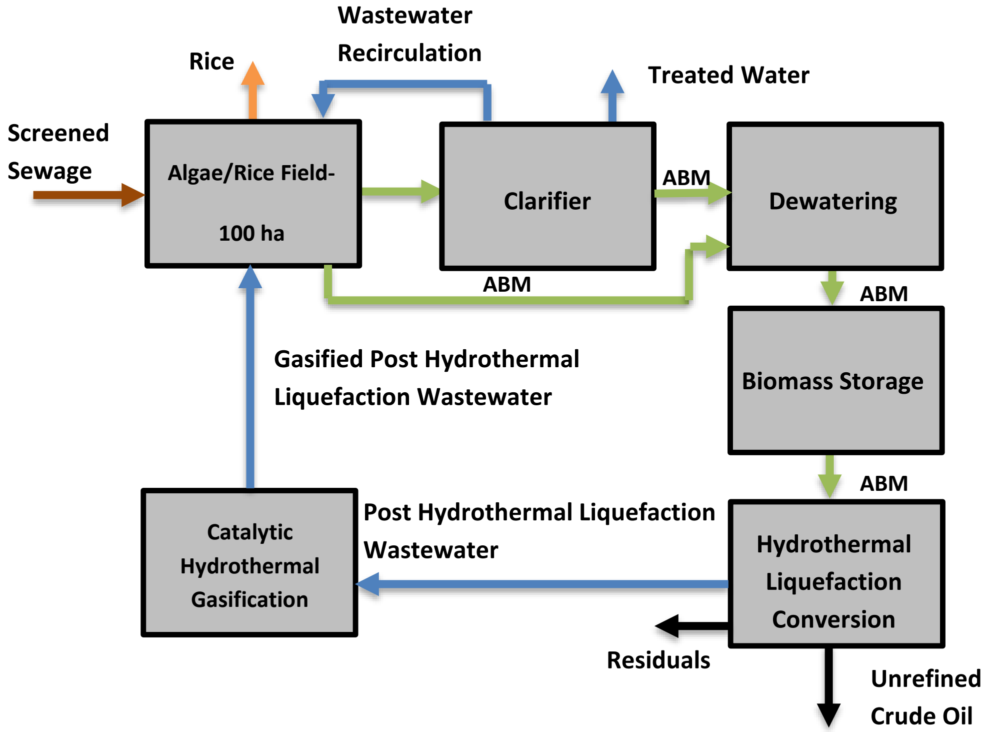

Figure 2-4: Co-Cultivation System Schematic

Chapter 3

Techno-Economic Analysis of the Proposed System and Experimental Verification

A summary of the parameters for this system is listed below. The various justifications for each parameter are discussed in Chapter 2.

Parameter	Selection
Location	United States/China
Light	Sunlight (full=2000 µmol photons m^{-2} s^{-1})
Daylight Temperature	26-28°C
Nighttime Temperature	20-22°C
Soil Type	Sandy/Clay Loam
Plant Spacing	22.5 cm x 22.5 cm
Nutrient Supply	Agricultural/Municipal Wastewater (350 mg L^{-1} Nitrogen)
Water Level	10 cm
Plastic Sheeting	HDPE Tarp
Land Area	100 hectares
Number of Rice Crop Cycles	2 (240 days)
ABM Harvest Method	Water Pumping (US), Broom Sweep (China)
ABM Harvest Frequency	Once every 20 days

Table 3-1: System Development Parameters Summary

To determine the costs of implementing a co-cultivation system, it is important to break down the costs into various categories:

- Growing and harvesting the algae biomass feedstock
- Converting the biomass to biocrude oil
- Upgrading the crude oil to a finished fuel
- Conducting catalytic hydrothermal gasification on the PHWW
- Credit for treating wastewater
- Credit for selling rice grain

- Minimum selling price of the fuel

One of the main goals of this study is to lower the cost of the algae biomass feedstock only, so only this component of the cost breakdown will be evaluated here.

3.1 Capital Costs

3.1.1 System Costs

Since the primary goal of this study is to decrease the cost to produce the algae biomass feedstock, the economics of conventional algae biomass production facilities must be looked at. This study's proposed system will then take aspects from conventional processes and adjust them to decrease the production cost. To compare this study's system to conventional algae biomass cultivation systems, the same scale must be used. The conventional algae biomass production system used in this study was defined in the study, "A Realistic Technology and Engineering Assessment of Algae Biofuel Production," by Lundquist et al., (2010). It discusses various techniques used to achieve an economically-favorable scenario for algal biofuel production. The Lundquist et al., (2010) study analyzed a 100 hectare algae growing facility, so all calculations for this study will be scaled to the 100 hectare size. A table of the costs associated with his approach to algal biofuel production is shown below.

Capital Cost	
Land (100 hectares)	$ 4,710,000.00
High Rate Ponds	$ 3,410,000.00
Drying beds	$ 2,420,000.00
Electrical	$ 1,900,000.00
Water piping	$ 1,660,000.00
2. Clarifier	$ 948,000.00
1. Clarifier	$ 420,000.00
Thickeners	$ 256,000.00
Buildings	$ 60,000.00
Silo storage	$ 54,500.00
Vehicles	$ 50,000.00
Road + fencing	$ 169,000.00
CO2 delivery	$ 594,000.00
Subtotal	$ 16,700,000.00

Table 3-2: Capital Cost of Producing Algal Biomass Feedstock (from Lundquist et al., 2010)

Since the Lundquist et al., (2010) study is the baseline for the economic analysis of this study, each component will be looked at individually. First, the cost of the high rate ponds used in the Lundquist et al., (2010) study composed of the wheel cost as well as cost for building the berms would be replaced with the cost of the plastic lining to grow the ABM in the co-cultivation system. The reason that cost for building the berms is not included in this study's cost estimation is that the berms will have been built by the rice farmer, and therefore paid by the rice farmer. There are no wheels in the co-cultivation system, so that is not included as well. As stated in the previous section, in this study's case, a plastic HDPE tarp will be laid out over the entire field to make collection of biomass easier. After looking at the cost per ton of the HDPE tarp, and the density of the tarp and the thickness (all reported in the previous section), it will cost for both the US and China \$223 acre^{-1} to use the HDPE tarp. The cost for installing the plastic sheet is calculated using the assumption that it would take one hour of work for the facility staff to place the plastic sheets on the ground. The amount of people required to place 100 hectares of plastic sheets is assumed to be the same amount of people required for water pumping (67 people) which is discussed in section 3.2.1. This means the cost for installation of the HDPE tarp in the US is $500 using the federal minimum wage of $7.25 hr^{-1} for 67 people working one hour. For China, 67 people working for 1 hour at China's federal minimum wage of $0.56 hr^{-1} is $38, or negligible. For 100 hectares, the total cost for the HDPE liners will be $55,600 for the US and $55,100 for China compared to $3,410,000 from the Lundquist et al., (2010) study. Already the cost of the ABM feedstock decreases significantly.

Another key factor in the feedstock cost is the drying beds used after the ABM has been harvested. According to Lundquist et al., (2010), this cost is based on the amount of space used for the drying beds. The amount of space used by the drying beds in this study is based on the amount of biomass produced from the proposed system. Since the system is a 100 ha field at a water depth of 10 cm, this translates to 100,000,000 liters of water containing a certain percentage of ABM at harvest time. This percentage is assumed to be .04% as discussed in the previous section. This means that there is 40,000 liters, or 40 m^3 of ABM that is heading to the clarifiers and eventually drying beds. This 40 m^3 of ABM would occupy 4000 m^2 or 0.4 ha of space for drying beds based on Lundquist et al., (2010) study's assumption that 1 m^3 of biomass occupies 100 m^2 of space. Using Lundquist et al., (2010) study's assumption that the cost for drying beds is $197,000 ha^{-1}, this will cost $78,800 for this system's drying beds.

The water piping is assumed to be the same as the Lundquist et al., (2010) study. This is because the cost for piping the ABM from the 100 ha field to the clarifiers to the thickeners and drying beds will be approximately the same as the Lundquist et al., (2010) system. The capital costs for the electrical components of the system will differ from the Lundquist et al., (2010) system because it is assumed that the electrical equipment for the clarifiers and drying beds, etc will be the same. Road and fencing will not be built in this system as they are not necessary for this facility and can decrease costs significantly.

The clarifier system will be based off the Lundquist et al., (2010) system in several key parameters. The first is that this system is aimed at producing ABM feedstock, not treating wastewater like the biofuel-emphasis case in the Lundquist et al., (2010) study. For this reason and based on the assumption from Lundquist et al., (2010), a primary clarifier for the wastewater before it goes into the algae/rice field will not be used. The wastewater will be pre-screened at the source of the water, so most solids will be removed from the water. This means that any leftover solids originally from the wastewater that could settle while it is in the field would release nutrients to the water essentially adding nutrients for ABM productivity. Second, the volume of the secondary clarifiers is different since the amount of water coming from the field to the clarifiers in this system is different than Lundquist et al., (2010)'s system. As stated previously, 100,000,000 liters or 100,000 m^3 of water/ABM is coming from the field. The secondary clarifier will settle the ABM, but in order to provide enough time for settling the clarifier has to be large enough to allow for settling to occur in 2.5 hours as suggested by the Lundquist et al., (2010) study. To achieve settling in 2.5 hours with 100,000 m^3 of water, this would require a volume of 10,416.7 m^3. Using Lundquist et al., (2010)'s assumption that a clarifier would cost \$36,700 1000 m^{-3} of settling tanks, this would cost this system \$382,000 for the secondary clarifiers.

The thickener cost will also differ with the Lundquist et al., (2010) study. The Lundquist et al., (2010) study used the assumption that 4 m^3 of thickener volume could handle a biomass volumetric flow of 24 m^3 day^{-1}. Using this assumption, the volume required for this system's biomass volumetric flow rate of 40 m^3 day^{-1} is 6.67 m^3. The cost assumed from the Lundquist et al., (2010) study that a thickener will cost $648,000 per 1000 m^3. Since the volume required for this system is 6.67 m^3, the cost for this system's thickeners is $4,320.

This system, unlike the Lundquist et al., (2010) system, will not incorporate buildings. Since the goal of this system is to produce a cost-effective fuel, buildings are considered to be unnecessary since they provide office space that is not necessary for this system. Administrative work can be done from the homes of the administrators rather than provide a space that will prove cost in-effective. Therefore, the cost for buildings for this system is $0.

To determine the amount of volume required for silo storage of the biomass after drying occurs, the total amount of dried biomass produced from the system must be determined from an experiment as described in the next section.

3.1.2 Algae/Bacteria Biomass Mixture Productivity

ABM productivity was measured at two stages in the rice growth. The first was when the rice plants were beginning to grow, so they do not occupy any canopy space. This is representative of how much ABM could grow during the times of the year when essentially only ABM is growing. The other stage is when the rice is fully grown and occupies the whole canopy. The figure below depicts the amount of photosynthetically active radiation (PAR) available beneath several rice plants.

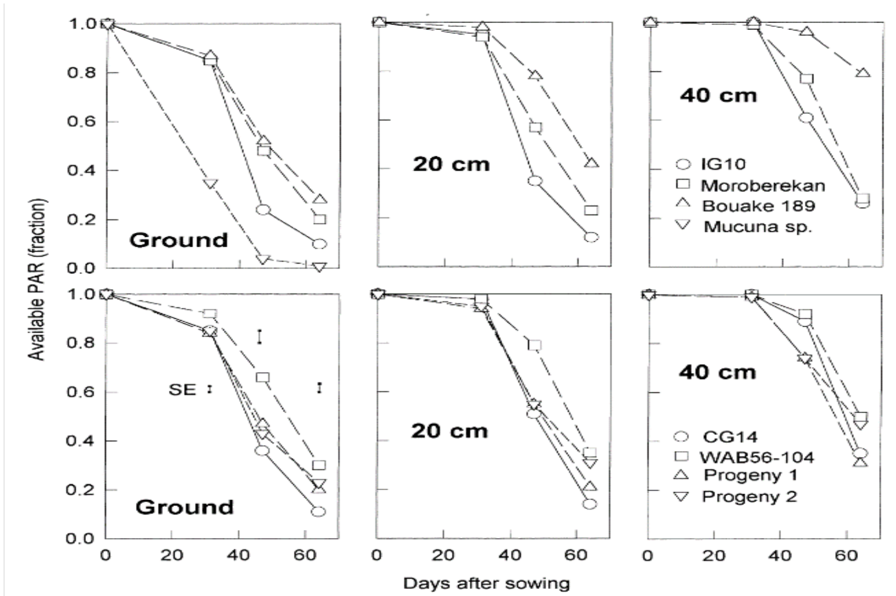

Figure 3-1: Time Courses of Diffuse PAR at Ground Level and 20 cm and 40 cm Heights (Fraction of Ambient PAR), for Seven Rice Cultivars (Including the Interspecific Progenies WAB450-24-3-2-P18 (1) and WAB450-I-B-B122-HB (2); Details in Table 1) and the Creeping Legume *Mucuna* Grown in Monoculture in Experiment 1. Vertical Bars Indicate Standard Error (SE) Across Cultivars. Mbe, Cote d'Ivoire, 1997 Wet Season. (Adapted from Dingkuhn et al., 1999)

The most important sets of curves in this figure is the "Ground" graphs as this would be the amount of sunlight available for ABM growth. Among the ground graphs, the bottom left graph will be used to determine a productivity curve as this graph has the larger amount of curves following the same relationship, so this is likely the truest relationship between shade and days after sowing for ground level. Thus, the graph of the productivity of ABM should look like this graph. To confirm this, ABM productivity was measured using the method for total solids test by the EPA (US EPA, 2001). The three productivities measured at two distinct stages of rice growth are shown in the figure below

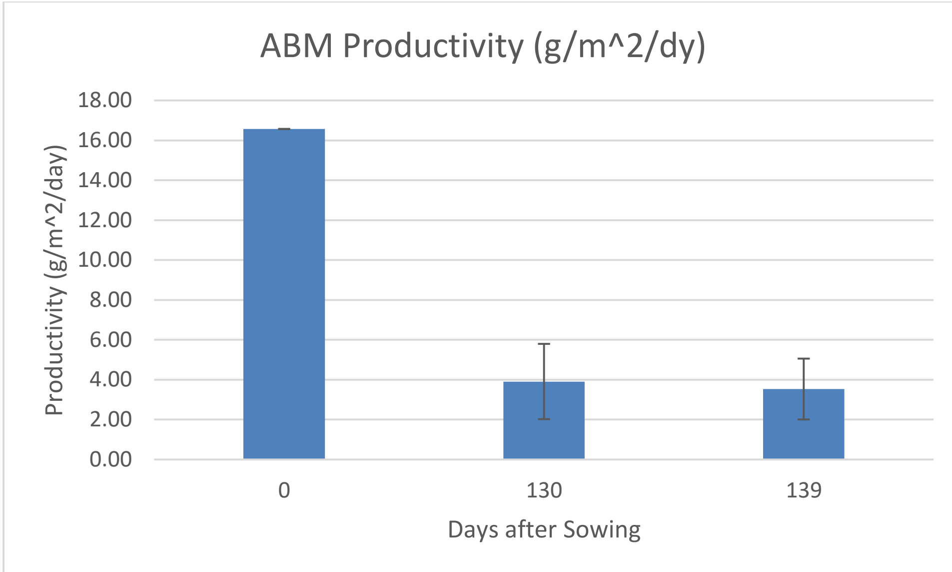

Figure 3-2: ABM Productivities at Three Distinct Stages of Rice Growth

The data show that ABM can grow up to 16.57 g m^{-2} day^{-1} which is very promising. The later stages of growth show that the ABM can have a productivity on average of 3.54 g m^{-2} day^{-1}, but this value may be overestimated based on the experimental setup. This is because the experimental setup of having rice plants inside the plastic boxes would lead to some light penetrating from the sides of the reactors whereas in a normal rice growing scenario, other rice plants would occupy that space, so light could not penetrate as easily. One additional thing to consider is that the overall rice growth took 168 days to grow which is 48 days longer than the average time it takes for rice to mature (120 days). Therefore, the productivities measured in this study at 130 and 139 days after sowing would correlate to the represent growth at approximately day 60 in figure 3-1. This is because at day 60 in the 120 day scale, the rice plant has entered the maturation phase where the rice begins to develop rice grains. The amount of shading provided by rice at the maturation stage is assumed to be the same as the amount of shade provided at the ripening stage (day 90 of the 120 day scale) since the ripening stage does not develop any more rice grains or panicles, but just ripens the existing rice grain. In the 168 day scale from this experiment, the ripening stage was noted to occur at day 130. Therefore, the amount of algae grown at day 130 of the experiment, the beginning of the ripening phase, is representative of algae grown at the beginning of the maturation stage, or day 80 of the experiment. This day in the experiment would correlate to Day 60 to the beginning of the maturation phase in the Dingkuhn et al., (1999) study. Using these correlations, the productivities for days 130 and 139 were adjusted to day 60 in the ABM productivity model. To model the ABM productivity over time, a productivity graph was produced using the computer program NLREG based on a cubic relationship. The cubic relationship had the largest r^2 value compared to other representations which is why the relationship was chosen to model the ABM productivity. The graph produced from that program is shown in the figure below.

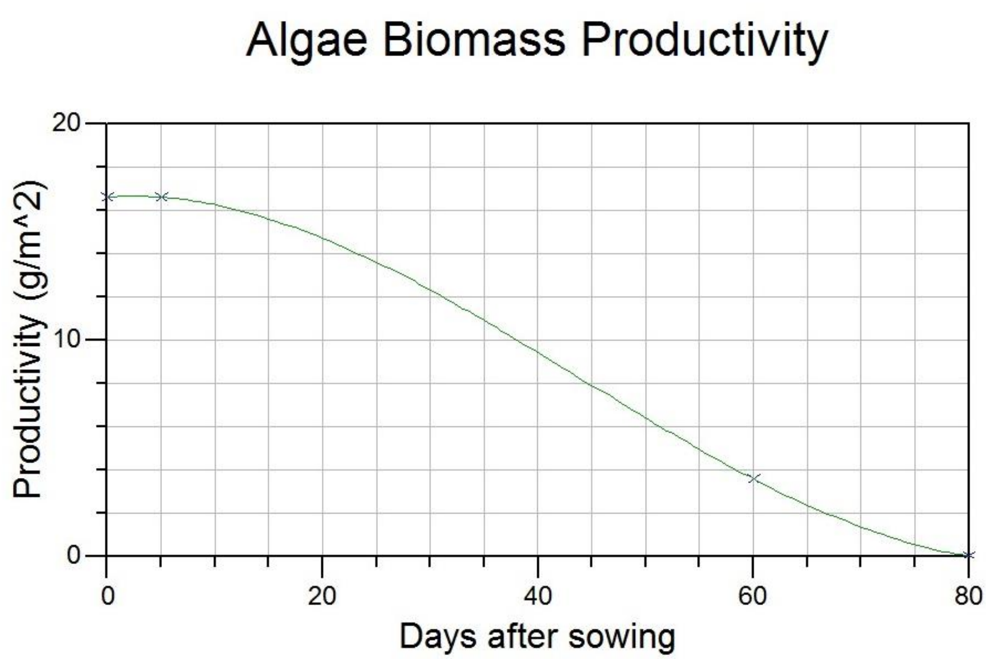

Figure 3-3: Algae Biomass Productivity Model Using NLREG

The equation that is represented from the above figure is shown below.

$$y = (5.9495106 * 10^{-5})t^3 + (-.00782023068)t^2 + .0376117758t + 16.57905 \quad \textbf{Eq. 1}$$

This relationship is representative of the kind of growth the ABM could experience over a span of 80 days, about one month short of the total length for a normal rice crop cycle (120 days). For example, in the beginning days of growth, the rice seedlings barely occupy any canopy space, so ABM would grow at its maximum rate for a long time. Then growth would begin to taper off as the rice grows higher and the leaves occupy more canopy space. Ultimately the ABM productivity would reach a low productivity at around day 60 as evident by Figure 3-1. At day 60, however, the ABM productivity would level out at 3.54 g m^{-2} day^{-1} until the end of the rice crop cycle (day 120). This is where the model from NLREG does not accurately depict the entire ABM growth over the entire rice growth because the ABM productivity model does not show the productivity approaching 3.54 g m^{-2} day^{-1} and maintaining that productivity until the end of the rice crop cycle.

To figure out how much biomass is produced, the growth curve model determined in Figure 3-3 using Eq. 1 was integrated over the first 60 days of growth in the rice crop cycle.

$$\left(\int_0^{60} (5.9495106 * 10^{-5})t^3 + (-.00782023068)t^2 + .0376117758t + 16.57905\right)$$

$$= 692.152 \frac{\frac{g}{m^2}}{cycle}$$

For the remaining 60 days of the rice crop cycle, a productivity of 3.54 g m^{-2} day^{-1} was integrated as depicted in the equation below.

$$\int_0^{60} 3.54 = 212.4 \frac{\frac{g}{m^2}}{cycle}$$

This means that over the course of one rice crop cycle, 904.6 g m^{-2} of ABM is produced. Using this productivity, the average ABM productivity over the rice crop can be calculated by dividing the productivity over 120 days which means the average productivity is 7.54 g m^{-2} day^{-1}.

Since the proposed system aims to grow ABM for 10 months, or 300 days, and rice is being grown for 240 of those days if it is double cropped, the productivity of ABM over the rice crop cycle must also be doubled. The remaining ABM growth in the 60 days of the proposed 10 months of growth will produce ABM using the average productivity calculated previously (7.54 g m^{-2} day^{-1}). In one year, ABM growth would occur at a rate of 7.54 g m^{-2} day^{-1} for the first 30 days without any rice co-growth. This is followed by 2 rice co-growth cycles over 240 days followed by the last 30 days of just ABM growth at 7.54 g m^{-2} day^{-1}. Overall, in one year, the amount of ABM produced was found below.

$$\int_0^{30} 7.54 + \left(\int_0^{60} (5.9495106 * 10^{-5})t^3 + (-.00782023068)t^2 + .0376117758t + 16.57905\right) + \int_0^{60} 3.54 + \left(\int_0^{60} (5.9495106 * 10^{-5})t^3 + (-.00782023068)t^2 + .0376117758t + 16.57905\right) + \int_0^{60} 3.54 + \int_0^{30} 7.54 = 2261.504 \frac{\frac{g}{m^2}}{year}$$

$$\frac{2261.504g}{\frac{m^2}{year}} * \frac{4046m^2}{acre} * \frac{1E-6\ ton}{g} = \frac{9.15\frac{ton}{acre}}{yr}$$

Only 85 percent of this biomass can be harvested based on an estimate from Lundquist et al., (2010)'s study which assumed a harvesting percentage of 91%. The reason less can be harvested is because of the loss of biomass in-between the rice stalks. A large scale field test would be required to confirm this estimation. After harvesting losses, the amount of ABM produced from a 100 ha field that will be used for fuel production is 1921 metric tons of dried biomass year^{-1}. 40 percent of this biomass will be converted to biocrude oil via hydrothermal liquefaction (Zhou et al., 2013).

