FINAL SUMMARY REPORT

DEVELOPMENT OF NUTRIENT AND WATER RECYCLING CAPABILITIES IN ALGAE BIOFUELS PRODUCTION SYSTEMS

California Polytechnic State University
San Luis Obispo, California

Advanced Algae Systems Program
Bioenergy Technologies Office
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Final Report

Development of Nutrient and Water Recycling Capabilities in Algae Biofuels Production Systems

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DEVELOPMENT OF NUTRIENT AND WATER RECYCLING CAPABILITIES IN ALGAE BIOFUELS PRODUCTION SYSTEMS

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EXECUTIVE SUMMARY

The objective of this two-year project was to develop and demonstrate methods of recycling of water and nutrients for the purpose of algal biofuels production. Recycling was accomplished both internal to the system and, in a broader sense, through import and reuse of municipal wastewater. Such an integrated system with wastewater input had not been demonstrated previously, and the performance was unknown, particularly in terms of influence of recycling on algal productivity and the practical extent of nutrient recovery from biomass residuals. The success of these practices would have profound effects on both the economics and environmental sustainability of algae biofuels.

BETO has set a goal of $3 per gallon of algae-derived gasoline equivalent (GGE) with a 5 billion gallon per year production by 2030. In terms of cost, over a 5-fold reduction is needed from the current projection of $18.63/GGE, and, therefore, the efficient use and recycling of resources is essential (Davis et al., 2012). In one projection, 28% of the operating cost of a large algae farm would be spent on nutrients and makeup water (urea, merchant CO_2, groundwater pump from 114 m) (Rogers et al., 2014).

Environmental lifecycle analyses find water recycling to be a “necessity” if algae biofuels are to contribute even a small percentage of US transportation fuel needs (Yang et al., 2011). The ability to recycle water many times will be critical to the success of algae biofuels (National Research Council, 2012). The recycling of nitrogen and phosphorus, both by reusing aqueous growth media and by the recovering these nutrients from processed algal solids, is “essential for the sustainable production of algal biofuels” (National Research Council, 2012).

The ability to recycle both water and nutrients depends on the technologies used to harvest and process the biomass into fuel. For example, in harvesting, if chemical coagulants are used, phosphate might be lost to precipitates and the blowdown rate increased due to salts in the coagulant. An even more crucial technology choice is the biomass-to-biofuel conversion process, which affects the disposition of the nutrients in the biomass. Nutrients can be lost in the biofuel intermediate or trapped in poorly degradable biomass residuals.

Many fuel conversion technologies have been investigated in projects supported by the BETO Advanced Algae Systems Program and others, including oil extraction from biomass, hydrothermal processing, and anaerobic digestion. Anaerobic digestion was selected for the present study because (1) it is a technology that is essentially ready for scale-up with algal biomass as the feed, (2) biogas is regularly purified to renewable natural gas (RNG) for vehicle fueling, providing substantial renewable fuel credits), and (3) it is already commonly used in the wastewater treatment industry—a potential early adopter of the investigated process. In contrast, oil extraction and hydrothermal liquefaction (HTL) are longer-term options, having high costs and intensive process requirements (e.g., biomass dewatering, use of hazardous chemical or high pressures, fuel upgrading needs, etc.) Given the importance of liquid fuels, an experimental study was included on growth of algae on recycled wastewaters from HTL production of biocrude from algal biomass.

For the present project, the envisioned process flow diagram for future full-scale facilities (Figure 1) has as inputs daily or continuous flow of wastewater to provide water, nutrients, and carbon and, during the peak summer growing season, input of CO_2 from external sources.

The main waste output is blowdown of unusable growth medium, which is raceway water that has accumulated excessive growth inhibitory compounds. (The extent of such inhibition was a major topic of this project.) The biofuel output is designated to be RNG, as discussed above.

Algae are harvested by sedimentation of bioflocculated culture, which may be enhanced as needed by use of chemical coagulant. Settler subnatant is thickened to 1-3% solids prior to anaerobic digestion.

Three internal recycling streams are used: CO_2 separated from the biogas is sparged into the raceways to provide carbon for autotrophic algae growth, supernatants from the algae settling clarifiers and algae slurry thickening tanks are recirculated to the raceway ponds for reuse, and anaerobic digester effluent (digestate) is pumped to the raceways to provide nutrients (e.g., nitrogen, phosphorus, minor nutrients).