3.1.3 Oil Production

Since the assumption of 40% oil conversion will be used, this means that 5635 barrels of oil year^{-1} will be produced from this 100 ha system that produced 1921 metric tons of ABM. Knowing that 5635 barrels of oil can be produced from a 100 ha facility, and a 100 ha facility can produce 400 tons of rice according to the Food and Agriculture Organization (United Nations Food and Agriculture Organization, 2016), the amount of oil that could be provided with this system can be calculated. Using Table 2-1's production numbers, there are 750,000,000 metric tons of rice grain produced in the world. This can be used to calculate how much oil could be produced in the world if the rice paddies produced ABM as well.

$$5635\,\frac{barrels}{100\ ha} * 100\,\frac{ha}{400\ tons\ rice} * 750{,}000{,}000\ tons\ rice$$

$$= 10{,}566{,}000{,}000\ barrels\ of\ oil$$

This means if every rice paddy in the world also grew ABM, 10,566,000,00 barrels of oil or 36% percent of the oil that is currently produced in the world (Energy Information Administration, 2016). This shows that the amount of oil produced from ABM increased from 1% of the US supply in the Lundquist et al., (2010) study to 36% of the world's supply.

Other constraints like nutrient availability must be considered to determine if this percentage is realistic. Since rice typically require 150 lbs N acre^{-1} (Wilson et al., 2014) and ABM can get 50 mg L^{-1} of nitrogen from wastewater (Food and Agriculture Organization, 2016), co-cultivation requires 480 lbs N ha^{-1} assuming a 10 cm water depth as shown in the following calculation.

$$\frac{50mg}{L} * \frac{100{,}000{,}000L}{100ha} * \frac{1g}{1000mg} * \frac{1lbs}{453.592g} = \frac{110\ lbs\ N}{ha}$$

$$\frac{150\ lbs\ N}{acre} * \frac{7.41\ acre}{ha} = \frac{370.65\ lbs\ N}{ha}$$

Using world rice harvested area (160,000,000 hectares) from Wailes, Eric J. and Chavez, Eddie C., (2012), the amount of nitrogen needed for world rice production is 77,000,000,000 lbs or 35,000,000,000 kg. Since this is the amount of nitrogen required to produce rice, it is important to look at how much nitrogen can be provided with wastewater and if there is enough. The world produces 1,500 km^3 or $1.5 * 10^{15}$ liters of wastewater every year (United Nations WWAP, 2003). Using the assumption from the United Nations Food and Agriculture Organization, this contains approximately 50 mg L^{-1} of nitrogen. This means that 75,000,000,000 kg of nitrogen are available from wastewater. This means that the amount of wastewater in the world can provide enough nitrogen for every rice field around the world. This means that the 36% oil supply provided by co-cultivation systems is not limited by land or nutrients.

Other factors like temperature could decrease the percent oil supply, but more research would have to be done to determine the locations at which temperatures could sustain a co-cultivation system.

3.1.4 Continued Costs

Using the productivity data from Figure 3-2, the volume required for storing this biomass after drying can be determined. Lundquist et al., (2010) assumed that a biomass volumetric flow rate of 34 metric ton day^{-1} would require 1700 m^3 of silo storage. As stated previously, this system will produce 1921 metric tons of dried biomass $year^{-1}$, so this will require a volume of 320 m^3 of silo storage. The Lundquist et al., (2010) study does not mention the price per volume for silo storage tanks, but the study states that it cost the feedstock facility (since the other half of the cost will be paid by the HTL conversion facility) $54,500 for a volume of 1700 m^3. Since it costs $54,500 for 1700 m^3 and this system requires 320 m^3, it will cost this 100 ha system $10,300 for silo storage tanks.

Since the goal of this system is to produce a cost-effective biofuel, vehicles will not be purchased as they provide a benefit that is not necessary to the production of fuel. Therefore, the cost for purchasing vehicles is $0.

There is no cost for CO_2 delivery because no CO_2 will be delivered to this system. Instead, a water pump will be used for shooting water down the field rows in the US. To purchase a pump that could pump 1000 gallons minute^{-1} (and more if required), an IS series electric centrifugal water pump from Alibaba.com would cost $1000, and this could provide up to 1760 gallons minute^{-1}. 1000 gallons minute^{-1} was chosen as the pumping rate to provide an adequate amount of flow as described in section 3.2.1. It was determined that 67 of these pumps would be required to pump enough water for a 100 ha field. This is based on the assumption that 2 of these pumps could provide enough power for a 3 ha field as discussed in the next section, therefore a 100 ha field would require 67. This would cost the system $67,000.

For China, brooms will be purchased instead of a pump to harvest the ABM as discussed in section 2.2.2. Since it was assumed that it will take 8 man hours (or 1 person) for a 0.6 ha field or 5 people for a 3 ha field, it will take 167 people to broom a 100 ha field. Brooms can be purchased for $12 a broom, so this will cost a 100 ha facility $2000.

The last component of the capital cost is the land. For this system the only land that has to be purchased is the land the clarifiers, thickeners, drying beds, offices, and silos require which is 1 ha as previously stated. The reason the land for the 100 ha of algae/rice field is not considered in the capital costs is because it is assumed that the rice farmer will pay for this land since their crop of rice is being grown in this 100 ha field. Lundquist et al., (2010) states that it costs \$15,000 ha^{-1} for land. Since this system is only paying for the 1 ha of land, this will cost the system \$15,000.

A table of the capital costs for the US and China are shown below.

Location: USA
Co-Cultivation

Capital Cost		Justification
Land (100 hectares)	$ 15,000.00	Land cost $15000/ha for agricultural land (100 ha paid for by rice cultivation)
HDPE Tarp	$ 55,600.00	$223/acre
Drying beds	$ 78,800.00	0.4 ha drying bed space
Electrical	$ 491,000.00	Lundquist et al., 2010
Water piping	$ 1,660,000.00	Lundquist et al., 2010
2. Clarifier	$ 382,000.00	2.5 hr HRT using ag ww prices
1. Clarifier	$ -	Any settling releases nutrients for algae/rice growth
Thickeners	$ 4,320.00	6.67 m^3 thickeners
Buildings	-	None Required
Silo storage	$ 10,300.00	504 m^3 silos
Vehicles	-	None Required
Road + fencing	-	None Required
Brooms	$ 67,000.00	67 Pumps @ $1000
Subtotal	$ 2,763,831.74	

Table 3-3: Capital Costs of Co-Cultivation in US

Location: China
Co-Cultivation

Capital Cost		**Justification**
Land (100 hectares)	$ 15,000.00	Land cost $15000/ha for agricultural land (100 ha paid for by rice cultivation)
HDPE Tarp	$ 55,100.00	$223/acre
Drying beds	$ 78,800.00	0.4 ha drying bed space
Electrical	$ 491,000.00	Lundquist et al., 2010
Water piping	$ 1,660,000.00	Lundquist et al., 2010
2. Clarifier	$ 382,000.00	2.5 hr HRT using ag ww prices
1. Clarifier	$ -	Any settling releases nutrients for algae/rice growth
Thickeners	$ 4,320.00	6.67 m^3 thickeners
Buildings	-	None Required
Silo storage	$ 10,300.00	504 m^3 silos
Vehicles	-	None Required
Road + fencing	-	None Required
Brooms	$ 2,000.00	$12/broom for 167 people
Subtotal	$ 2,698,380.91	

Table 3-4: Capital Costs of Co-Cultivation in China

3.2 Operating and Maintenance Costs

3.2.1 System Costs

Like the previous section, the operating and maintenance costs will be based on the Lundquist, et al., (2010) study. The table below lists the operating and maintenance costs in the Lundquist et al., (2010) study.

O&M Cost		
Algae facility staff	$	748,000.00
Maintenance	$	333,982.00
Electricity purchase	$	322,200.00
Administrative staff	$	187,500.00
Insurance	$	90,000.00
Outside lab testing	$	25,000.00
Vehicle maintenance	$	7,500.00
Lab & office supplies	$	6,250.00
Employee training	$	5,000.00
Subtotal	$	1,725,432.00

Table 3-5: Operating and Maintenance Costs of Producing Algal Biomass Feedstock (from Lundquist, et al., 2010)

This study's costs will differ from the Lundquist et al., (2010) costs in several key areas. First is the algae facility staff. This is composed of the collection facility staff (the people operating the clarifiers, thickeners, drying beds, and silos) and the people in the field harvesting the ABM. According to Lundquist et al., (2010), the collection facility staff members will be paid on average a salary of \$41,000 yr^{-1}. Since the Lundquist et al., (2010) study assumed that 14 people were needed to operate a 100 ha facility, and the only operators in this study are for the collection facility of 1 ha, only 1 collection facility operator will be required. In addition to the collection facility operator is the operators for harvesting the ABM in the field. Since the harvesting method differs based on the location, the number of harvesting staff will differ. For the US, since there will be 67 pumps being used as discussed in the previous section, there will be 67 people in the field performing the harvest. Since there are 15 harvesting days as discussed in section 2.2.2, this means 67 people will be working for a total of 15 days throughout the year. Using the US federal minimum wage of \$7.25 hr^{-1}, this will cost the facility \$58,300 for the harvesting staff assuming an 8 hour work day. All algae facility staff are assumed to get benefits which will be 30% of the total wages as assumed in the Lundquist et al., (2010) study. The collection facility staff, the harvesting staff, and the 30% benefits adds up to \$140,000 for the 100 ha US facility.

For China, since more people are required to broom the 100 ha facility, the cost will be different. As discussed in section 3.1.4, 167 people are required to physically broom the 100 ha field. This harvesting staff will also be working a total of 15 days out of the year for harvesting, the same as the US system. They will be paid the lower end of China's minimum wage of \$4.49 day^{-1} since this is manual labor. This totals out to \$11,200 for the harvesting staff. The same salary of 1 person for the collection facility will be used, except at a ratio of China's minimum wage to the US federal minimum wage. This means that the collection facility staff member will be paid a yearly salary of \$3,170. Using the same 30% benefits of salaries, the total algae facility staff cost for China is \$20,900.

The maintenance cost is calculated as 2% of the capital cost, as assumed by the Lundquist et al., (2010) study. This totals to \$55,300 for the system.

The electricity purpose cost is 3 fold. First is the cost of pumping water in the collection facility. The Lundquist et al., (2010) study assumed an energy requirement of 2000 kWh day^{-1}, and this assumption will be used for this study since the collection facility will operate the same way as the Lundquist et al., (2010) study. One other component of the electricity cost is the cost of pumping the ABM that has been pushed into the trenches all the way to the collection facility. This study will use the same energy requirement that the Lundquist et al., (2010) study used, 1250 kWh day^{-1} as the same mechanism of pumping the ABM from the 100 ha field to collection facility will be used. The cost rate per kWh for China and the US industrial prices were found to be \$0.134 kWh^{-1} for China and \$0.065 kWh^{-1} for the US (Comerford et al., 2016). These two parasitic energy requirements charged at their respective costs for a total of 300 days for the year will cost \$63,400 for the US and \$131,000 for China. This is the only cost associated with electricity for China, while the US has another component: water pumping in the field for harvesting. Using the following equation below, the cost to pump water to the field was calculated.

$$C = \frac{0.746 * Q * h * c}{3960 * \mu_p * \mu_m} \quad \textbf{Eq. 2}$$

Where

$$C = cost\ per\ hour\ (USD)$$

$$Q = volume\ flow\ (US\ gpm)$$

$$h = head\ (ft)$$

$$c = cost\ rate\ per\ kWh\ \left(\frac{USD}{kWh}\right)$$

$$\mu_p = pump\ efficiency\ (0-1)$$

$$\mu_m = motor\ efficiency\ (0-1)$$

Since there are 67 pumps for a 100 ha field, the volumetric flow rate required for 1 pump has to be calculated from a 3 ha field. The volumetric flow rate was calculated using the following parameters: a water depth of 10 centimeters as stated in the previous section, the area required to cover the whole acreage of the rice field (3 ha), and the time at which would be desired to pump all of the water into the field (14 hours). The flow rate found was 943 US gallons $minute^{-1}$. The pump efficiency and motor efficiency were assumed to be 90%. The head, or the height at which the water would have to be pumped upwards was assumed to be 10 meters at maximum based on the fact that rice fields are generally very flat, so water really is not needed to pump in the vertical direction. It was found that the cost for pumping the water would be $0.14 hr^{-1} in the US. This is equivalent to saying it costs $10.05 ha^{-1} in the US to operate 1 pump for shooting pressurized water down the rows as well as pumping in fresh wastewater. For the 100 ha facility, this will cost $67,300. Therefore, the total cost for purchasing electricity for the US and China is $131,000.

The administrative staff salaries are divided in half between the biomass facility and conversion facility as described in the Lundquist et al., (2010) study. The Lundquist et al., (2010) study states there will be 1 plant manager who will be paid $114,000 yr^{-1}, 1 supervisor of the operators paid $93,600 yr^{-1}, 1 lab manager paid $62,400 yr^{-1}, and 1 admin/secretary paid $17,700 yr^{-1}. All of these employees will also be paid benefits which will be 30% of the total salaries from these administrative personnel. This totals up to $375,000, but from the biomass facility perspective, $187,500. This cost will be the same for this study for the US. For China, they will be paid at a ratio of the higher end of China's minimum wage of $9.78 day^{-1} to the US federal minimum wage of $58 day^{-1}. This means the administrative costs for China total up to $31,600.

The costs for insurance, outside lab testing, and employee training for this study will be assumed to be the same as the Lundquist et al., (2010) study: $90,000, $25,000, and $5,000 respectively.

Since this system will not use vehicles, the costs for vehicle maintenance are $0.

The costs for lab & office supplies will be similar to the Lundquist et al., (2010) study, except that this study has a smaller collection facility and buildings required for lab testing. Therefore, less supplies will be needed. The cost for this component was scaled down for the amount of biomass produced from this system compared to the Lundquist et al., (2010) system. Therefore, the costs for lab & office supplies are $2,540 for this study.

Tables for the operating costs of this system for the US and China are shown below.

Location: USA

O&M Cost		**Justification**
Facility staff	$ 140,000.00	1 Collection facility operator + 67 Water Pumping staff
Maintenance	$ 55,300.00	2% Capital Cost
Electricity purchase	$ 131,000.00	Water Pumping cost+Collection facility electricity
Administrative staff	$ 187,500.00	Lundquist et al., 2010
Insurance	$ 90,000.00	Lundquist et al., 2010
Outside lab testing	$ 25,000.00	Lundquist et al., 2010
Vehicle maintenance	$ -	No Vehicles
Lab & office supplies	$ 1,610.00	Scaled down for decreased biomass productivity
Employee training	$ 5,000.00	Lundquist et al., 2010
Subtotal	$ 635,219.41	

Table 3-6: Operating and Maintenance Costs of Producing ABM Feedstock in US

Location: China

O&M Cost		**Justification**
Facility staff	$ 20,900.00	1 Collection facility staff paid at minimum wage + Brooming staff
Maintenance	$ 54,000.00	2% Capital Cost
Electricity purchase	$ 131,000.00	Water and algae pumps at collection facility
Administrative staff	$ 31,600.00	Lundquist et al., 2010 paid at China Wages
Insurance	$ 90,000.00	Lundquist et al., 2010
Outside lab testing	$ 25,000.00	Lundquist et al., 2010
Vehicle maintenance	$ -	15% of vehicle capital cost
Lab & office supplies	$ 1,610.00	Scaled down for decreased biomass productivity
Employee training	$ 5,000.00	Lundquist et al., 2010
Subtotal	$ 358,670.95	

Table 3-7: Operating and Maintenance Costs of Producing ABM Feedstock in China

Taking into consideration the ABM yield from the 100 ha field, the costs per ton of biomass produced and per gallon of gasoline equivalent are shown below for the US and China.

Biofuel Yield	
Biomass Harvest (mt/yr)	1921
Oil Production (barrel/yr)	5635
Cost per ton Biomass Harvest ($/ton)	$495.26
Cost per gallon oil produced ($/gal)	$3.9

Table 3-8: Yield and Prices for ABM in the US

Biofuel Yield	
Biomass Harvest (mt/yr)	1921
Oil Production (barrel/yr)	5635
Cost per ton Biomass Harvest ($/ton)	$347.41
Cost per gallon oil produced ($/gal)	$2.8

Table 3-9: Yield and Prices for ABM in China

Comparing the two tables, it is evident that operating in China is cheaper than in the US, so this means that operating in China makes the most sense. The US, on the other hand, is higher, but the cost for the feedstock (is lower per gallon than the value reported in the Lundquist et al., (2010) study, but higher per ton ($488 ton^{-1} and $6.61 gallon^{-1}). This means that although it is more expensive to do this in the US, it is still cheaper than other literature-reported feedstock costs in terms of cost per gallon.

To determine how much of a decrease in cost and where the costs were reduced, the cost breakdown between the different systems must be compared in the figures below.

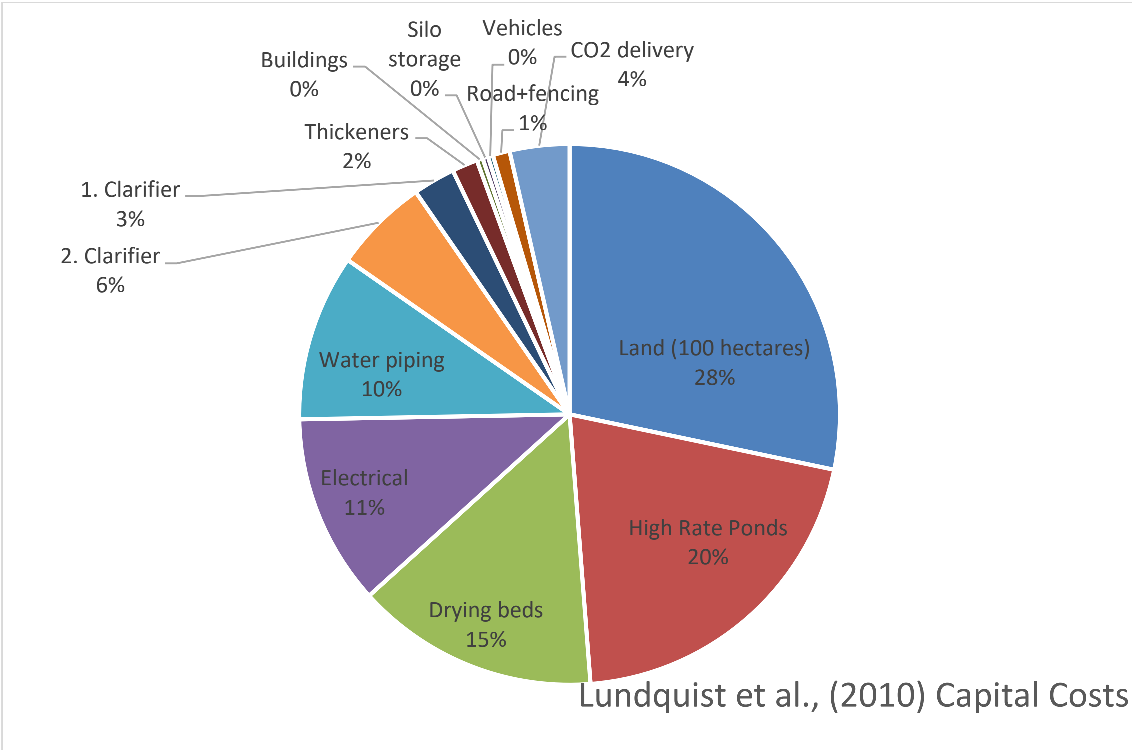

Figure 3-4: Cost Breakdown of Lundquist et al., (2010) Capital Costs

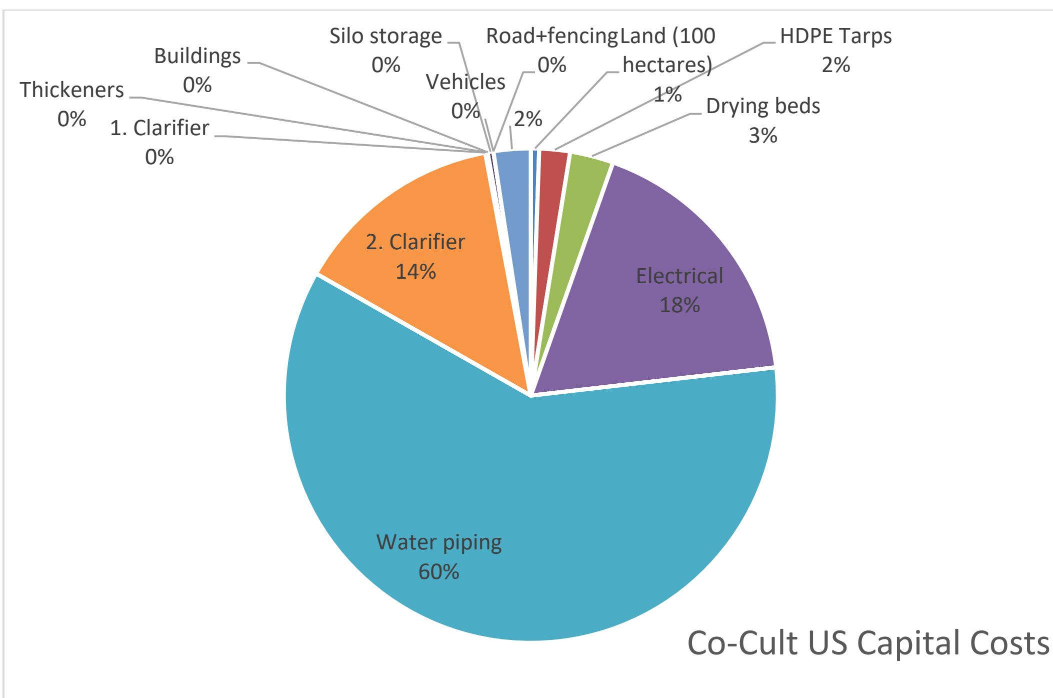

Figure 3-5: Cost Breakdown of Co-Cultivation in the US Capital Costs

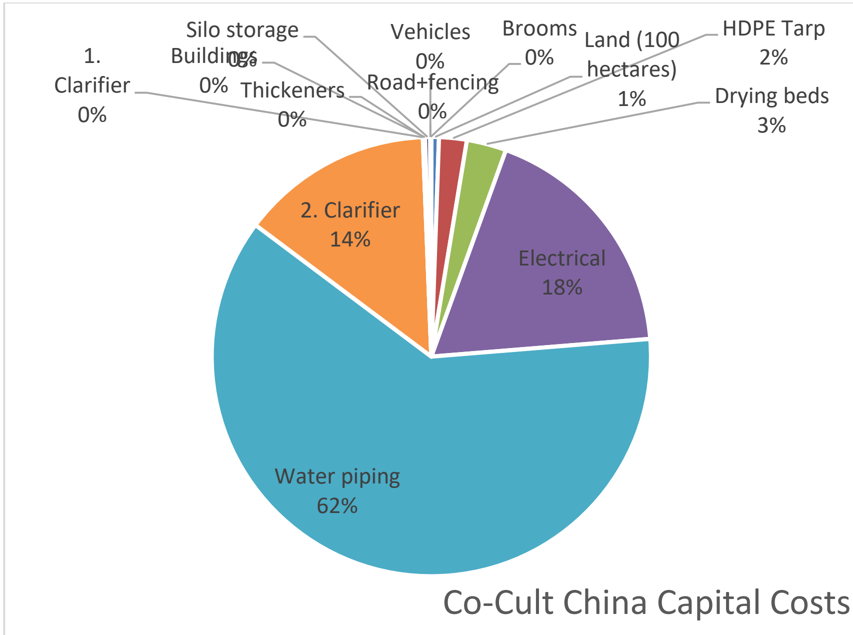

Figure 3-6: Cost Breakdown of Co-Cultivation in China Capital Costs

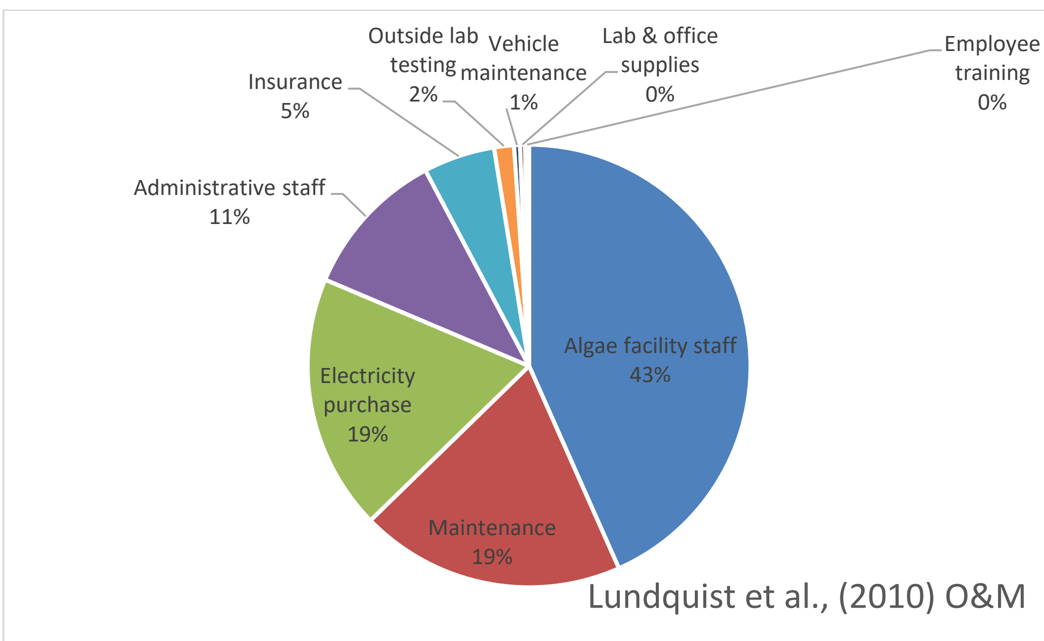

Figure 3-7: Cost Breakdown of Lundquist et al., (2010) Operating & Maintenance Costs

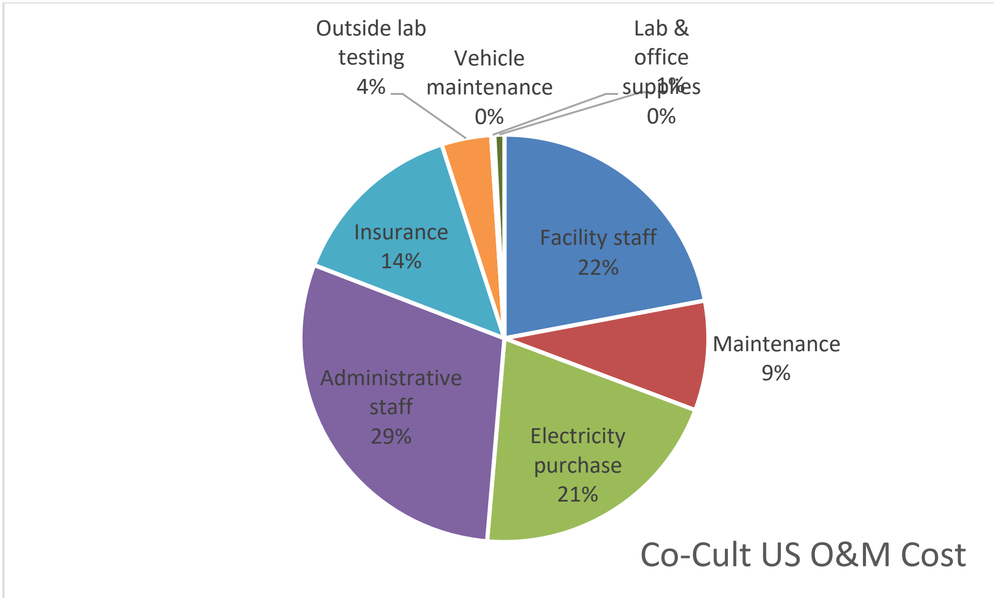

Figure 3-8: Cost Breakdown of Co-Cultivation in US Operating & Maintenance Costs

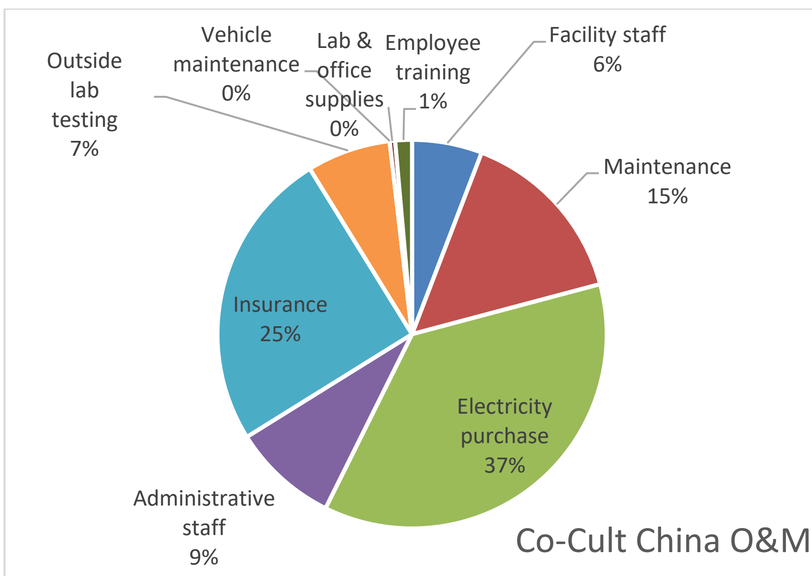

Figure 3-9: Cost Breakdown of Co-Cultivation in China Operating & Maintenance Costs

First, it is evident that the cost for land was formerly the largest fraction of the capital cost of algal biofuel production (28%). The percentage for land decreases to almost 0% for the co-cultivation systems. The high rate ponds component was the second largest fraction of the capital cost (20%). Though the co-cultivation system does not use high rate ponds, it uses a HDPE tarp instead, and this cost is only 2% of the total capital cost. For operating and maintenance costs, the administrative staff was almost half of the costs for the Lundquist et al., (2010) study, and that is reduced to almost 30%, now almost equivalent to the facility staff, for the co-cultivation case in the US. In China, however, this is further reduced to 9% due to the lower cost of labor. In this case, electricity cost is the largest fraction of the O&M cost due to the increased cost of industrial electricity in China. This makes sense because the aim of the China system was to use as much manual labor as possible since it was cheaper than the electricity cost, so whatever electricity that has to be used would make up most of the O&M costs.

These cost comparisons indicate that as far as algal biofuels are concerned, the cost for the feedstock is not as large of a factor as before (Jones et al., 2014). To see how this reduction in feedstock cost impacts the overall cost of producing fuel from algae, a cost breakdown of the different components in algal biofuel production is shown below.

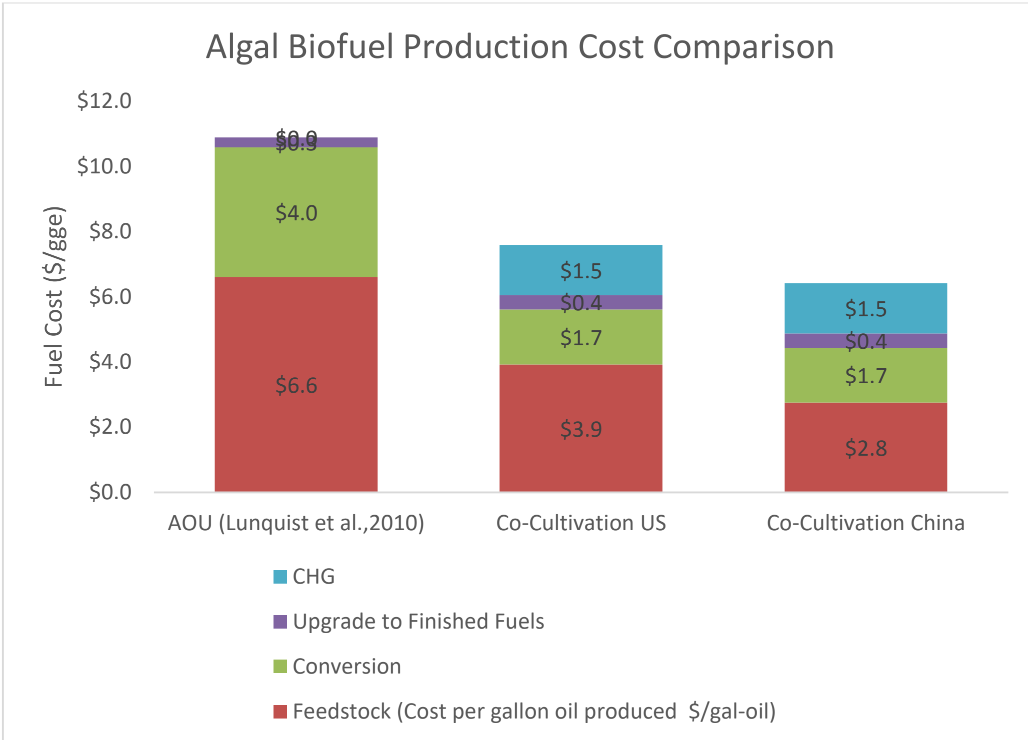

Figure 3-10: Algal Biofuel Cost Comparison of the Different Growing Scenarios

These are all costs before the byproducts are considered. Already, the cost for producing the fuel has been decreased from $10.9 gge^{-1} to $7.6 and $6.4 gge^{-1} for the co-cultivation systems in US and China respectively mostly due to the decreased feedstock cost. The byproducts for these scenarios include treated wastewater, and in the case of co-cultivation, rice grain. These byproducts will give credits toward the production facility decreasing the overall production cost. To see how much rice grain can be produced and the effects of growing rice with algae and wastewater, an experiment was conducted.

3.2.2 Greenhouse Experiment

To determine how their co-growth affects each other, a growing experiment was conducted inside a greenhouse. This experiment will look at how normal rice growth without the presence of ABM compares to rice grown in various wastewaters and the ABM they produce.

3.2.2.1 Objectives

The objective of this experiment is to determine how rice yield is affected by the presence of ABM as well the use of either swine manure lagoon effluent wastewaters or post hydrothermal liquefaction wastewaters in the rice paddy standing water.

3.2.2.2 Methods

3.2.2.2.1 Light Conditions

This experiment was set up at the Plant Care Facility Greenhouses at the University of Illinois at Urbana-Champaign and ran from January 21st, 2016 until July 8th, 2016. High Intensity Displacement (HID) lights were turned on between the hours of 6am and 10pm to establish a 16-hour photoperiod so as to supply extra light since the growing season was occurring during some winter months which would provide less sunlight. According to the Illinois State Water Survey's Illinois Climate Network data, the table below shows the average daily solar radiation over the whole month at the location of this experiment (Illinois Climate Network, 2016).

Month	Average umolphoton/m2/sec
January (11 days)	435
February	570
March	693
April	972
May	1090
June	1433
July (8 days)	929

Table 3-10: Average Daily Solar Radiation Over a Month During Growth Period

According to this table, it is evident that the winter months provide less sunlight than what is normally recommended for plant growth which is sunlight at 2000 μmol-photons m^{-2} sec^{-1}(Sharp, R.E. and Boyer, J.S., 1986) A diagram of the layout of the HID lights in the greenhouse as well as several solar radiation readings within the greenhouse is shown below.

Taken: 8/1/16 2:30PM CDT

Outside solar irradiance readings: U-184.84

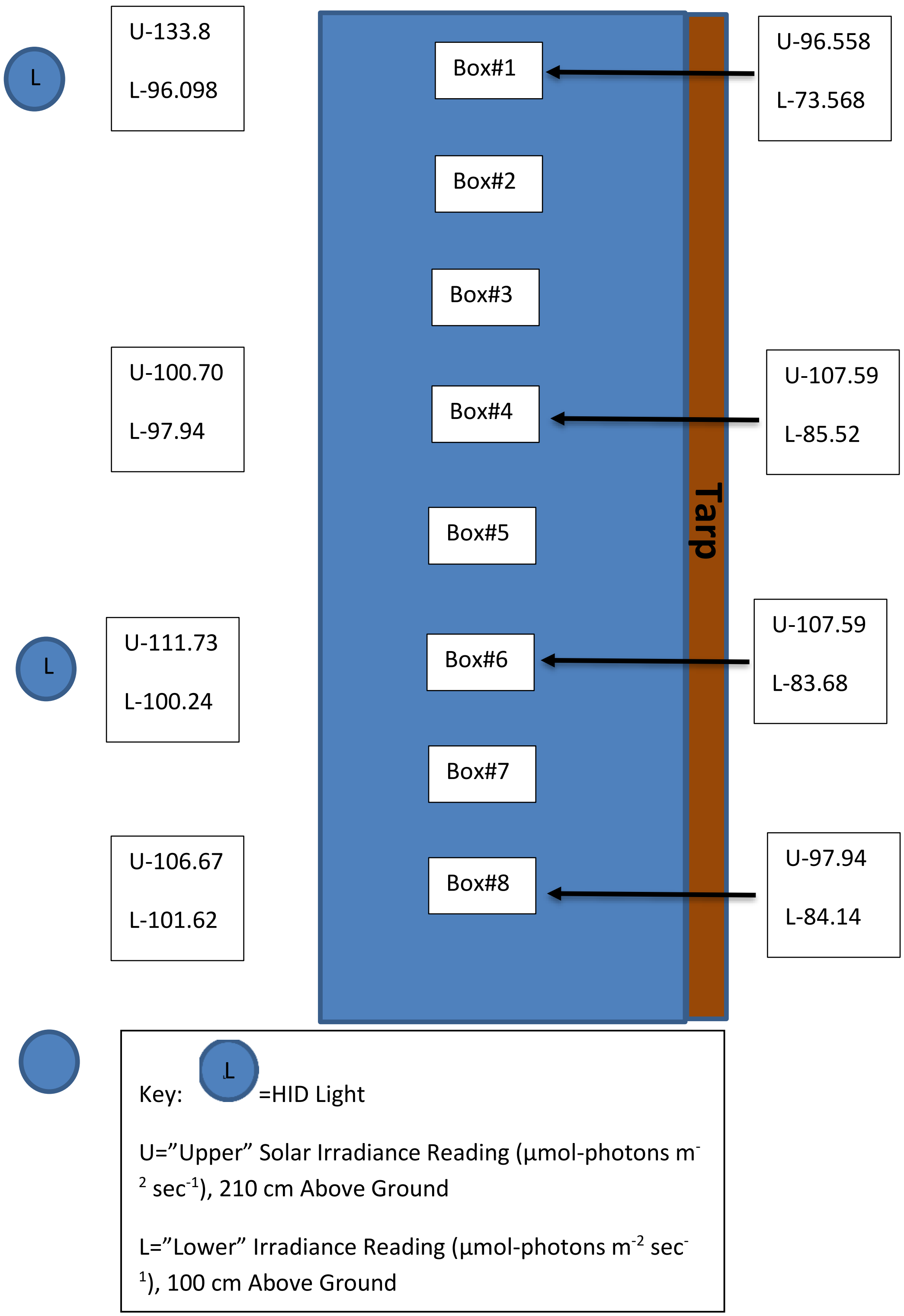

Figure 3-11: Greenhouse Solar Irradiance at 2:30PM CDT

Taken: 8/1/16 6:13PM CDT

Outside solar irradiance readings: U-16.09

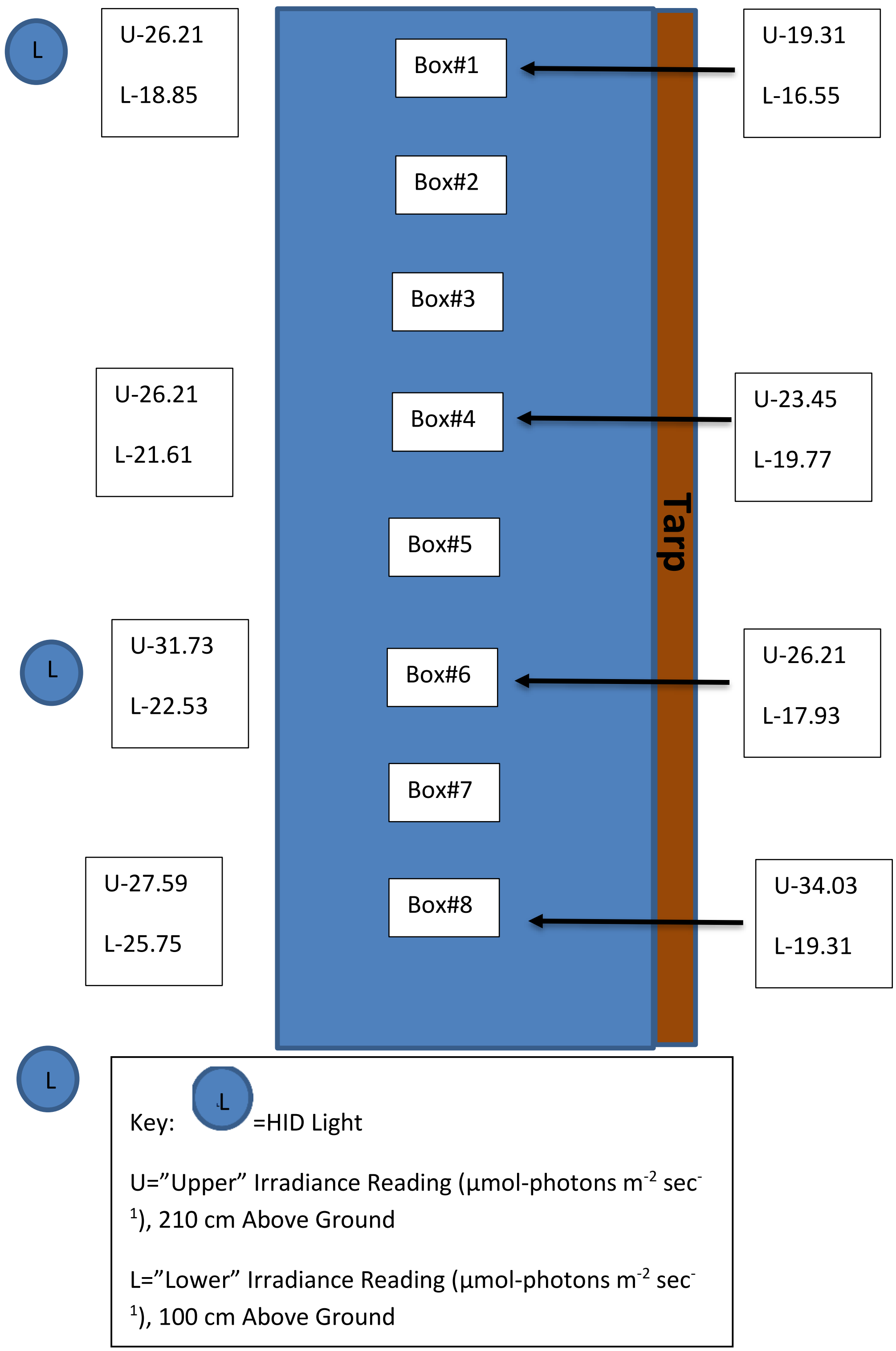

Figure 3-12: Greenhouse Solar Irradiance at 6:13PM CDT

These data were measured in the beginning of August, when the greenhouse facility had put whitewash on starting May 17th. These figures show that the greenhouse with whitewash severely restricted the amount of sunlight available to the rice to nearly 10% of what the sun provided. Overall, it is evident that the rice plants received less sunlight than normal over the course of the growing period which would explain why it took the plants longer to achieve harvestability.

3.2.2.2.2 Temperature Range

The temperature range selected for the experiment was based on the temperature range recommended for rice growth: 26-28°C during the day and 20-22°C at night as previously stated. Taking that into consideration, the temperature during the day inside the greenhouse was set to 26.67°C and 24.44°C at night. Since it was a greenhouse, the temperature was regulated to be the same every day.

3.2.2.2.3 Soil Selection

Since the preferred soil for rice growth is a clay-like material as stated in section 2.1.4, one must consider the reasons why rice grows well in this soil. The main reason is that clay-like soil will retain water for an extended period of time. For this reason, the soil used was placed inside clear plastic boxes with thin plastic sheeting placed on the inside as a protective barrier. The plastic boxes were purchased from a local farm supply store in Urbana, Illinois.

The soil consisted of screened garden compost from the Landscape Recycling Center in Urbana, Illinois and a drummer silty clay loam from Urbana, Illinois (USDA, 2016). The garden compost was mixed thoroughly with the drummer soil to achieve homogenization in the pile. To ensure the boxes had a representative sample of soil, soil was taken from three locations within the mixed soil: one from the east side, middle and west side of the pile and added to Box 1. This process was repeated for all 8 boxes. Achieving further soil homogenization was done by remixing each box's soil with other boxes. This process consisted of removing 1/3 from the first box and placing it into a separate "intermediate" container. Then the 1/3 of the second box's soil was removed and added to the first box. This first box was then remixed thoroughly. The "intermediate" container's soil was then added to the second box and this box was then remixed thoroughly as well. This process was repeated for all 8 boxes two times over to ensure the soil in each box was representative of the other. After homogenization, the soil was manually aerated by using a hoe as well as shovel. This was done to ensure ease of plant root movement through the soil. Soils were kept at indoor temperatures at all times before and during box preparation.

3.2.2.2.4 Soil Depth

The soil combination was added to each box and was then homogenized several times over. After the soil was broken up using a hoe, excess soil was removed from the top to achieve a soil height of 20 cm. This is to ensure the roots have enough room to spread out. A minimum 10 cm of space between the soil line and the top of the container for space for 10 cm of water was the desired amount of space, but since space was limited and to prevent over spilling, 5 cm of water was used. The diagram below shows a representation of the height of the soil compared to the height of the box.

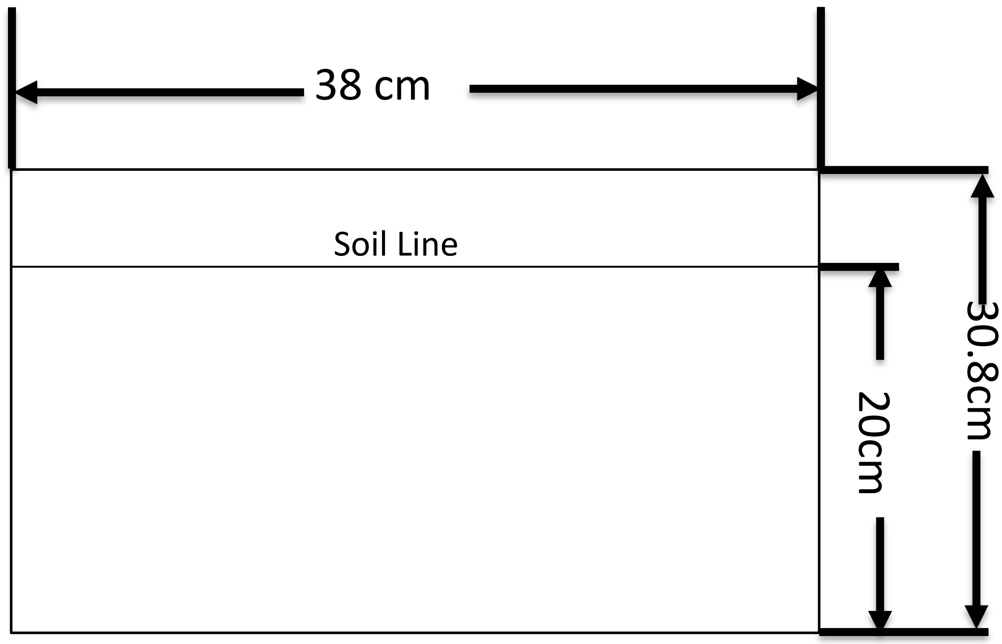

Figure 3-13: Side View of Box Containing Soil and Rice Seeds

3.2.2.2.5 Rice Seed

Cocodrie rice seed was obtained from Mississippi State University's Delta Research and Extension Center. Cocodrie is considered a semi-dwarf long-grain rice and has a relatively short growing season (Wilson et al., 2014) that was selected due to several desirable characteristics. A relatively short-growing season variety was desired that had shorter and thinner stalks. The shorter growing season would theoretically allow a rice farm to complete two cycles of growth so as to double the rice yield of the short growing season rice variety. The shorter stalks would also allow for more light to penetrate through to the water so as to allow more algae to grow.

3.2.2.2.6 Seed Pre-Germination

For this experiment, a pre-germinated seeding rate of 13 seeds ft^{-2} was used. Therefore, 200 seeds were placed in a tupperware container with room temperature water for 24 hours and then placed on a damp paper towel spaced so that each seed was not touching another. The paper towel was then rolled up and placed inside a plastic Ziplock bag for 1 week to allow the seeds to sprout. The seeds were then taken out of the plastic bag and placed in the soil holes in the boxes as described in the next section.

3.2.2.2.7 Plant Spacing

In each planting box, two holes with a two centimeter depth were dug out of the soil 22.5 cm apart using fingers. A diagram below shows the layout view from the top of the boxes. Five pre-germinated seeds were placed in each hole of each planting box.

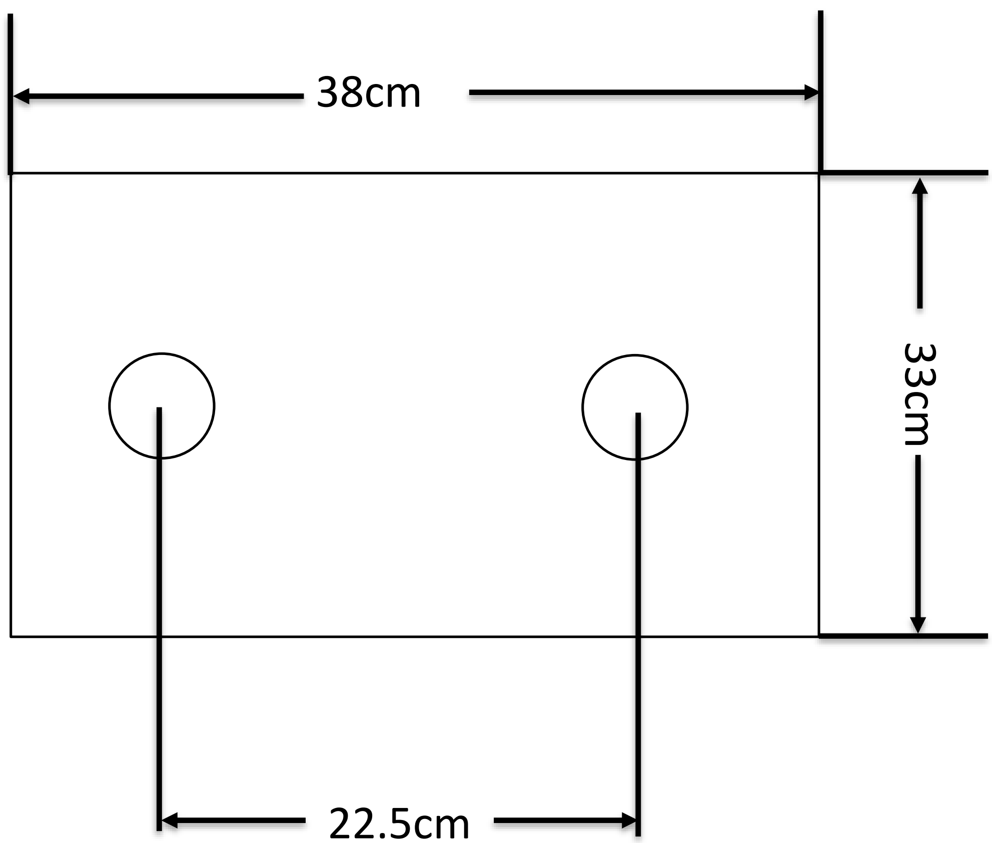

Figure 3-14: Top-Down View of Box Containing Soil and Rice Seed

A picture below shows the prepared boxes after the seeds were placed in their holes.

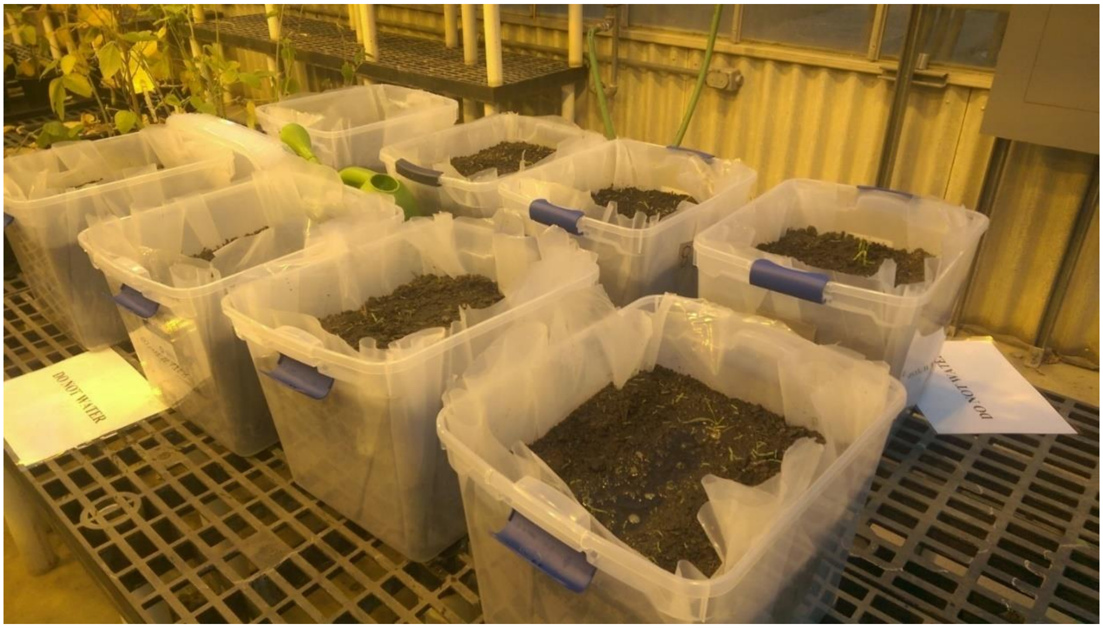

Figure 3-15: Initial Greenhouse Set Up

3.2.2.2.8 Nutrient Supply

The way water and nutrients were supplied to the system were key variables for this experiment. First, a condition representing “normal” rice growth was defined as using tap water with synthetic fertilizer and without the presence of ABM. Second, since the proposed system uses plastic sheeting as a way to harvest the ABM, the effects of plastic sheeting (while still using tap water) on rice growth must be monitored, so that is the second condition. A third growing condition used a fairly dilute wastewater to provide nutrients for ABM growth. And the final experimental condition was used to demonstrate the Environment-Enhancing Energy Process by using a diluted post-hydrothermal liquefaction wastewater (PHWW) to grow the ABM. This experiment was designed to demonstrate the ability to grow rice and ABM together, process the ABM to make fuel, and reuse the wastewater from the fuel production process to grow more ABM. Each condition had 4 replicates with each box representing a duplicate. Each condition had 11.5 g of synthetic fertilizer applied 41 days after sowing based on the recommendation from the Arkansas Rice Handbook of applying 105 lbs of nitrogen acre^{-1} before flooding (Roberts et al., 2014). The synthetic fertilizer was Hyponix 13-13-13 N-P-K fertilizer made from ammonium phosphate, potassium chloride, and urea. When adding the initial amount of wastewater to the wastewater conditions, this will in effect add more nutrients to these conditions providing an unfair advantage to these conditions. Additionally, rice crop fertilization typically occurs in two stages, one before flooding and one mid-season according to Roberts et al., (2014). Taking this into consideration, since 3.135 g of Nitrogen would be added from the initial wastewater dosing as discussed in the next section, 24.1 g of the Hyponix fertilizer had to be added to the conditions that will not receive wastewater. This was applied 61 days after sowing. A table of the different conditions is listed below.

Treatment	**Synthetic Fertilizer**	**Plastic**	**Tap Water**	**Algae/Bacteria Mix (ABM)**	**Swine Manure Lagoon Effluent**	**PHWW**
Water + No Plastic	Yes	No	Yes	No	No	No
Water + Plastic	Yes	Yes	Yes	No	No	No
Swine + Plastic	Yes	Yes	No	Yes	Yes	No
Swine/PHWW + Plastic	Yes	Yes	No	Yes	Yes	Yes

Table 3-11: Greenhouse Experimental Conditions

The figure below depicts how the plants were laid out in the greenhouse before the wastewater was added.

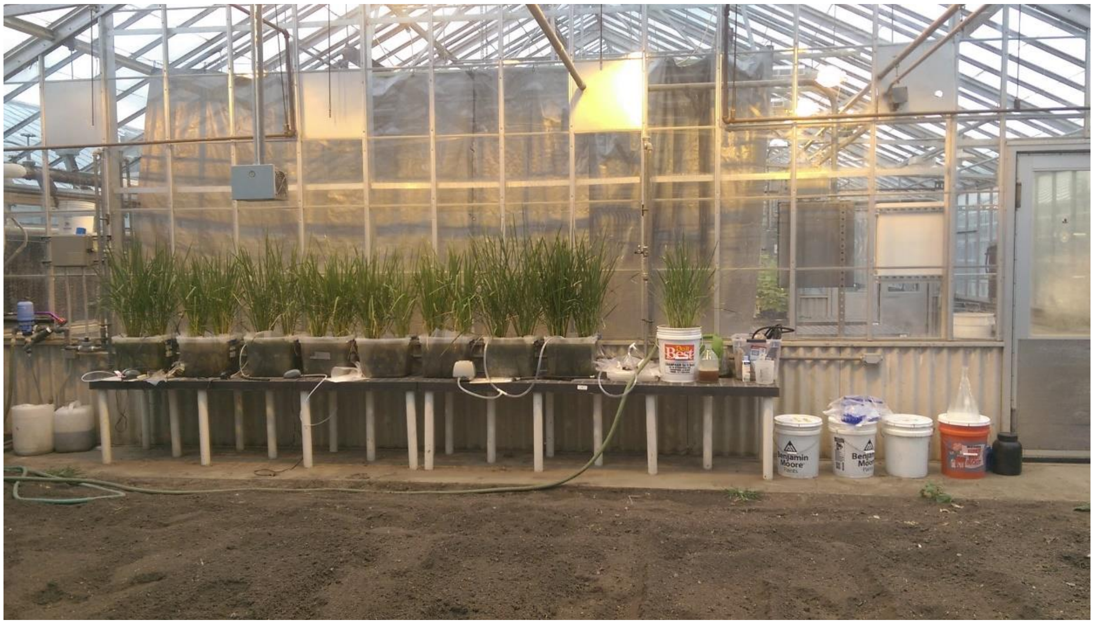

Figure 3-16: Experimental Conditions Layout, Boxes 1-8 (1 to the left)

3.2.2.2.9 Water Feeding

The next step in the experimental design is to determine how much water should be added and when. The recommended minimum fertilizer application dedicated for solely algae growth was 350 mg L^{-1} of nitrogen, and it was determined that 500 mg L^{-1} of nitrogen would provide better growth. To find a wastewater that was that concentrated, a local source of liquid portion of animal manure (LPAM) was selected. This could provide 1183 mg L^{-1} of total Nitrogen. A table below shows the characteristics of the LPAM.

	LPAM	
all units in mg L^{-1}	Typical	Range
TS	8778	3405 - 16160
VS	3995	1141 - 8875
TSS	10	0 - 50
COD	15690	2004 - 81172
TN	1183	500 - 2440
NH3-N	6437	1640 - 9898.85
P	197	105 - 225
K	520	
Na	160	
Ca	48	
Fe	12	
Zn	0.21	
Cu	<0.01	

Table 3-12: LPAM Characteristics

In order to provide the desired 500 mg L^{-1} of TN, the LPAM had to be diluted with a much more dilute water while still trying to use a source of wastewater. Filtered swine manure lagoon effluent from the local swine farm in Urbana, IL provided 22 mg L^{-1} of total nitrogen, so this was the water used to dilute the LPAM. Each box having 5 cm deep of water was desired as stated in section 3.2.2.2.4, so this meant that each box had 6.27 liters of water. It was then determined that in order to provide 500 mg L^{-1} of TN from a water that has 1183 mg L^{-1} of TN and one that has 22 mg L^{-1} of TN, in a 6.27 liter space, that meant that 2.58 liters of LPAM and 3.7 liters of swine lagoon effluent could provide the desired amount of nitrogen for ABM growth. This was for the conditions that had wastewater but no PHWW. For the treatment with PHWW, a 500 mg L^{-1} of TN was desired, but this time, half of the nitrogen would come from the PHWW and half from the LPAM/lagoon effluent. Below is a table of the characteristics of the PHWW.

COD/ mg L^{-1}	TN/ mg L^{-1}	NH_4^+-N/mg L^{-1}	TP/ mg L^{-1}
840-118,000	4,752-8,651	1,860-7,070	3-1,068

Table 3-13: The Characteristics of the PHWW from Swine Manure (from Appleford, J.M., 2004)

Based on these characteristics, and assuming a TN concentration of 10,000 mg L^{-1} for PHWW, it was determined that 157 ml of PHWW, 4.8 liters of swine lagoon effluent, and 1.33 liters of LPAM were required to provide 500 mg L^{-1} of TN for each box. After the initial dosing of nutrients to the reactors, swine lagoon effluent was added every day to the regular wastewater conditions in the amount of 1.2 liters as this was the amount that was taken up by the rice plants. To find the amount of water evaporating over time, a side box of just soil was placed with 5 cm of water above it, and no decrease in height of the water was measured over the course of a week. The addition of 1.2 liters of swine lagoon effluent was done in order to provide some amount of nutrients continually (22 mg L^{-1} TN) for ABM growth, but still have a relatively low amount so as not to cause an excess of nitrogen which could potentially harm rice growth as reported from Singh, K.K. and Mishra, L.C. After a week of adding swine lagoon effluent, a floating ABM species began to grow in the wastewater reactors that was not there during inoculation which will be discussed in section 3.2.2.2.10. An insignificant amount of evaporation occurred throughout the experiment. For the PHWW conditions, the same amount of nitrogen had to be added to the reactors as the regular wastewater conditions but with a much more concentrated wastewater. To accomplish this, it was determined that since 1.2 liters of swine lagoon was added over a week period, this would add 185 mg of TN. To provide this with

PHWW and swine lagoon effluent, 15 ml of PHWW was added once during the week for the duration of the experiment while 1.1 liters of swine lagoon effluent was continually added to the PHWW reactors every day. Since fresh wastewater had to be added every day, this in effect added more nutrients to these reactors relative to the reactors that did not have wastewater. The wastewater reactors got 7.198 g of Nitrogen over the course of the growing season while the regular water reactors received 4.6108 g of Nitrogen over the course of the growing season.

3.2.2.2.10 Algae Inoculation

At first, mixed-culture algae from a previous student's work was inoculated into a 15 liter photobioreactor (PBR). This culture consisted mostly of chlorella species algae as depicted below.

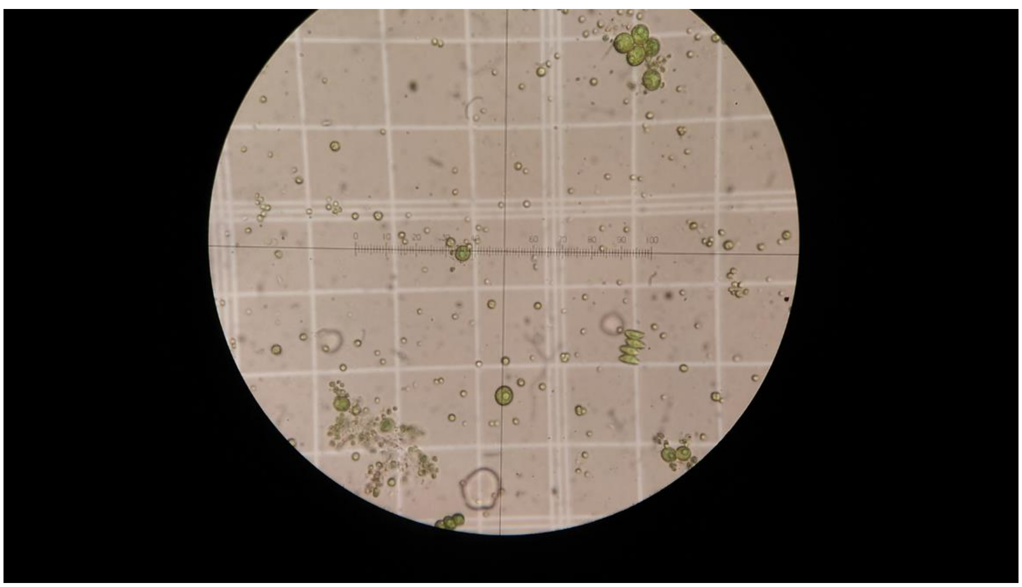

Figure 3-17: Photobioreactor Mixed Algae Species Under Microscope

This reactor was slowly scaled up until it reached 15 liters of 1000 mg L^{-1} concentrated algae. The figure below shows the algae PBR used in this study for growing algae seeding cultures to be added to the rice cultivation boxes.

Figure 3-18: Mixed Species Algae Photobioreactor

Unfortunately, the culture then experienced some very stark pH changes that killed off a large portion of the algae. The culture was then brought back to health until it achieved a density of 1000 mg L^{-1} again. The figure below depicts what the algae looked like under a microscope.

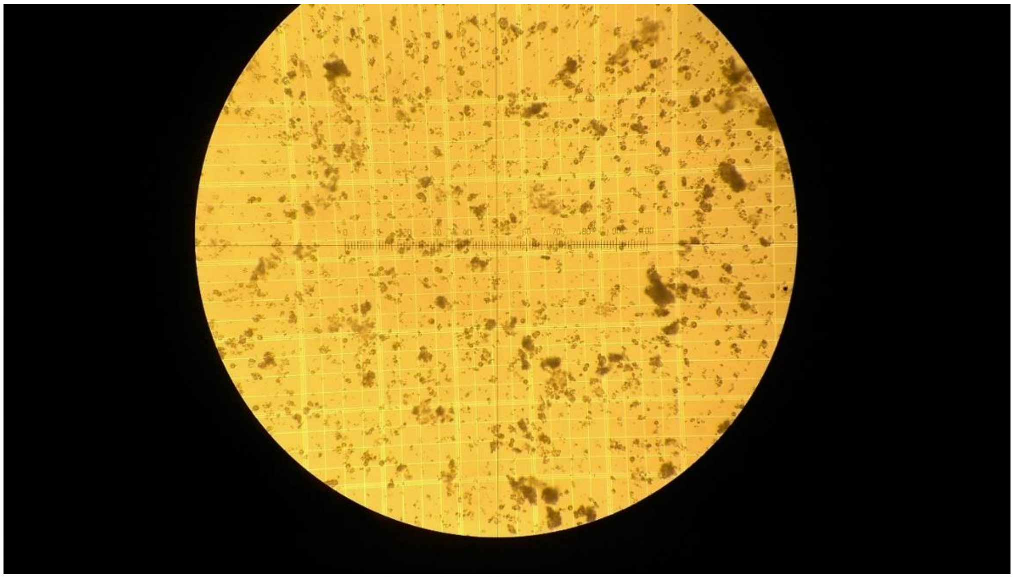

Figure 3-19: Algae After Recovery Attempt

This was then inoculated into the greenhouse 6.27 liter reactors at a concentration of 600 mg L^{-1} or a 60% dilution ratio. The initial dose of wastewater was added as a part of this inoculation. After one week of adding swine lagoon effluent, a floating ABM species started to take dominance over the pre-inoculated species. This wild ABM grew in each of the containers and was then collected so as to make sure each scenario had the same amount (or lack thereof) of biomass. The ABM was then placed in the rice reactors that ABM was supposed to grow in (i.e. the wastewater rice reactors). In order to understand what the ABM species was composed of, the ABM species grown for 57 days in reactor 1 (swine lagoon effluent) and reactor 8 (PHWW) were submitted for CHN analysis to the Microanalysis Lab at the University of Illinois. A table of the CHN characteristics of this ABM is listed below.

Element	Swine Lagoon ABM AVG (%)	STDEV	PHWW ABM AVG (%)	STDEV	Theoretical (Sudhakar, K. and Premalatha, M., 2015)
C	26.23	0.551543	29.825	1.873833	25
H	3.42	0.014142	4.04	0.311127	8
N	3.59	0.226274	4.355	0.544472	5
O	28.2		23.22		12

Table 3-14: CHN Characteristics of the Different Algae Biomass Used in this Study

The oxygen concentration was calculated as the difference as suggested by Hampel, Kristen, 2013. This table clearly shows that the PHWW-grown ABM has a higher percentage of C, H, and N. The only issue is that the sample was far less consistent than the swine lagoon effluent-grown ABM. Overall, the C values were higher than previously reported algae species C content. This would suggest that the fuel produced from this study's ABM would be of a higher quality since it has more carbon compared to the *Scenedesmus sp*. from Sudhakar, K. and Premalatha, M, (2015)'s study. This would imply that the PHWW-grown ABM in this study would produce an even higher quality fuel since its carbon content is even higher than the swine lagoon ABM. The ABM grown in this study, however, have a lower hydrogen content which could have implications on the amount of fuel produced as discussed in the previous section.

3.2.2.3 Results and Discussion

3.2.2.3.1 Rice Plant Height Growth

In order to determine if the rice plant was growing well over time, the height of the rice plants from the bottom of the stems of the plants to the tallest leaf blade were measured. Since the common method for measuring the height of the plant leaves is normally done at the end of harvest and takes a considerable amount of time, a non-standard method was used to measure the heights to ensure this data could be collected quickly. 2 of the tallest leaf blades from each "section" -or what was formerly a hole for the seeds- were measured from each box. This was done about every 2 weeks over the course of the growth period. The figure below depicts that growth curve.

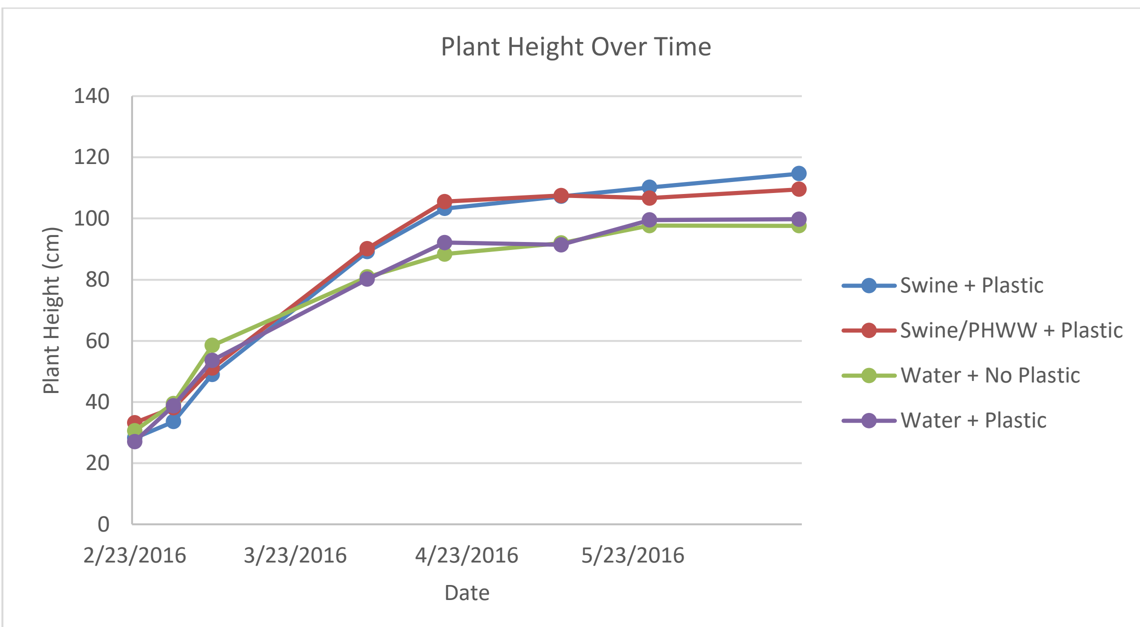

Figure 3-20: Rice Plant Heights Over Time

In this graph, there are a few points where it looks like the plant height decreased as time progressed. The reason for this is most likely due to sampling technique. As the rice plant grows, leaves start to fall over as they start to yellow. Newer rice stalks begin to take over and grow taller. This falling over of the leaves is most likely the reason why some data points look like the plant got smaller because as the leaves fall, other stalks that stand straight are measured and they might not be as tall as the leaf that fell over. Overall, it is clear that the treatments with wastewater are the tallest at the end of the growth cycle. A more accurate representation of the yields of each condition will be discussed in the next section.

3.2.2.3.2 Rice Grain Yield

To determine how the rice growth was affected by the different conditions being tested, the Standard procedure for determining yield components at harvest by the International Rice Research Institute was used. The most important components of the yield to look at are 1000 grain weight and yield. All of the data will be compared to data found in the literature about Cocodrie rice yields. The literature yield was determined based on the yield you would get from Cocodrie if the seed was dispersed at the same rate that the experiment was. Since 10 seeds were planted per box, that results in a 15 seeds ft^{-2} seeding rate. Although this is on the low end of seeding rates, this is acceptable because the seeds were pre-germinated.

Cultivar	Seed Weight	Seeds/lb	Number of Seed/Sp. Ft								
			10	15	20	25	30	35	40	45	50
			Seeding Rate, lbs/A								
Cocodrie	25.6	17,734	--	--	49	61	74	86	98	111	123
Francis	22.8	19,912	--	--	44	55	66	77	88	98	109
Jazzman	25.2	18,016	--	--	48	60	73	85	97	109	121
JES	26.5	17,132	--	--	51	64	76	89	102	114	127
Jupiter	25.8	17,597	--	--	50	62	74	87	99	111	124
Mermentau	23.3	19,460	--	--	45	56	67	78	90	101	112
Presidio	24.3	18,683	--	--	47	58	70	82	93	105	117
Rex	27.6	16,449	--	--	53	66	79	93	106	119	132
RT CL XL 729	21.79	20,835	21	31	42	--	--	--	--	--	--
RT CL XL 745	21.70	20,922	21	31	42	--	--	--	--	--	--
RT XL723	21.14	21,476	20	30	41	--	--	--	--	--	--
RT XL753	20.50	22,146	20	30	39	--	--	--	--	--	--
Roy J	22.9	19,825	--	--	44	55	66	77	88	99	110
Taggart	27.4	16,569	--	--	53	66	79	92	105	118	131
Templeton	22.7	20,000	--	--	44	54	65	76	87	98	109
Wells	25.2	18,016	--	--	48	60	73	85	97	109	121

Table 3-15: Seeding Rates for Different Seeds per Square Foot Based on Seed Weight (from Wilson et al., 2014)

This table reveals the weights of different cultivars, and is evident that the cultivar used in this experiment, Cocodrie, has a very similar seed weight to the Wells cultivar. This will be important in determining the yield of Cocodrie later on. After taking these various seeding rates into consideration, the yield of each cultivar can be found. Wilson et al., (2014) reports the values for only a few of these cultivars, and that table is shown below.

Seed Rate	**Grain Yield**			
	Bengal	**CL161**	**Francis**	**Wells**
Lbs/acre	**Bu/acre**			
45.0	154	135	132	141
67.5	160	136	136	144
90.0	159	143	155	145
112.5	163	146	141	147
135.0	161	149	144	147
LSD	22			

Table 3-16: Influence of Seeding Rate on Grain Yields of Five Rice Varieties Averaged Across Five Locations in 2004 and 2005 (Wilson et al., 2014)

Since this table does not have Cocodrie rice yields listed, one of the four cultivars must be chosen and the one that is most similar to Cocodrie is Wells, as addressed in the previous table. With this in mind, looking at the yield of Wells given the seed rates, Cocodrie's yield at a similar seed rate can be determined. Since the seed rate used in the experiment was slightly smaller than the ones listed here (15 seeds ft^{-2} is 38 lbs $acre^{-1}$ for Cocodrie), the yield must be determined via linear extrapolation. The extrapolation equation is as follows:

$$\frac{67.5lbs/acre - 45lbs/acre}{144bu/acre - 141bushels/acre} = \frac{45lbs/acre - 38lbs/acre}{141bu/acre - x\ bu/acre}$$

$$x = 140.066 \frac{bushels}{Acre}$$

$$140.066 \frac{bushels}{acre} * 45 \frac{lbs}{bushel\ of\ rice} * \frac{1\ metric\ ton}{2204.62lbs} * 2.47105 \frac{acre}{1hectare}$$

$$= 7.06 \frac{metric\ ton}{hectare}$$

This is the yield that the experimental data will be compared to.

The most important factor in determining the growth of the rice plant is the yield or the amount of grain per unit area the rice can produce. The figure below shows the yield of the different treatments.

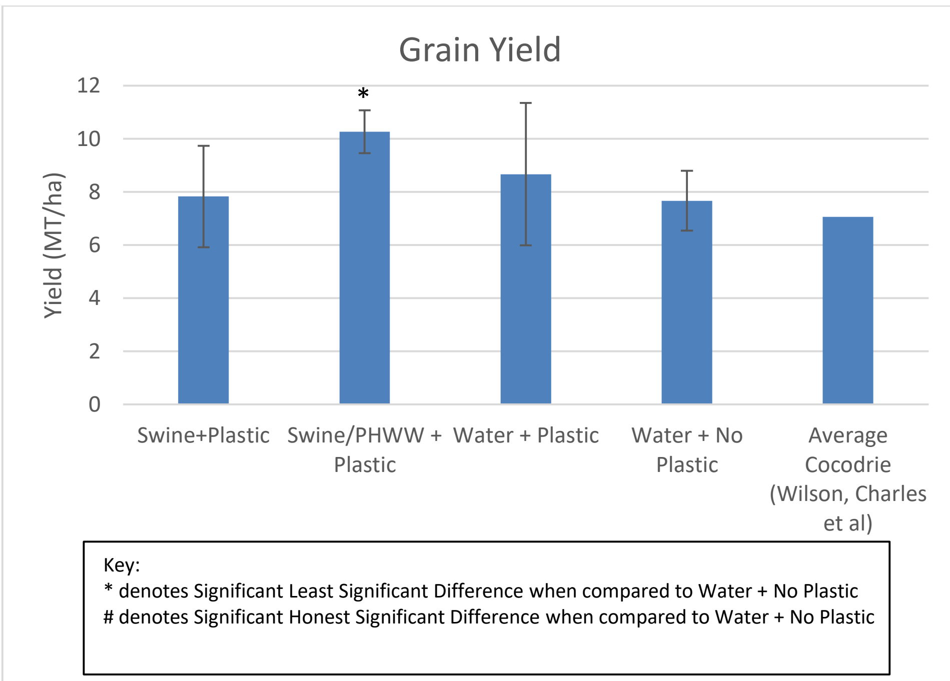

Figure 3-21: Rice Grain Yield Between Different Treatments

From this figure, the data do not show a clear trend when comparing regular water addition and wastewater/ABM. Overall the different treatments had a higher yield compared to literature-reported yields. This could be because of the smaller spacing between the rice plants (22.5 cm x 22.5 cm), or more likely because this experiment was in a greenhouse where conditions are optimal for plant growth. Greenhouses do not have wind which can have harmful effects to the seed establishment, and blow other things into the rice that could weigh on the rice and ultimately kill the rice plant. Other benefits of being in a greenhouse is the more consistent temperature conditions. Sometimes temperatures in nature will go to extremes which can be harmful to plants, which could decrease their yield in nature (the literature yields) while the experimental yields were not affected by temperature extremes. Looking past the overall experimental yields relative to the literature yields, it is important to discuss the relation amongst the different treatments relative to each other. At first glance, it appears that the yield from the Swine/PHWW with plastic treatment has a larger yield than all other treatments. This would suggest that whatever additional nutrients that were in the PHWW were beneficial to the rice grain yield. The Water + Plastic treatment seems to have the second highest yield and a much larger yield compared to the regular growth with tap water and no plastic. On the other hand, the Water plus Plastic treatment does have the largest amount of standard deviation meaning that this yield could be highly speculative. Intuitively this does not make sense why plastic would enhance the yield of the rice. It is therefore essential to conduct a statistical analysis with ANOVA on this data as the variation might have an impact on the results. All ANOVA analysis for the experiment was conducted on the data using the Analysis Toolpak in Microsoft Excel. The table below shows the ANOVA analysis on the data.

Yield (MT/ha) ANOVA
Anova: Single Factor

Yield

Groups	Count	Sum	Average	Variance	Stdev
Swine+Plastic	4	31.32	7.83	3.67	1.91
Swine/PHWW + Plastic	4	41.10	10.27	0.65	0.81
Water + Plastic	4	34.68	8.67	7.20	2.68
Water + No Plastic	4	30.68	7.67	1.27	1.13
Average Cocodrie (Wilson, Charles et al.)			7.06		

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	17.0634	3	5.6878	1.779477	0.204531	3.490295
Within Groups	38.356	12	3.196333			
Total	55.4194	15				

Table 3-17: ANOVA Analysis on Grain Yield

The F score relative to the critical F score and p-value all suggest that these data are not statistically different. Post-hoc analysis was conducted after each ANOVA test. The two post-hoc tests used were the least significant difference (LSD) and Tukey's honest significant difference (HSD). Statisticians believe that the HSD test is more accurate in determining statistical significance, but the LSD test is still used commonly. The LSD test showed that the increase in the Swine/PHWW mix grain yield was statistically significant. In contrast, the HSD test revealed that it was not according to its criteria. However, since the LSD test showed that the Swine/PHWW mix grain yield was significant for its criteria, it can be inferred that the Swine/PHWW mix can increase the grain yield. Overall, it is evident that the data show that co-cultivation of rice and algae does not decrease the rice yield compared to growing rice by itself while showing a potential for Swine/PHWW-grown rice to increase yield. Therefore, at worst, the yields of all the rice were the same. To determine the quality of the rice grain, thousand grain weight must be found.

The figure below shows the thousand grain weight, or the weight of 1000 individual rice grains, based on the components of yield.

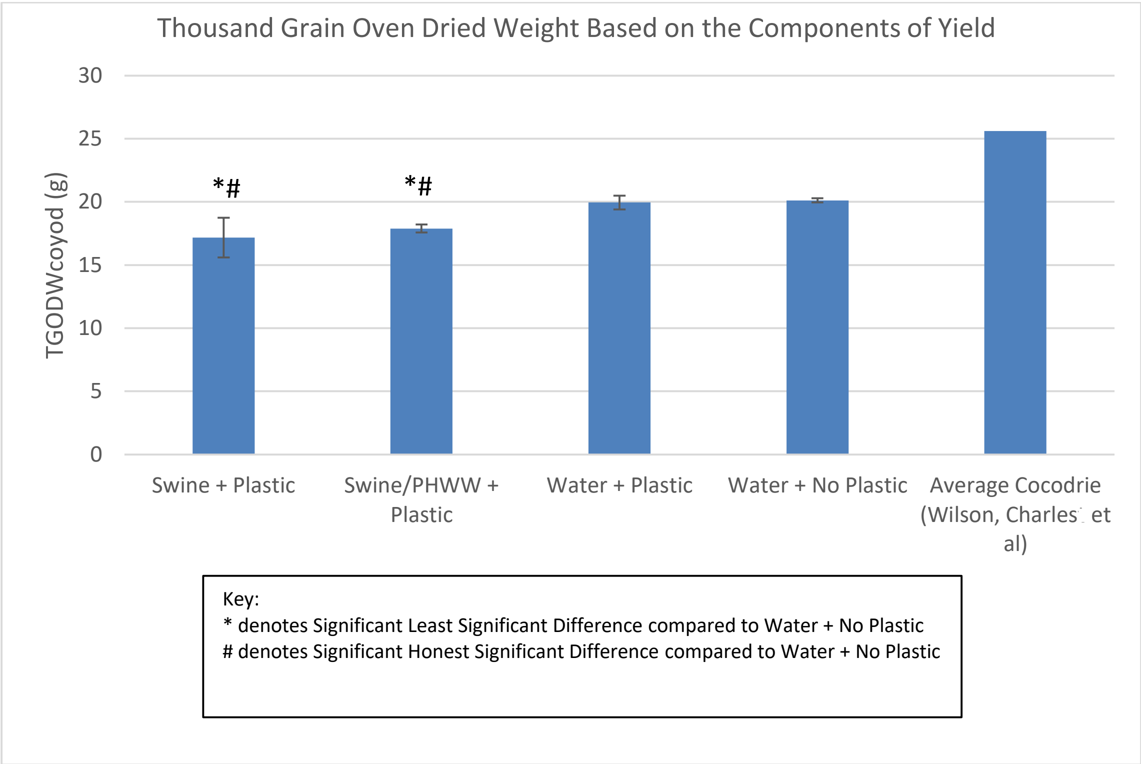

Figure 3-22: The Effect of the Different Conditions on Thousand Grain Oven Dried Weight Based on the Components of Yield (TGODWcoyod)

First off, it is evident that the overall thousand grain weight of the experiment is considerably lower than that reported by Wilson et al., (2014). The difference, however, is that this thousand grain weight is based on the components of the yield. This means that the method of obtaining the 1000 grain weight is different from my samples compared to the Wilson et al., (2014) literature since the Wilson et al., (2014) method dries the material to 14% compared to the components of yield method used in this study where the material is dried to 80 degrees over 4 days. For this reason, this study's sample grains will be more dried out and therefore lighter than the Wilson literature grain. This along with the fact that they received less sunlight than normal as described in Table 3-10 would explain the difference between this study and the Wilson et al., (2014) study.

When comparing the experimental conditions with each other, it is evident that the treatments that did not have wastewater and therefore, ABM, have a higher 1000 grain weight. Whether this is a statistically significant difference will be revealed by ANOVA, LSD, and HSD analysis. The results from this data set are shown below.

Thousand Grain ANOVA
Anova: Single Factor

Thousand Grain Oven Dried Weight based on the components of yield

Groups	*Count*	*Sum*	*Average*	*Variance*	*Stdev*
Swine + Plastic	4	68.65	17.16	2.45	1.57
Swine/PHWW + Plastic	4	71.55	17.89	0.10	0.32
Water + Plastic	4	79.78	19.94	0.29	0.54
Water + No Plastic	4	80.47	20.12	0.03	0.17
Average Cocodrie (Wilson, Charles et al.)			25.6		

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	26.22761	3	8.742538	12.15744	0.000599	3.490295
Within Groups	8.629319	12	0.71911			
Total	34.85693	15				

Table 3-18: ANOVA Analysis on the 1000 Grain Weight

This tables shows that from both the p-value (.000599) being much less than .05 and the F score (12.157) being higher than the critical F score (3.4903) that these data are statistically different. This means that it can be said with relative certainty that the presence of wastewater and ABM will decrease the 1000 grain weight of the rice. The sampling size does not hinder the statistical significance of the data. The difference in the thousand grain weight between the swine and Swine/PHWW is very small, so further analysis must be conducted to determine if the difference is enough to justify making the claim that Swine/PHWW mix increases the thousand grain weight of the rice relative to swine wastewater treatment.

Other yield components other than yield and 1000 grain weight can be used to confirm these data. The first that will be looked at is the Number of Panicles m^{-2}. Panicles are the parts of the rice plant that have the actual rice grains, so these data are representative of how much rice grain it can produce (although not directly the amount of rice grain). The figure below shows these data.

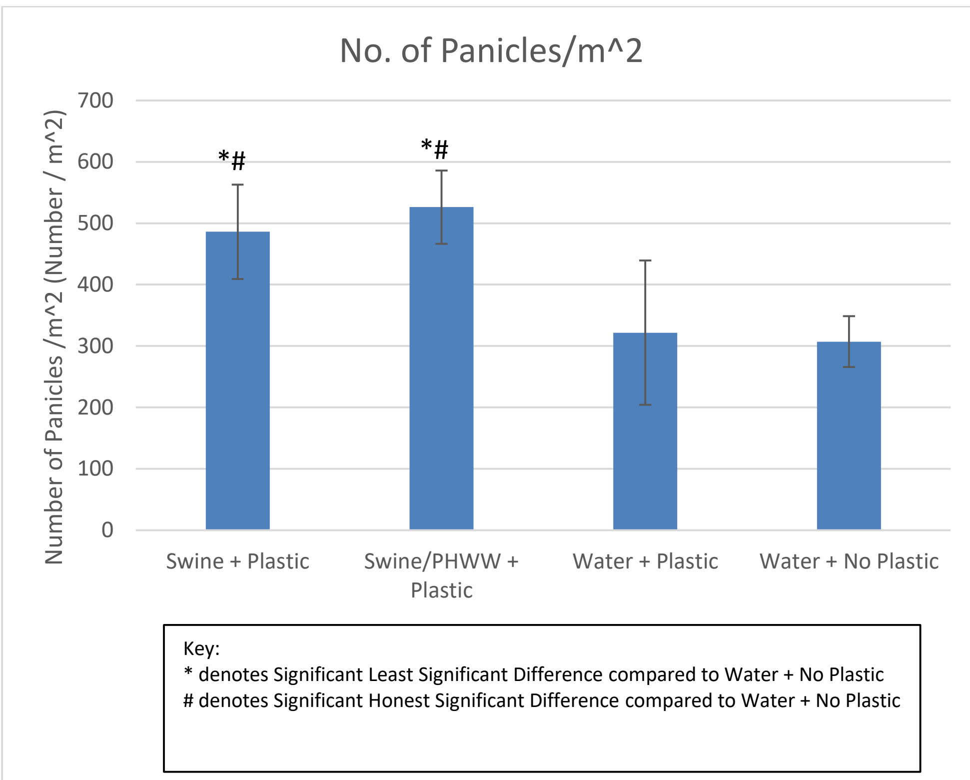

Figure 3-23: Number of Panicles m^{-2}

Here, it looks like the wastewater treatments produce more panicles per unit area with relatively small variability. This appears to indicate that the wastewater-grown rice produces more rice stalks that carry rice grains. In order to confirm this, ANOVA, LSD, and HSD analysis were conducted on the data in Microsoft Excel.

Number of Panicles m^{-2} ANOVA
Anova: Single Factor

Number of Panicles m^{-2}

Groups	*Count*	*Sum*	*Average*	*Variance*	*Stdev*
Swine + Plastic	4	1944.44	486.11	5895.69	76.78
Swine/PHWW + Plastic	4	2105.26	526.32	3562.35	59.69
Water + Plastic	4	1286.55	321.64	13821.92	117.57
Water + No Plastic	4	1228.07	307.02	1709.93	41.35

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	150941.2	3	50313.74	8.053457	0.003312	3.490295
Within Groups	74969.65	12	6247.47			
Total	225910.9	15				

Table 3-19: ANOVA Analysis of Number of Panicles m^{-2}

The F-score being larger than the critical F score and the p-value indicate that these data are statistically significant. This means that the conclusion that the wastewater and ABM treatments did have a higher number of rice stalks that produced grain. It even can be suggested that Swine/PHWW-grown rice can produce more grain than the swine wastewater treatment. This is most likely due to the PHWW having more nutrients than the pure swine wastewater that will allow it to produce more seed.

Another way to determine the relative productivity of the rice plant is by looking at the number of filled spikelets (or grains) per panicle. Filled spikelets are the grains considered in determining the yield of the crop whereas the unfilled spikelets are not considered. Determining this number will help determine the relative health of the plant. A graph of the number of spikelets per panicle is shown below.

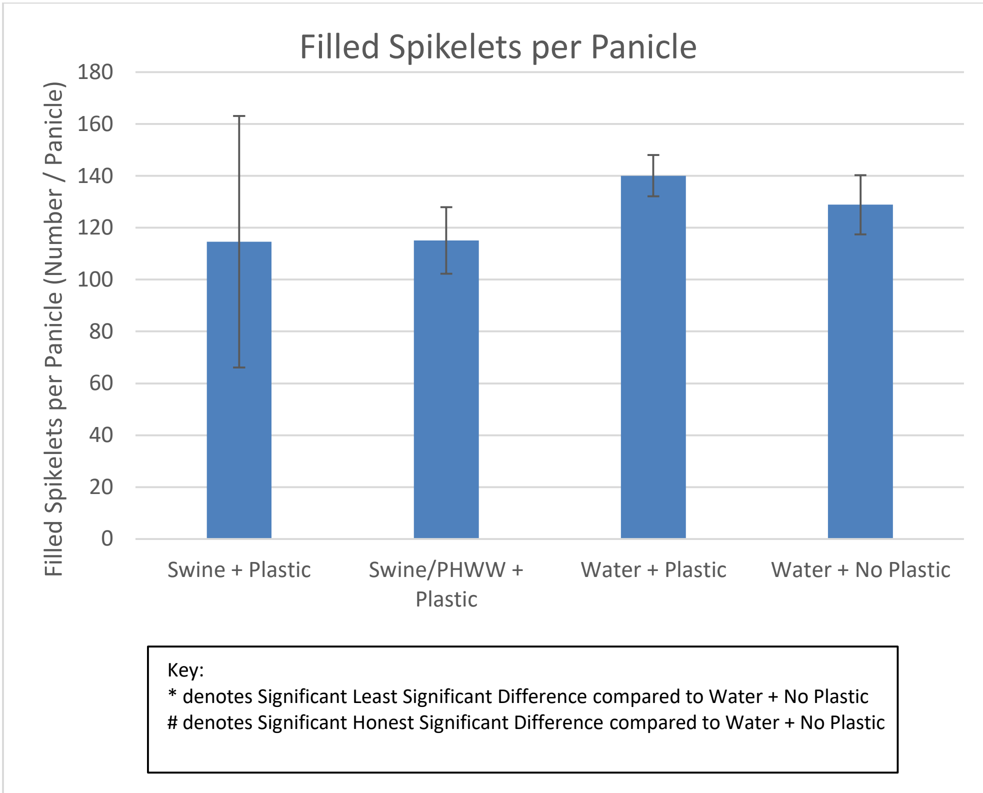

Figure 3-24: Filled Spikelets per Panicle

From this graph, it appears that the treatments that did not have wastewater had more filled spikelets per panicle compared to the treatments with wastewater. This would suggest that the presence of wastewater does indeed hinder the development of the rice spikelets within the panicle which has been reported from previous literature. The next step to confirm this is by conducing ANOVA, LSD, and HSD on it. The ANOVA table is shown below.

Filled Spikelets Panicle^{-1} ANOVA
Anova: Single Factor

Filled Spikelets Panicle^{-1}

Groups	*Count*	*Sum*	*Average*	*Variance*	*Stdev*
Swine + Plastic	4	458.31	114.58	2354.61	48.52
Swine/PHWW + Plastic	4	460.36	115.09	164.35	12.82
Water + Plastic	4	560.37	140.09	64.03	8.01
Water + No Plastic	4	515.54	128.89	131.06	11.45

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1796.990333	3	598.9967777	0.882809878	0.4774892	3.490295
Within Groups	8142.139674	12	678.5116395			
Total	9939.130007	15				

Table 3-20: ANOVA Analysis of Filled Spikelets per Panicle

This table shows through the F-score and p-value that these data are not statistically different from each other. This means that either the number of filled spikelets per panicle was relatively the same or that there were not enough replicates to determine a statistical difference. This again confirms the overall yield of the plants being considered the same at worst.

One other way to look at the quality of the rice growth is to look at the number of filled grains per unit area. Looking at the previous data, the treatments that had wastewater and ABM produced more panicles, but those panicles produced less filled grains, so the amount of filled grains per unit area should be investigated. The figure below shows this relation.

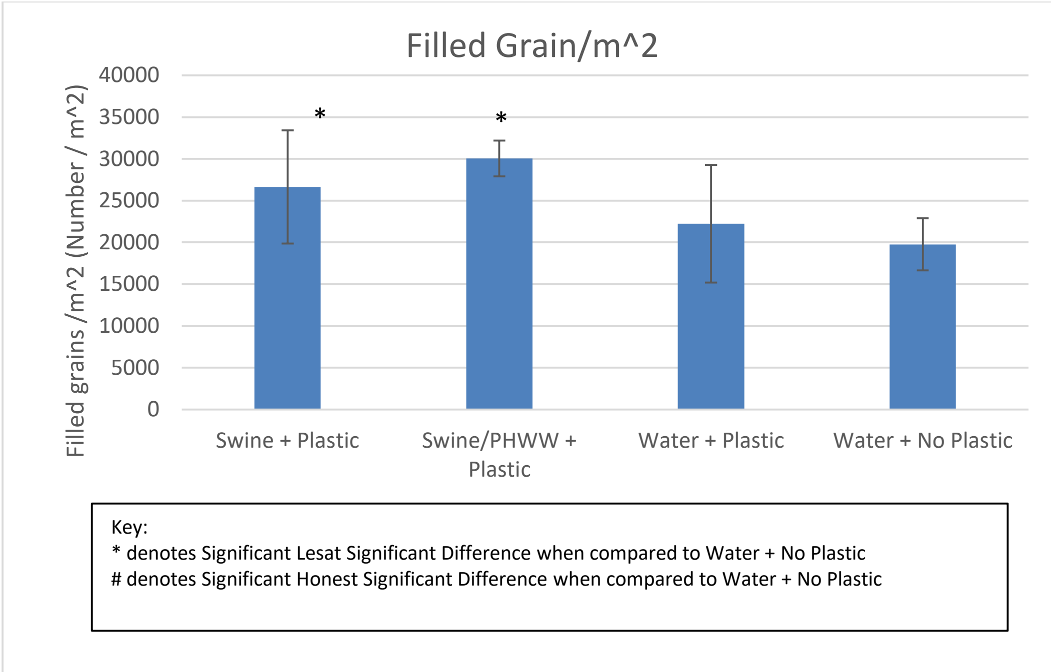

Figure 3-25: Filled Grains m^{-2}

This figure shows that although the number of filled spikelets per panicle were lower for the treatments with wastewater, the fact that so many panicles were produced in those treatments overcompensated for the deficiency. ANOVA, LSD, and HSD analysis were conducted to make sure this was statistically different.

Filled Grains m^{-2} ANOVA
Anova: Single Factor

Filled Grain m^{-2}

Groups	*Count*	*Sum*	*Average*	*Variance*	*Stdev*
Swine + Plastic	4	106580	26644.99	45949500	6778.61
Swine/PHWW + Plastic	4	120216.2	30054.04	4550782	2133.26
Water + Plastic	4	88976.9	22244.22	49444593	7031.69
Water + No Plastic	4	79057.3	19764.33	9763493	3124.66

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.51E+08	3	83784470	3.054807	0.069702	3.490295
Within Groups	3.29E+08	12	27427092			
Total	5.8E+08	15				

Table 3-21: ANOVA Analysis of Filled Grains m^{-2}

Unfortunately, the p-value and F-score both show that these data are not different enough to be considered statistically different from each other, although it is very close to being significant. After LSD and HSD tests were conducted, it was revealed that the treatments with wastewater and ABM were significant for LSD, not HSD. Therefore, it can be said that when wastewater and ABM are used, there is the potential for more filled grains per unit area, but at worst case, they will produce the same amount. This again confirms the overall yields of the plants being considered the same while having a potential for more yield with wastewater being used.

Another way to look at how productive the rice plant was is to look at the Percent of Filled Grains Spikelets $^{-1}$ By Number. This will determine the filling efficiency of the plants relative to each other. The table below shows that relation.

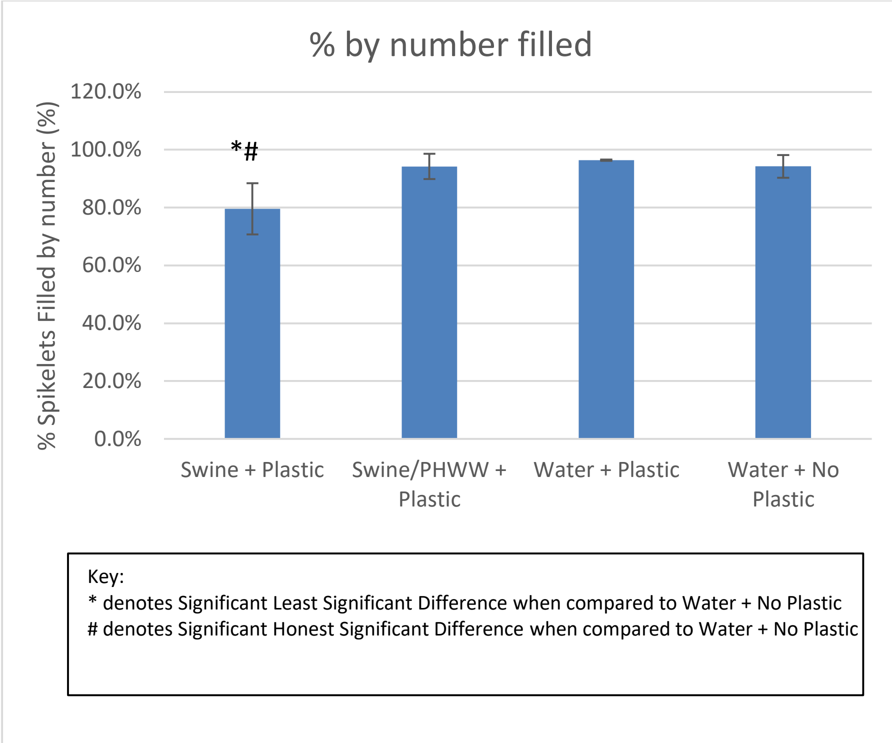

Figure 3-26: % By Number Filled Grains

This figure at first glance shows that the Swine Plus Plastic treatment had the lowest percentage of filled grains relative to the other treatments although it has the highest variability. This would suggest that the swine wastewater hinders the filling of grains, but that adding PHWW helps the filling. To determine if this data was statistically different, ANOVA, LSD, and HSD analysis were completed. The table below shows the ANOVA analysis.

% By Number Filled ANOVA
Anova: Single Factor

% By Number Filled

Groups	*Count*	*Sum*	*Average*	*Variance*	*Stdev*
Swine + Plastic	4	3.18	79.6%	0.01	0.09
Swine/PHWW + Plastic	4	3.77	94.2%	0.002	0.04
Water + Plastic	4	3.86	96.4%	4.63E-06	0.002
Water + No Plastic	4	3.77	94.2%	0.002	0.04

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.072212	3	0.024071	8.557248	0.002611	3.490295
Within Groups	0.033755	12	0.002813			
Total	0.105967	15				

Table 3-22: ANOVA Analysis of % By Number Filled Grains

This analysis reveals that the data is statistically different from each other since the F score and p-value are high and low respectively. After LSD and HSD were completed, it was found that only the Swine Plus Plastic treatment was statistically different compared to the normal treatment of Water Plus No Plastic. The other treatments can be considered statistically the same as the normal treatment including the swine wastewater/PHWW mix. This can be confirmed by the colors of the rice grains which will be discussed in section 3.4.4.3.3. One would expect that since the Swine wastewater hindered the filling of grains that the Swine/PHWW mix would also hinder the filling, but the data suggest that this is not the case. One possible reason is that the Swine/PHWW mix had other heavy metals in it that these colored the rice grains to appear filled. Since the % By Number Filled Grains is based off visual sight of a filled versus unfilled grain, the heavy metals could have darkened the grains to appear filled even though they were not. This along with previously mentioned literature stating that yields (and therefore % Filling) could decrease or not be improved by the addition of wastewater would further enhance the conclusion that wastewater does in fact hinder the filling of grains even though the Swine/PHWW mix did not yield that result. The Swine Plus Plastic treatment had considerably lower % Filled grains which would decrease the overall yield which does confirm the results from the grain yield analysis. The increase in the grain yield for the Swine/PHWW mix over the Swine treatment could be due to this coloring of the rice grains from the heavy metals in the Swine/PHWW mix. Ultimately, these data suggest that either the PHWW added extra nutrients that helped fill the rice grains better, or more likely that the PHWW darkens the rice grains rather than fills them. Further analysis should be conducted to investigate which conclusion can be made.

3.2.2.3.3 Rice Grain Color

To further investigate the effects of the treatments on the rice grain, the color of each treatment was looked at. The figure below depicts what each of the grains looked like compared to each other.

Treatment	Picture of Rice Grain
Swine + Plastic	
Swine/PHWW + Plastic	
Water + Plastic	
Water + No Plastic	

Table 3-23: Rice Grain Color from the Various Treatments

From this picture, it is evident that the treatments with the Swine/PHWW treatments are darker than every other container. This corresponds with the theory previously stated that the PHWW added a darker color to the rice grains which would in effect increase the % Filled grains since that percentage was based off of visual counting as suggested by the IRRI's procedure.

3.2.2.3.4 Rice Grain Heavy Metals Analysis

After looking at the colors of the rice grains and noticing the darker color in the PHWW-grown rice grains, looking at the heavy metals in the rice grain was imperative. Rice grains from the Swine + Plastic, Swine/PHWW + Plastic, and Water + No Plastic treatments were sent in for heavy metal analysis looking for the metals Calcium, Cadmium, Copper, Mercury, Potassium, Magnesium, Manganese, Sodium, Lead, and Zinc as suggested by the Kang et al., (2007) study which compared rice grown in wastewater with rice conventionally-grown. The Kang et al., (2007) study wanted to investigate both the yield of the rice when wastewater was used as well as the potential hazardous effects the rice grains could have when consumed. To complete this analysis, this study's rice grains were sent with no replicates to the Microanalysis lab at the University of Illinois at Urbana-Champaign. The table below shows the ICP-Heavy metal analysis results from the various rice grains.

	Metal Concentrations in Rice Grain (mg/kg)									
Treatment	**Ca**	**Cd**	**Cu**	**Hg**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Swine + Plastic	93.30	0.04	0.90	0.04	4304.20	1730.80	17.40	18.2	0.11	32.10
Swine/PHWW + Plastic	95.10	0.10	2.55	0.34	4673.70	1675.65	17.60	17.85	0.15	36.70
Water + No Plastic	97.90	0.03	10.80	0.00	4398.70	1685.20	32.15	11.20	0.12	34.80
Kang, et al	60.00	0.30	0.00	1.30	2415.00	405.00	83.65	87.00	1.85	10.30
US FDA		0.30		0.05					0.25	
China MCF		0.20	50.00	0.02					0.50	
EU		0.20							0.20	
Hong Kong		0.10		0.50					6.00	

Table 3-24: Heavy Metal Concentrations in Rice Grain from Various Treatments

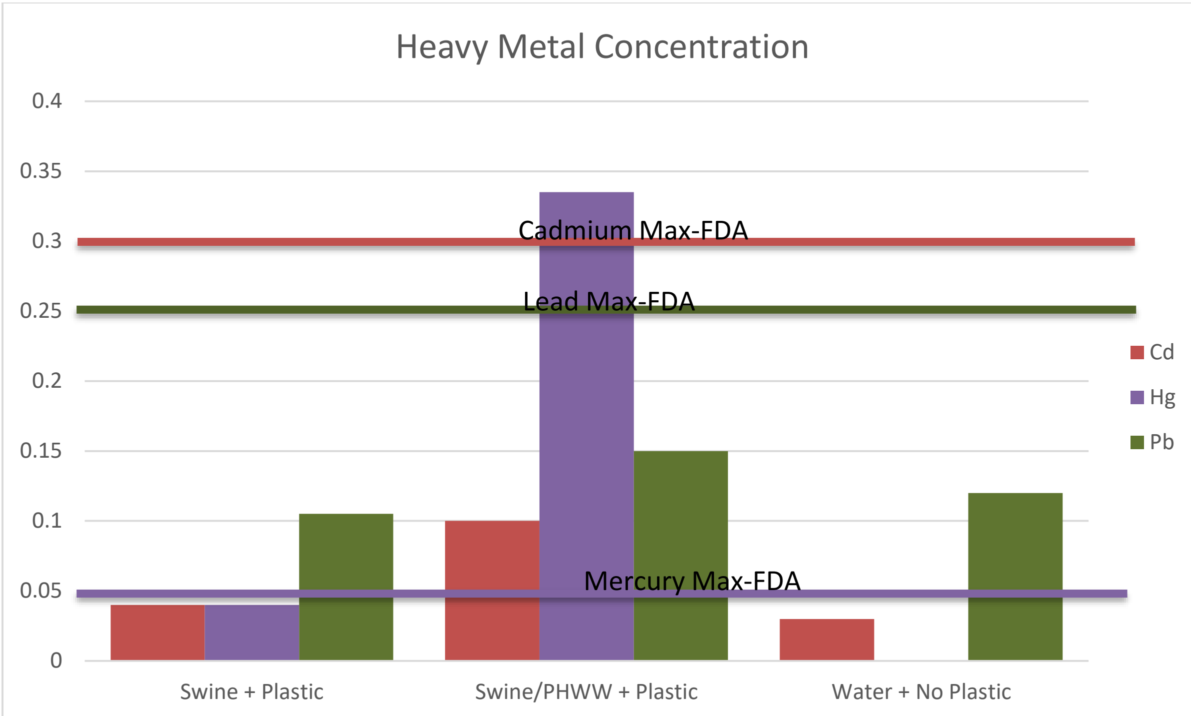

Figure 3-27: Comparison of Heavy Metal Concentrations Between the Treatments

The standards for the different regions vary. The US FDA standards are for rice protein (US FDA, 2016). The China MCF standards are the Maximum Levels of Contaminants in Foods standards for various food commodities in China (Clever, Jennifer, and Jie, Ma, 2015). The European Union has its own standards for food consumption, but does not include a mercury maximum level for cereal grains like rice (Directorate-General Health and Consumer Protection, 2004). The Hong Kong standards come from Hong Kong's Chapter 132V Food Adulteration Regulations. Using these standards, this table shows that the metals from the grains in the experiment are well within the range for normal rice grain metals. One exception was the concentration of Cadmium in the Swine/PHWW was fairly high, but still below the threshold for rice grains. Long-term effects of taking in more cadmium in the diet would need to be studied to determine if this has a negative effect. One other exception is the level of mercury in the rice grains from both the swine treatment as well as the swine/PHWW treatment. They both are larger than the acceptable amount. This is a concern because the Swine/PHWW grain was 1675% larger than the maximum allowable amount of mercury in rice for the US. The increase in heavy metals in the rice grains can come from several sources ranging from the wastewater they grew in, the ABM that grew in each treatment, or the anaerobic conditions caused by the plastic. Since the ABM grown in the two different treatments is composed of the same material, it is most likely not the ABM. Since both the Swine + Plastic and the Swine/PHWW + Plastic treatments had plastic causing anaerobic conditions, it is most likely not this condition that would explain the increase in mercury from Swine + Plastic to Swine/PHWW + Plastic. Therefore, the most likely source of the metals is the wastewater they grew in. To confirm that the wastewater was the source of the harmful heavy metals, ICP-Heavy Metals analysis was conducted on the Swine/PHWW mix containing 2.4% PHWW. To conduct the analysis, the water sample had to be filtered so as to not clog tubes. The results from the test are shown below.

	Heavy Metal Concentrations in PHWW Water (PPM)									
	Ca	**Cd**	**Cu**	**Hg**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
2.4% PHWW Mix	83.1	0.1	0	0	126.1	42.2	53.7	52.5	1.1	0.2

Table 3-25: Heavy Metal Concentrations in Filtered 2.4% PHWW (* indicates below limit of detection)

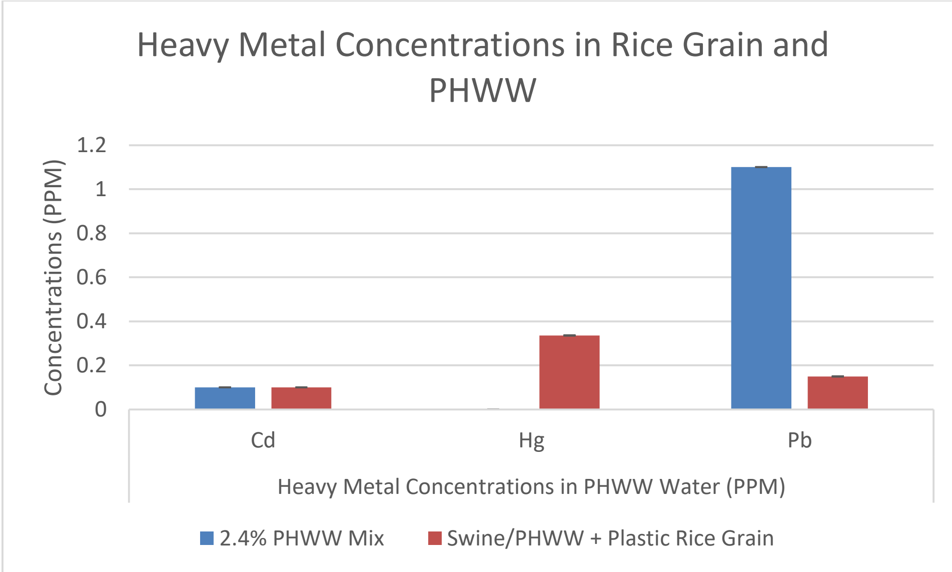

Figure 3-28: Comparison of Heavy Metals in Swine/PHWW Rice grain and PHWW

This shows that the mercury concentration in the rice grain did not necessarily come from the PHWW. What is misleading is that this test had to filter the water to conduct the analysis, which is not representative of the water that was used to grow the rice, therefore this analysis does not represent what actual nutrients the rice grain received. Tiny particles are contained in the PHWW that most likely contain the heavy metals, and these were filtered out in the analysis conducted by the Micro-analysis lab for liquid sample. This means that it is not possible to find the concentration of mercury in the water sample using ICP analysis. Taking this into consideration, it is still believed that the reason for the increase in heavy metals is due to PHWW rather than the other proposed reasons. This analysis does, however, show that the rice grain from Swine/PHWW is unsafe to eat and some mitigation could be required if the water is the source of the heavy metals. The Swine treatment's mercury concentration is on the borderline, so more studies should be done to confirm that its level of mercury is too large. Every other metal concentration is within the food safety regulations that are currently in place.

3.2.2.4 Rice Yield Conclusions

The purpose of the greenhouse experiment was to determine that ABM and wastewater did not harm rice grain yields while also providing insight as to how much rice grain could be produced. This was done to determine how much byproduct credit could be given to the overall biofuel production cost. Since the overall rice grain yield was statistically the same as each other at worst while showing the potential for Swine/PHWW-treated rice yield to increase, it can be inferred that ABM growth and wastewater do not decrease the yield of the rice plants. The overall yield of the greenhouse experiment was higher than the literature-reported yields for Cocodrie, but as explained previously, this is most likely due to being inside a greenhouse constantly providing optimal conditions for growth. Therefore, to determine the amount of credit given, the yield of the literature-reported Cocodrie will be used (7.06 metric tons ha^{-1}). In this system, there will be two rice crop growth cycles, so the yield of the rice will double. According to the Food and Agriculture Organization, rice crop production costs \$359 ha^{-1}. Since this system is growing two crops of rice, this cost will double. The Food and Agriculture Organization also says that rice is sold at \$210 metric ton^{-1}. Since this system produces 7.06 metric tons ha^{-1}, this system can sell for \$2,250 ha^{-1} which means the rice farmer will get a net profit of \$1,890 ha^{-1}. To compare this to the ABM production cost, this equates to \$92.75 gge^{-1} for the 100 ha facility.

The other byproduct credit yet to be considered is the wastewater treatment credit.

3.2.3 Water Quality Analysis

The ABM that was grown in the greenhouse experiment was grown in wastewater as described previously. This ABM was consuming some of the nutrients from the wastewater as it grew. The concentrations of the nutrients in the wastewater were monitored over time. First, the Ammonia treatment will be considered. Ammonia water quality was analyzed using the Hach Nessler Method 8038. The figures below depict the quality of the water based on ammonia concentration over time and the percent removal of ammonia from the various feedstock wastewaters.

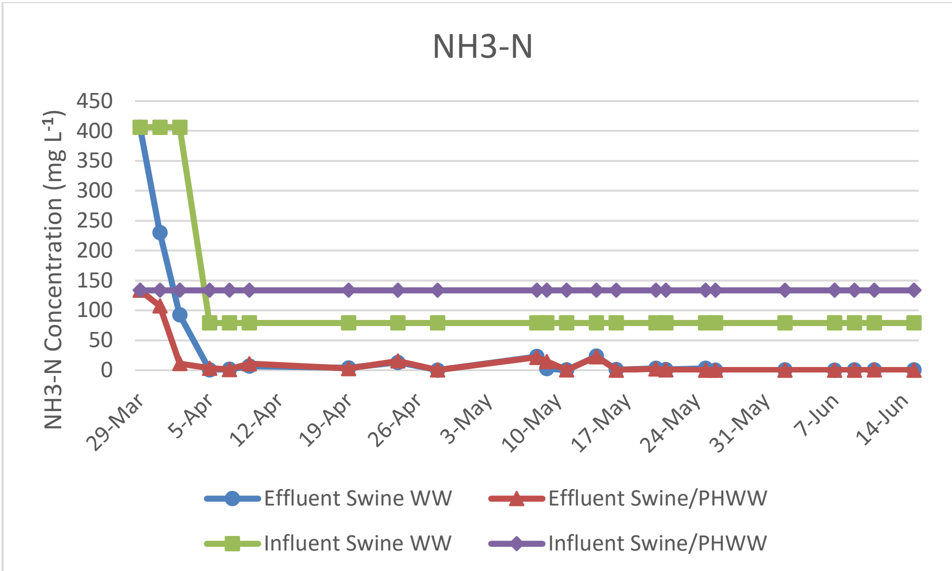

Figure 3-29: Ammonia Concentration Over Time

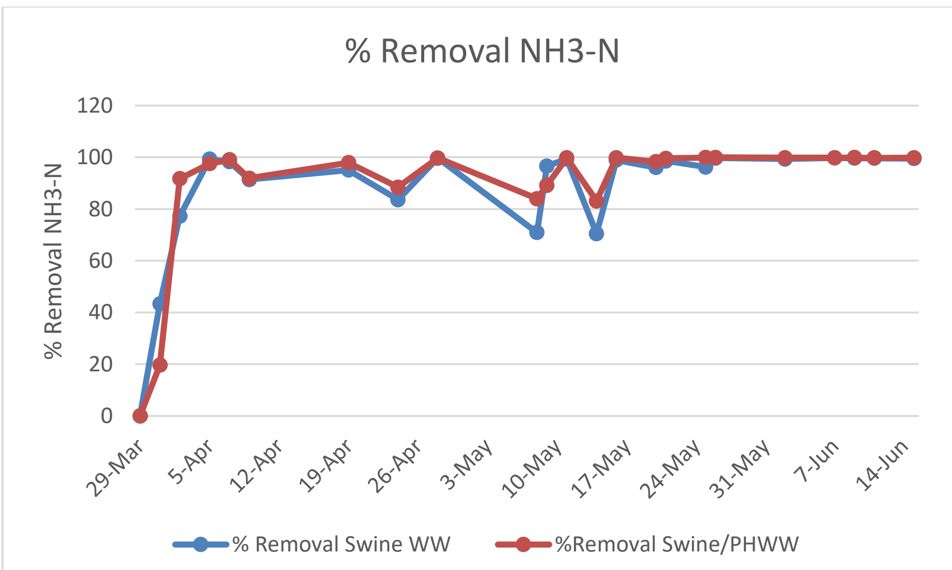

Figure 3-30: Percent Ammonia Removal Over Time

According to the data, all treatments had positive percent removal meaning that ammonia was not produced during the growth of the ABM. This data is backed up by the fact that when ABM grow, they undergo the process of nitrification. The treatments with only swine wastewater had a more consistent removal of ammonia most likely due to the fact that the Swine/PHWW treatment was dosed with the PHWW once a week resulting in a shock treatment whereas the Swine wastewater treatment was a consistent batch. This would explain the delay in the percent removal evident in the dips in the graph depicting % removal in the Swine/PHWW mix. This data suggests that the ABM that grew in the Swine + Plastic treatments removed more ammonia faster than the ABM that grew in the Swine/PHWW mix. The Swine + Plastic ABM can remove up to 99% of ammonia while the Swine/PHWW Plus Plastic ABM can also remove 99% but the time it takes to achieve the same removal is 3 days while the Swine treatment only takes a day. A reason for this delay in treatment could be because of the high concentration of nutrients and potential harmful metals in the PHWW. To further investigate the relationship between highly concentrated wastewaters like PHWW and algae, a study was conducted to determine a relationship between the two.

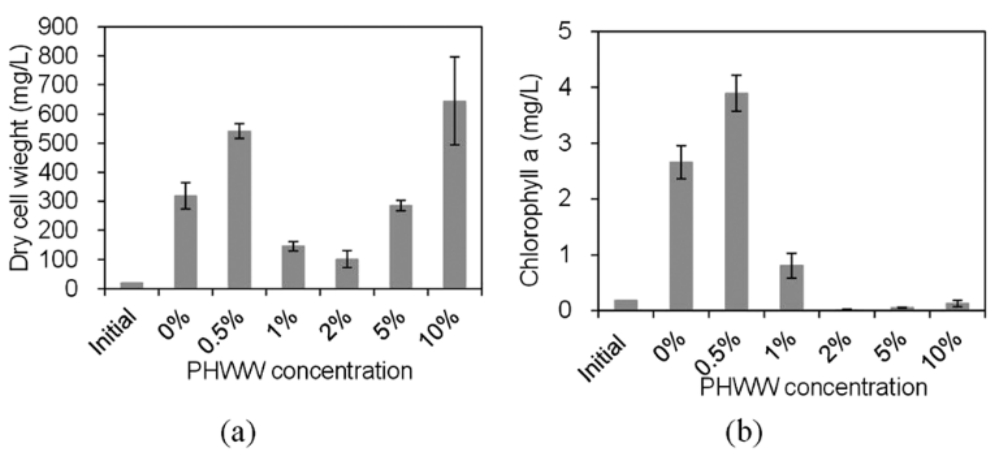

Figure 3-31: Biomass Production in Filtered Municipal Wastewater Spiked with Various Doses (0-10%) of Post-HTL Wastewater (PHWW-Spirulina) After 10 Days of Cultivation. (a) Total Biomass Production Presented as Dry Cell Weight. (b) Autotrophic Biomass Production Presented as Chlorophyll a Concentration. Error Bars Represent the Standard Deviation (n=3). Adapted from Zhou et al., (2013).

The figure shows that the autotrophic biomass (the ones that produce higher concentrations of chlorophyll a) grew well in up to 0.5% concentrated PHWW but that 1% was too high of a concentration and hindered the growth of the algae biomass. Once heterotrophs dominated the amount of the species (where chlorophyll a was not produced), increasing the concentration did not hinder the growth of the algae. Heterotrophic growth will not be considered a major factor in this study since the concentration of PHWW was so low, so only the autotrophic growth is of concern. Clearly the Zhou et al., (2013) study shows that autotrophic growth will decrease at high concentrations of PHWW due to the contaminants in the PHWW, so this would explain the inability to remove ammonia and other nutrients since the algae would grow slower. The Zhou et al., (2013) study seems to be confirmed by this study's delay in treating ammonia which can be correlated to the growth of the ABM. Since the ammonia removal was delayed for the PHWW treatment cases, this is explained by the ABM's hindered growth with PHWW.

Other sources of nitrogen for ABM growth can come from nitrate present in the feedstock. Nitrate was measured via the NitraVer5 HACH cadmium reduction method. The figures below depict the nitrate concentration over time and the corresponding removal from the various feedstock wastewaters.

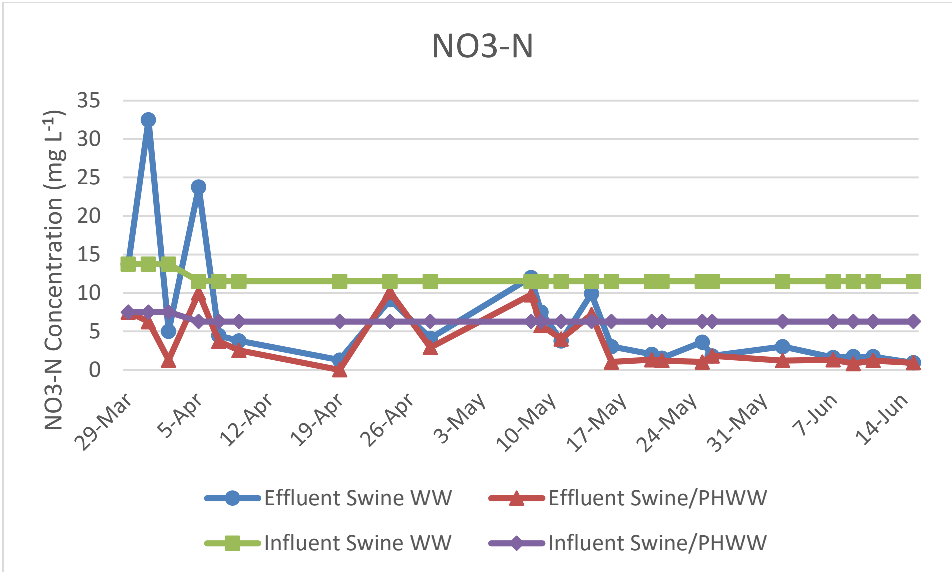

Figure 3-32: Nitrate Concentration Over Time

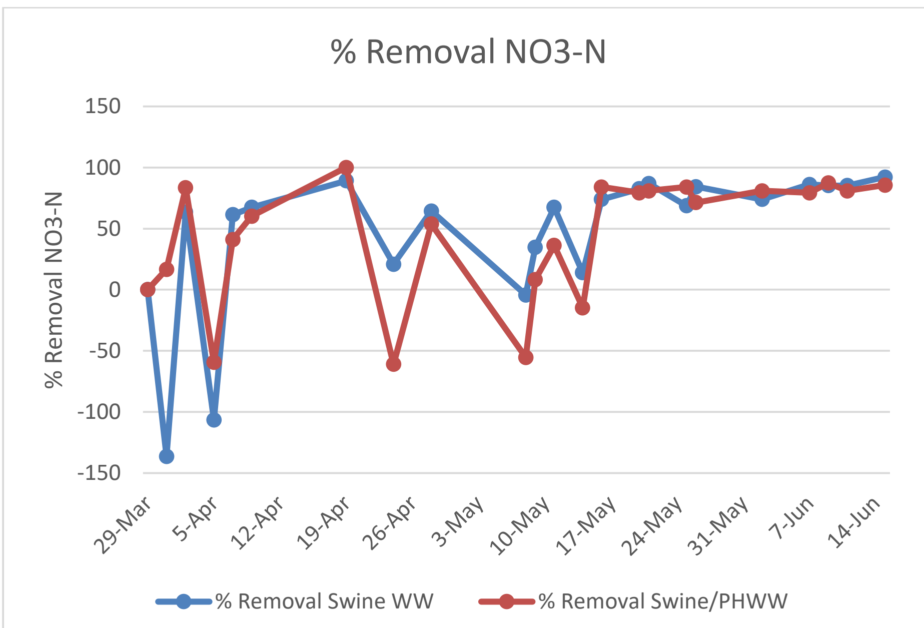

Figure 3-33: Percent Nitrate Removal Over Time

According to the data, the ability of the ABM to remove nitrate is not as clear as the ability to remove ammonia. This is partly due to the complicated process of nitrification that occurs in the ABM. Nitrification is the process where ammonia undergoes oxidation and becomes nitrate, a form of nitrogen that most plants prefer for growth (Mezzari et al., 2013). The figure below depicts the nitrogen cycle.

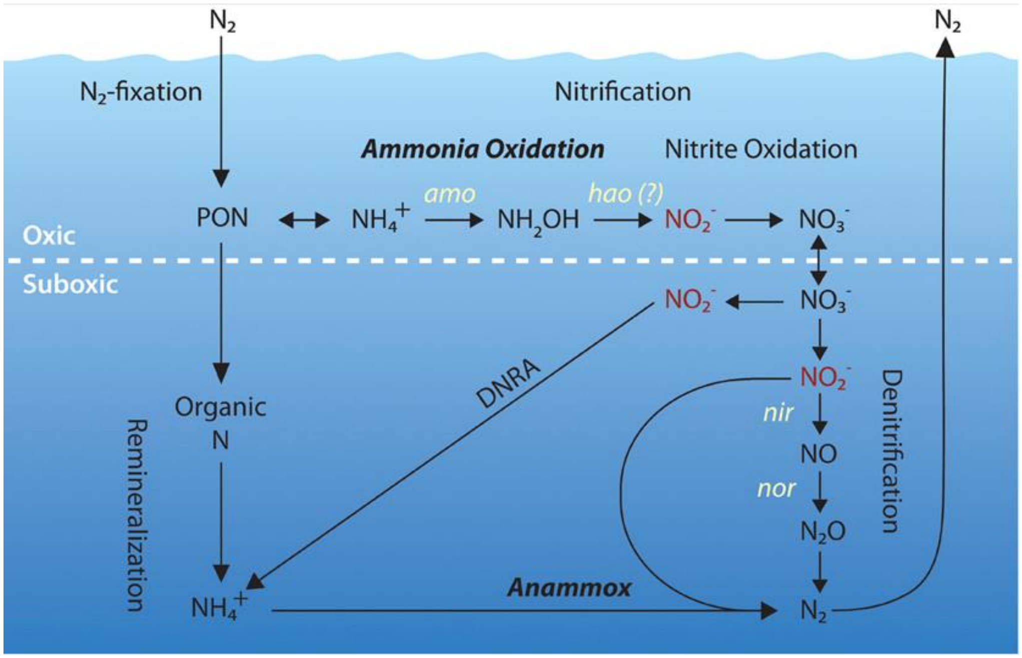

Figure 3-34: The Nitrogen Cycle (from Francis et al., 2007)

In this cycle, the ABM would carry out the nitrification process into nitrate which would then be used by the rice plants as a source of nitrogen. This conversion from ammonia to nitrate by the ABM and nitrate uptake by the rice plants means that the level of nitrate will fluctuate from being produced by the ABM to be reduced overall since the rice plant is consuming it. The production and consumption of nitrate would explain why the percent removal graph varies from negative percent removal to all removal. This would also suggest that after May 17th when the nitrate removal levels out to 85-92%, the uptake of the nitrate by the rice plants occurs faster than the production of nitrate from the ABM. This could be explained by the fact that the ABM would not be growing as fast in the later months since the rice plant occupies more canopy space at this stage of growth. Since plants like rice prefer nitrate as their source of nitrogen, they would consume the nitrate from the feedstock and the ABM immediately in effect showing no presence of nitrate in solution (Killpack, S.C. and Buchholz, D., 1993). This means that the periods of negative percent removal of nitrate were when the ABM was growing faster (and therefore producing a lot of nitrate) than the rice could uptake it. The symbiotic relationship between the ABM and the rice plants makes monitoring nitrate quality complicated, but this clearly shows a lot of room for improvement. If the ABM can convert ammonia into nitrate to grow biomass, then the rice would consume the nitrate to grow, this would mean that the rice would need less fertilizer which could then decrease costs associated with fertilizer application. This has large implications on the rice farming industry and should be further investigated in a future study.

Other than nitrogen, there are other sources of nutrients involved in the experiment. One of those is organics. The soluble chemical oxygen demand (sCOD) was measured according to Hach Method 8000. The sCOD concentration over time and sCOD percent removal from the various feedstock wastewaters figures are shown below.

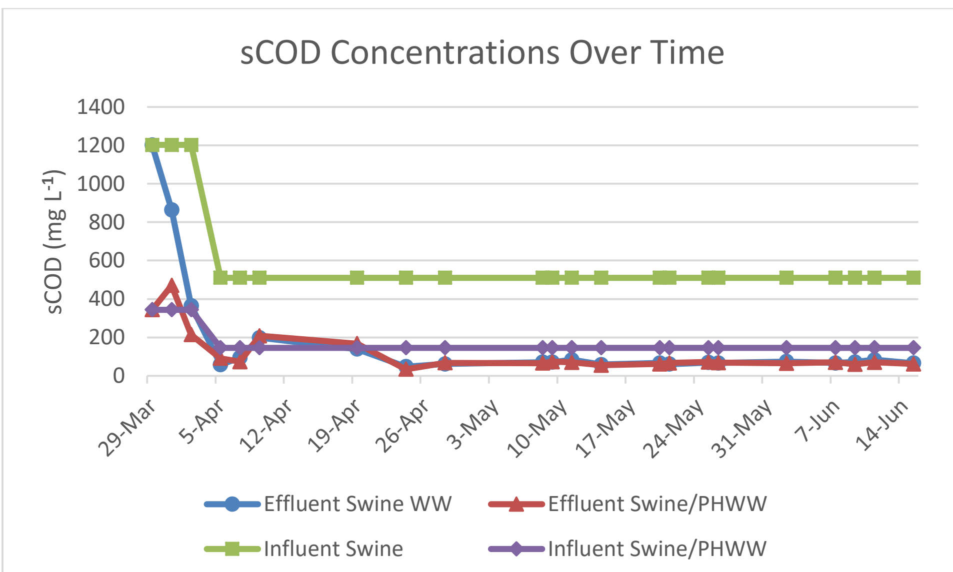

Figure 3-35: Chemical Oxygen Demand Concentration Over Time

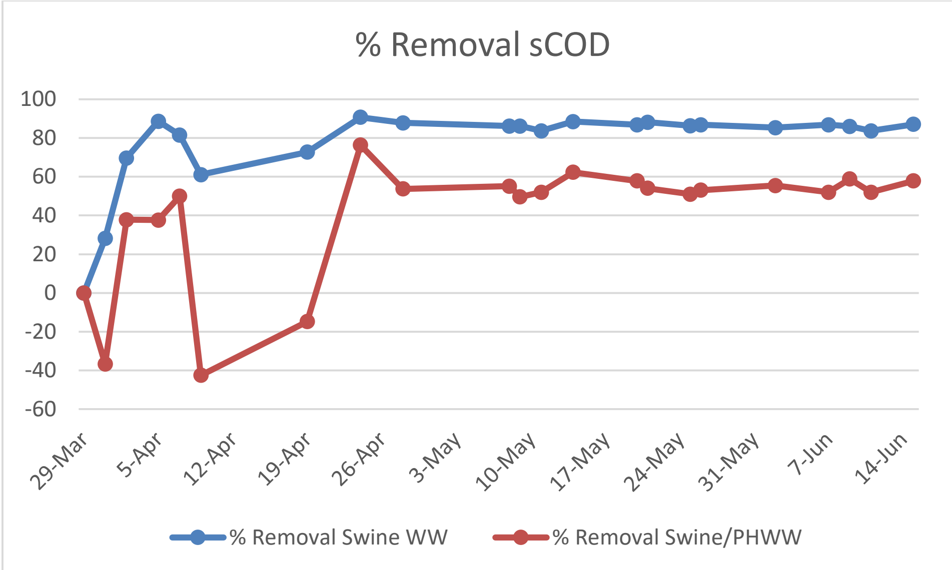

Figure 3-36: Percent Removal of Chemical Oxygen Demand Over Time

These figures show positive results. In this system, both the ABM and rice are consuming the organics in the water. The rice plants break them down because in a rice paddy, methanogens grow inside the soil since they have very little oxygen (due to the flooding of water) and break down the organics in the water and produce methane (Intergovernmental Panel on Climate Change, 1996). The Swine WW percent removal of sCOD was consistently in the positive meaning more organics were being consumed than being produced. The Swine/PHWW percent removal did achieve negative percent removal on a few instances. The reason for this is not clear and more experiments would have to be conducted. Most likely it is due to a detection limit since the actual concentration of the treated water compared to the feedstock was only 20-50 mg L^{-1} higher, and the lowest the spectrophotometer can read is 30 mg L^{-1}. Overall, the ABM removed up to 90% of sCOD in the Swine FS while only up to 58% of sCOD in the Swine/PHWW FS. This confirms the ABM's inhibited ability to remove nutrients from the Swine/PHWW compared to the Swine WW.

3.2.3.1 Conclusions

This data show that the ABM can remove up to 99% of ammonia, 92% of nitrate, and 90% of sCOD. These removal rates will be used to relate to the large scale 100 ha facility. Each of these treatments can give a credit towards the production cost of the biofuel. For total nitrogen removal (TN), the credit is worth \$1.91 lb^{-1} TN according to the Lundquist et al., (2010) study. In this case, it will be assumed that TN only consists of ammonia and nitrate. Using the influent concentrations of ammonia and nitrate (79 and 11.5 mg L^{-1} respectively), the total pounds of nitrogen was calculated for the 100 ha facility assuming a water depth of 10 cm. This was calculated to be about 20,000 lbs TN. This is representative of the amount of TN every two weeks. To determine how much of this TN was removed by the ABM, the average of the average removal of ammonia and nitrate was found. The average amount of TN removed by the ABM was calculated to be 72%. Removing 72% of the 20,000 lbs of TN is about 14,400 lbs TN removed every two weeks. The total amount per year for a 10-month year is about 288,000 lbs TN. Using the credit assumed previously of \$1.91 lb^{-1}, the facility will get \$550,000 for the 100 ha facility. This is equivalent to a credit of \$2.27 gge^{-1}.

Another treatment credit can be given for the amount of BOD removed. BOD is approximately 50% of sCOD. This relation will be used to find the BOD from the sCOD values. Since the influent sCOD concentration was 510 mg L^{-1}, the BOD is assumed to be 255 mg L^{-1}. For the 100 ha facility at a water depth of 10 cm, this is 25,500 kg of BOD in the water every two weeks. The average sCOD removal, and therefore BOD removal, was calculated from the sCOD removal rates in this study. This removal is 85% of BOD, therefore the amount of BOD removed every two weeks is 21,600 kg. The amount per year for 10 months is 432,000 kg. The credit for removing BOD is \$1.23 kg^{-1} according to the Lundquist et al., (2010) study. For the 100 ha facility, this is \$532,000 or \$2.19 gge^{-1}.

Taking these credits into account, the production facility will get in total \$4.46 gge^{-1} credit for treating the wastewater. This in addition with the credit from the rice production decreases the overall production cost significantly.

3.3 Cost Comparison

After taking all of the costs and credits into account, the table below depicts how much the fuel will cost overall and compare it to the Lundquist et al., (2010) study.

	AOU (Lundquist et al., 2010)	Co-Cultivation US	Co-Cultivation China
Feedstock (Cost per ton Biomass Harvest \$/ton-biomass)	\$488.0	\$495.0	\$347.0
Feedstock (Cost per acre \$/acre)	\$89,706.3	\$23,509.0	\$16,491.0
Feedstock (Cost per gallon oil produced \$/gal-oil)	\$6.6	\$3.9	\$2.8
Conversion	\$4.0	\$1.7	\$1.7
Upgrade to Finished Fuels	\$0.3	\$0.4	\$0.4
CHG	-	\$1.5	\$1.5
Balance of Plant			
Total Cost before Byproduct Credit (\$/ gal)	**\$10.9**	**\$7.6**	**\$6.4**
Byproduct Credit			
WW Treatment Credit (BOD removal) (\$/gal oil produced)	\$9.0	\$4.5	\$4.5
Electricity Credit (\$/gal oil produced)	\$1.5		
Rice Product (\$/gal oil produced)		\$92.8	\$92.8
Rice Product (\$/acre)		\$4,670.2	\$4,670,2
Minimum Selling Price (\$/gallon oil)	**\$0.4**	**\$3.1**	**\$1.9**

Table 3-26: Cost Comparison of the Different Systems in the Study

As discussed previously, the cost of the fuel had decreased from \$10.9 gge^{-1} to \$7.6 and \$6.4 gge^{-1} for the co-cultivation systems in the US and China respectively. This cost represents the cost before credits were given. The Lundquist et al., (2010) study receives a credit for treating the wastewater as well as producing electricity from an anaerobic digester. This study receives a credit for treating wastewater. Even though this study does not receive as much credit for treating the wastewater as the Lundquist et al., (2010) study does due to the decreased water treatment efficiency and lower wastewater flow rate, the overall production cost is significantly less than the Lundquist et al., (2010) study. In fact, the wastewater treatment credit of \$4.5 gge^{-1} pays for the production cost of the ABM biomass feedstock entirely. This however is not enough of a credit to pay for producing the fuel when incorporating HTL conversion, upgrading, and CHG. Therefore, this suggests that even though biomass feedstock cost decreased significantly, more work needs to be done to decrease the costs of the fuel production process to make algal biofuels cost competitive. Using current gasoline prices of \$2.15 gallon^{-1} (EIA, 2016) and the production cost of the fuel from ABM as \$3.1 and \$1.9 gge^{-1} for the US and China respectively, a US rice farmer would lose \$0.95 gge^{-1} while a Chinese farmer would make \$0.25 gge^{-1}. These ABM fuel costs would decrease the US rice profits from \$92.8 gge^{-1} to \$89.6 while increasing the Chinese rice profit to \$93.05 gge^{-1}. This shows that at current fuel prices, Chinese farmers should implement a co-cultivation system, while the US rice farmers need to wait until the price of fuel goes up again.

Chapter 4

Conclusions

The goal of this study was to find a way to decrease biomass feedstock production costs and prove that this system could work. First, a co-cultivation system was developed. HDPE tarp would be laid out over the rice field to make collection of ABM more efficient. Municipal or agricultural wastewater would be used to deliver nutrients to both the rice and ABM. Harvesting would occur either through a water pump shooting water down to push biomass into a trench or using brooms to sweep the biomass into the trench. The ABM would then be collected and transported to a clarifier and eventually dried out and sent to a hydrothermal liquefaction facility to convert it into biocrude oil. This oil could then be used for various fuel purposes. Rice will have two cycles of growth (assuming 120 days for one cycle) totaling up to 8 months. ABM would be growing with the rice for these 8 months and an additional 2 months out of the year without the rice.

To determine how much of an effect on the cost of fuel production from ABM grown with rice, a techno-economic analysis was conducted. Using a previously well-established study by Lundquist et al., (2010), several changes were made to their analysis to decrease costs for a co-cultivation system of the same size (100 ha). In order to determine how much ABM could be produced in the co-cultivation system, several biomass growth rates were found, experimentally. It was shown that ABM could achieve a growth rate of 17 g m^{-2} day^{-1} when no rice plants are present and 3 g m^{-2} day^{-1} when rice plants significantly occupy the canopy at 130 days of growth (out of 168 total days of rice growth). Based on these two data points and a correction for the longer growing season in the experiment, a growth curve was established to determine the amount of ABM that could be produced in the system assuming 120 days of rice growth. It was determined that 1921 tons of ABM could be produced over the year for the 100 ha facility.

The co-cultivation system uses hydrothermal liquefaction as its oil conversion process. It takes the grown ABM as well as the leftover rice straw and converts it into biocrude oil. To determine how much oil could be produced from the grown biomass in the system, it was assumed that 40% of the biomass could be converted to biocrude oil as suggested by previous literature that inoculated a high-conversion species of algae. This would mean the 100 ha facility could produce 5635 barrels of oil per year.

Continuing with the economic analysis from the Lundquist et al., (2010) study, the harvesting of ABM mechanism was changed for the co-cultivation system. Based on the location of the proposed system where rice is normally grown, a different method of harvesting ABM would have to be used. The reason for different harvesting methods is because of the difference in labor and electricity costs for the two regions. For the US, a water pump would shoot pressurized water down the crop rows to collect the ABM at the edge of the field. In China, people would sweep down the rows with brooms to collect the ABM at the edge of the field. This harvesting mechanism is cheaper than the Lundquist et al., (2010) study, and cuts the cost of the feedstock significantly.

Since the method of growing ABM in a rice field is different, different materials are used in the field. Namely, an HDPE tarp is used to make collecting of ABM easier. Using an HDPE tarp cuts the cost down significantly. Having the rice farmer pay for the 100 ha land that the rice and algae are being grown in also decreases the cost a large amount. The only land that the ABM facility will pay for is the land for the collection facility that processes the grown ABM.

The collection facility where processing and drying of the ABM is carried out also costs less than the conventional process in the Lundquist et al., (2010) study. Since the amount of biomass produced from this system is less, the amount of equipment to process and dry the ABM is less. This also decreases costs for the ABM facility.

Ultimately, it was found that if a system growing rice and algae in the US was implemented, the feedstock cost of the ABM would increase from \$488 ton^{-1} to \$495 ton^{-1} and drop from \$6.60 gge^{-1} to \$3.9 gge^{-1}. If this system were implemented in China, it would decrease even further to \$347 ton^{-1} or \$2.8 gge^{-1}.

In both the Lundquist et al., (2010) study and this study, credits are given for the byproducts produced in the systems. In the co-cultivation system, rice grain is a byproduct. To determine how much rice grain could be produced in this system and if grain yields were affected by the wastewater in the system and growth of algae, an experiment was conducted. To test the co-growth of the system, 4 different treatments were set up: one with Swine manure lagoon effluent wastewater and plastic; one with a mix of Swine manure lagoon effluent, PHWW, and plastic; one with regular tap water and plastic; and one control treatment with tap water. They were harvested at 168 days of growth after seeding. The yield of the rice when grown with ABM and without it had varying results. First, the data suggest that the thousand grain weight was lower for the treatments that had ABM and wastewater compared to the treatments that received tap water and had no ABM growth. The data show that the treatments with ABM and wastewater do compensate for this disadvantage by producing more panicles m^{-2}, so this helps compare the yields between the different treatments. That is why it is shown that the yields between the various treatments were statistically the same while the Least Significant Difference test suggests that Swine/PHWW-treated rice could increase the grain yield. This increase in yield did not pass the Honest Significant Difference test, but this still shows the possibility for Swine/PHWW mix to increase the grain yield. It was also shown that the wastewater treatments had lower grain filling percentage most likely due to the increase in nutrients compared to what rice normally receives.

This means that although the treatments that had wastewater and grew ABM had a lower thousand grain weight, they produced more grains, so the yield was the same compared to the regular treatments. This means that it makes sense for farmers all over the world to grow their rice with ABM to produce two crops, one for food consumption and one for biofuel production.

After heavy metal-ICP analysis of the rice grains, it was shown that the treatments that had received wastewater had a higher cadmium and mercury concentration. The cadmium concentration was within safe limits for consumption, but is higher than normal especially in the Swine/PHWW treatment. A long term study should be done to confirm this is not harmful for human consumption. The mercury concentration in the Swine treatment was higher than the control, and is slightly lower than the safe amount. More studies would have to be conducted to determine the chronic effects of consumption of this rice grain. On the other hand, Swine/PHWW-treated rice grains had a significantly higher mercury concentration, one that is too high for safe human consumption. This means that rice grown in Swine/PHWW mix is not safe to eat, but the Swine treatment is.

Assuming no harmful chronic effects in the Swine manure lagoon effluent-grown rice grains, this experiment showed that rice yield was not hindered by the growth of ABM while using wastewater. Therefore, the overall yield of 7 metric tons ha^{-1} reported from the literature could be used as the yield for one cycle of rice growth. Since this system proposes two cycles, this would mean 1400 metric tons of rice are produced in the 100 ha field in a year. This equates to a value of \$92.8 gge^{-1}.

To decrease the cost of the fuel production, a water treatment credit is given to the system. To determine how much of a treatment could be given, water quality analysis was conducted on the water that was used in the rice co-growth experiment. Water quality analysis suggests that the ABM and rice can remove up to 99% of ammonia in the water, and do it on a consistent basis. Nitrate results suggest that it could be removed up to 92% by the rice and ABM, but that it takes longer for the ABM to consume the nitrate compared to ammonia. sCOD results show that the COD could be removed up to 90%. These results suggest that an ABM and rice bioreactor system could reduce ammonia, nitrate, and sCOD levels significantly. Since water treatment facilities will pay to remove nitrogen and BOD, this 100 ha facility can get a credit of \$4.5 gge^{-1}.

After the credits are included in the fuel production cost, the cost of producing the fuel from the ABM was found to be \$3.1 and \$1.9 gge^{-1} for the US and China respectively. At current fuel prices of \$2.15 gallon^{-1}, this means that US rice farmers would lose some profit with co-cultivation while Chinese farmers would gain profit. If the price of fuel continues to rise, this would mean that even the US rice farmers could gain profit as long as the price is above \$3.1 gallon^{-1}. The feedstock cost was decreased significantly (from \$6.6 from the Lundquist et al., (2010) study to \$3.9 and \$2.8 for co-cultivation), but the cost of HTL, upgrading, and CHG increase the overall cost

Paired with a low cost is the scale of a co-cultivation system. Since there is a lot of land dedicated to rice production currently, when these lands are converted to co-cultivation systems, 36% of the world's crude oil supply could be supplied via this system. The amount of wastewater in the world can provide enough nutrients to co-cultivation systems, so this percent oil supply is not limited by nutrients nor land. Other factors like particular climate could decrease this percent supply, but more research would have to be done to determine this percentage. This has large implications for the global fuel economy.

This lower cost and high oil supply in addition to the experimental results showing no decrease in yield for rice grown in wastewater with algae prove that this co-cultivation system can work in a cost-effective manner and that more than one third of the world's oil supply could be provided if rice farmers implemented a system like this.

Chapter 5

Recommendations for Future Work

Since this proof of concept showed promising results, additional larger scale studies need to be conducted to confirm this finding. A larger scale rice/algae field would confirm the rice grain yield results that showed no harmful effects. Being outdoors would also confirm general yields reported from literature. It would also confirm the assumption that 85% of the grown algae could be harvested via brooms or water pumping.

Another area for future work could investigate the limiting factors involved in determining how much oil could be supplied from the system. This study looked at the amount of land and amount of nutrients supplied through wastewater, but other factors like temperature and light could limit the potential amount of oil that can be provided.

In addition to the large scale study, small batch studies could also be conducted to fine tune the parameters of the experiment. One of these parameters is the concentration of PHWW in the wastewater. It was found that a 0.23% PHWW/Swine mix can lead to a too high concentration of mercury in the rice grain that it is not acceptable for human consumption, although more tests are needed to confirm these results. To mitigate this affect, the concentration of PHWW could be adjusted to see how much PHWW could be used before the rice grain becomes unsafe to consume. Aeration could be another component of the co-cultivation system. Using a small batch experiment, pumping in some form of CO_2 as an additional source of carbon for ABM growth could increase ABM productivity. This would also allow the rice farmer to blow in warm air during the colder months to increase ABM productivity even more. Economic analysis would have to be conducted to determine how much aeration could be provided until the cost of producing the fuel outweighed the value of the fuel.

With these additional studies, it is clear that algae biofuel production cost can be reduced by the co-cultivation of rice and algae and more rice farmers should implement a system like this.

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Appendix A: HTL Tests

Hydrothermal Liquefaction tests were conducted to determine the quantity and quality of the bio-crude raw oils produced from rice straw, rice straw co-liquefied with ABM, and pure ABM. The reason rice straw and co-liquefaction were included in this experiment was to show that the rice straw byproduct could also be used to produce more fuel. The conditions for the reactions were based on a rice straw HTL study (Singh et al., “Hydrothermal liquefaction of rice straw”, 2015). First, the effect of temperature on the conversion of rice straw to oil was investigated. Then the effect of conducting HTL on pure rice straw versus pure ABM versus a combination of the two was also investigated. This is to show that if the system were implemented on a rice farm that the farmer could produce oil from not only the ABM, but also the leftover rice straw. The effect of liquefying them both at the same time is also important because this would determine if the ABM would have to be processed separately from the rice straw. All experiments were only conducted once. The table and figure below lists the conditions that the HTL experiments were conducted at as well as the toluene soluble fraction and bio-oil yield from each condition. The oil yield was calculated based on a dry, ash-free basis. “FS” represents feedstock.

Sample	ABM FS Dry Ash-Free Weight (g)	Rice Straw FS Dry Ash-Free Weight (g)	Temperature (deg C)	Reaction Time (Minutes)	Bio-Oil Yield (%)
Rice Straw 275	0	7.322771	275	60	1.81%
Rice Straw 300	0	7.161252	300	60	7.87%
ABM300	8.431868314	0	300	60	5.12%
ABM Rice Straw	3.217440768	3.46626	300	60	8.60%

Table A-1: Hydrothermal Liquefaction Conditions and Yield

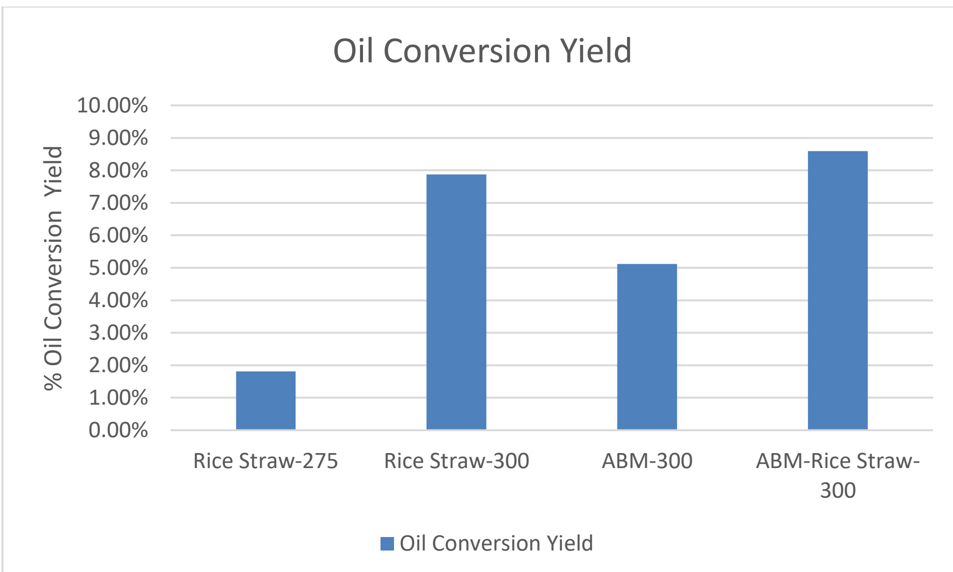

Figure A-1 Oil Yield from HTL of Rice Straw and ABM

First, this figure shows that increasing the temperature of the reaction of the rice reaction from 275 degrees C to 300 significantly increases the oil yield. Interestingly enough, a reaction with just ABM has a lower yield than the cellulosic biomass of rice straw. Having a rice and ABM mixture seems to increase the yield respective to pure ABM feedstock while decreasing the yield respective to pure rice straw. The yields for pure rice straw and rice straw/ABM mixture are relatively the same, though. The reason that the ABM/rice mixture increases the yield relative to pure ABM could be due to the fact that the increase in amount of ash from the rice straw might promote oil conversion (Chen et al., 2016). All yields are relatively the same as previous literature-reported yields for both macroalgae (7-20%) (Singh et al., “Hydrothermal liquefaction of macro algae”, 2015; Zhou et al., 2010) and rice straw (17-21%), (Singh et al., “Hydrothermal liquefaction of rice straw”, 2015; Tekn, Kubilay & Karagoz, Selhan, 2013). This shows that although the yields were lower than the Zhou et al., (2013) study algae, they are in line with other reported literature. This is mostly due to the fact the Singh et al., (2015) study had a similar feedstock of macroalgae compared to this study’s feedstock ABM. This shows that the ABM that grew naturally was a feedstock not preferable for HTL conversion. If the ABM from this study was grown in the 100 ha facility, crude oil production would not be a viable option.

To investigate the bio-oils further, CHN analysis was conducted on them to determine the quality. The results from the CHN analysis are shown below.

Element	ABM 300	ABM Rice Straw	Rice Straw 300	Rice Straw 275	Theoretical (Elliott, D.C., et al., 2013)
C (%)	69.285	71.815	71.47	71.345	70
H (%)	7.815	7.575	7.46	7.735	7
N (%)	3.08	2.605	2.645	2.255	3
O (%)	19.82	18.005	18.425	18.665	20
HHV (MJ/kg)	31.029	31.866	31.511	31.817	30.08

Table A-2: CHN Analysis of Bio-oil Produced from HTL of various feedstocks

These results show that the quality of the oil from each feedstock is very similar to oil produced from previous studies. The higher heating value (HHV) was then calculated using the formula (Sudhakar, K. and Premalatha, M., 2015):

$$HHV\left(\frac{MJ}{kg}\right) = 0.3383C + 1.422\left(H - \frac{O}{8}\right) \qquad \textbf{Eq. 3}$$

The higher heating values from the various feedstocks are very close to one another that not much can be said about whether having a mixed feedstock or using a lower temperature really has an effect on the quality of the oil. This means that the rice farmer could liquefy the ABM with the rice straw at the same time without sacrificing the quality of the oil. This coupled with the fact that liquefying the ABM with the rice increases the conversion yield relative to pure ABM means that it makes sense for rice farmers to produce oil from a mixture of the rice straw and ABM and could save time and money by liquefying them at the same time without sacrificing quantity or quality of the oil.

Comparing the HHVs from the feedstocks to the literature-reported data, it is evident that they are relatively the same. This means that although the conversion percentage is smaller than what would be expected, the oil product is generally the same quality so it could still be used for biofuel purposes as previously proposed. But since the yield is very low, it is not recommended that this feedstock be used for oil conversion. Instead, this problem could have been fixed by inoculating a macroalgae with better oil conversion capabilities. Since the ABM used in this study was not inoculated, this meant that the macroalgae grown was wild, and the oil content was therefore not controlled. If oil was the main purpose of the ABM like it is in this study, ABM should be inoculated. In regards to the economic analysis of this study, the oil yield of 5% from the experiment will not be used. Instead, the oil yield from the Zhou et al., (2013) study will be used (40%) since this was a biomass meant for oil conversion.