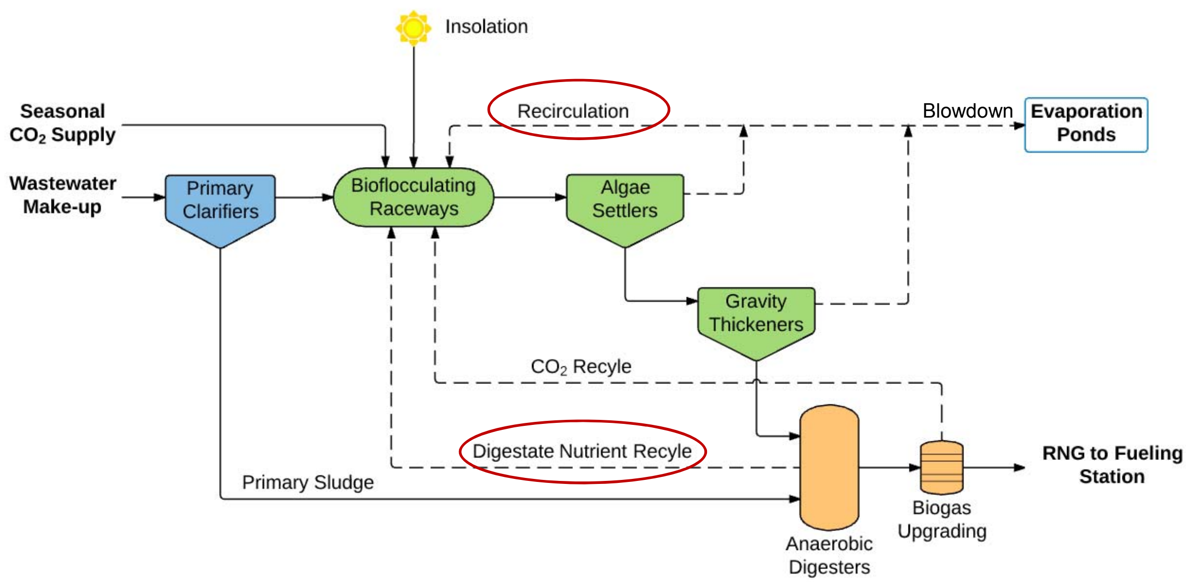

Figure 1. *The process flow diagram for the envision algae biofuel production system. The paths that were the main focus of the present study are circled. The battery limits for the techno-economic and lifecycle assessments conducted for the present study include all unit operations shown, including the evaporation ponds.*

An unusual aspect of this process design is the recycling of whole digestate, as opposed to recycling of only the liquid fraction of the digestate, which is more commonly proposed and studied. Returning whole digestate has the advantages of potentially increasing the solubilization and reuse of particulate nutrients and the provision of additional organic carbon to promote heterotrophic and/or mixotrophic biomass production in the raceways.

The envisioned full-scale digesters are modeled after covered lagoon digesters, which are common at confined animal facilities. These unmixed, unheated digesters were selected for their low cost compared to the mixed, heated, above-ground tank digesters often used at urban municipal wastewater treatment plants. Although digestion is slower in unheated digesters, the low-cost construction (plastic lined earthwork) allows for long retention times which compensate to a large extent for the low digestion rate.

Overall, the present project focused mainly on exploring the effects of digestate and supernatant recycling to the raceways (the two paths circled in Figure 1) in terms of biomass productivity and nutrient recycling efficiency. Through long-term lab- and pilot-based research, the main project accomplishments and conclusions were the following, described in order from preliminary experiments to pilot-scale experiments:

1. Bench-scale pre-treatment of algal biomass did not sufficiently increase methane yield or nutrient solubilization during anaerobic digestion to warrant incorporation of pre-treatment into the pilot plant. The trialed pre-treatments were high-pressure orifice homogenization, sonication, and two types of heat treatment.
2. Solubilization of biomass particulate nutrients by lab anaerobic digesters ranged from 20% to nearly 60% for N and 40-65% for P. Subsequent aerobic degradation of the anaerobically digested biomass simulated aerobic raceways receiving whole digestate and resulted in an additional 20-55% N solubilization and an additional 20% P solubilization.
3. Comparisons of laboratory and pilot digesters showed that laboratory digesters were reasonable proxies for pilot-scale.
4. Pilot-scale anaerobic digesters were designed, installed, and operated to digest algal biomass. Nutrient re-solubilization by the digesters was monitored and whole digestate was successfully used as a fertilizer in pilot algae raceways.
5. Unheated, unmixed digesters resulted in greater methane yield and nutrient solubilization than heated, mixed digesters, presumably due to longer the solids residence times in unmixed digesters. The unmixed, unheated pilot digesters yielded 0.16 L_{CH4}/g volatile solids (VS) introduced with 0.15 g VS/L-d organic loading and 16^{o}C average temperature. A conventional heated mixed lab digester yielded 0.22 L_{CH4}/g VS with 0.25 g VS/L-d and 30^{o}C. The highest yield (0.30 L_{CH4}/g VS) was achieved by the unmixed lab digesters operated at a constant 20^{o}C. All digesters were operated with a 40-d hydraulic residence time.
6. In general, 50-75% of initial particulate N and P could be solubilized during anaerobic digestion and available for subsequent rounds of algae cultivation.
7. Bench-scale experiments demonstrated the recovery from hydrothermal liquefaction (HTL) wastewater of carbon via anaerobic digestion and of nutrients to grow algae. To satisfy the nitrogen demand of algae cultivation, HTL wastewater would be diluted 400-fold, which was found to eliminate inhibition of algae growth by HTL wastewater.
8. Anaerobic digestion methane yield was lower for algal biomass containing coagulants such as would be used to aid harvesting or dewatering. Depending on doses, starch-based coagulant decreased yield by 10-14% and aluminum chlorohydrate decreased it by 14-26%. The lowest yield was 0.28 L CH_4/g volatile solids introduced to the digesters.
9. Algae harvested from raceways operated with recycled water had methane yields 13% higher than algae from raceways operated with both recycled water and nutrients provided by whole algae digestate. The slightly lower yield was expected due to the presence of previously digested biomass from the digestate fertilizer.
10. Defined media was replenished with nutrients and recycled repeatedly in sequential batch growth of *Chlorella sorokiniana* (DOE 1412). This laboratory study tested for inhibition and accumulation of inhibiting compounds (allelopathic or auto-inhibitory substances), information that would help estimate the blowdown ratio needed for an integrated system (Figure 1). In these laboratory experiments in which water was recycled a total of five times, each successive round of reuse resulted in an average 4±3% reduction in log-phase specific growth rates. However, linear-phase growth inhibition was only observed in the final fifth round of reuse.

11. No decline in productivity was detected after 15 rounds of water recycling with nutrients provided by whole digestate in lab cultivation. Lab testing allowed for steady light and temperature, thereby increasing the ability to statistically detect any inhibition.
12. An initial pilot inhibition study was commenced early in the project. Wastewater growth media was reused once while productivity was monitored. Media reuse was accomplished with triplicate sets of 33-m^2 raceways operated in series. First-round gross productivity (based on effluent biomass flow) averaged 23 g/m^2-day annually while second-round gross productivity averaged 19 g/m^2-day annually. In terms of net productivity (based on raceway effluent biomass minus influent biomass), the first-round productivity averaged 15 g/m^2-d and second-round averaged 13 g/m^2-d during June-September operation. The higher productivity in the first-round ponds was likely due to heterotrophic/mixotrophic growth on the wastewater organic matter.
13. In a culminating pilot experiment, coagulant was used to decrease the carry-over of unsettled algae into subsequent rounds of growth. Over nearly 8 months, 93% of the media (or the equivalent of 14 rounds of water re-use) was recycled without significant loss in culture productivity compared to control raceways. The ponds receiving both recycled water and nutrients had net productivities of 14-24 g/m^2-d during fall and mid-summer, respectively.
14. Techno-economic analysis of the proposed facility found the minimum fuel selling price to range from $7.01/gallon gasoline equivalent without revenue other than fuel to $3.85/GGE with revenue from wastewater treatment fees and LCFS and RIN (Low Carbon Fuel Standard and Renewable Identification Numbers) credits.
15. Life cycle assessment indicated GHG emissions of 40.7 g CO_2/MJ fuel and a net energy ratio (energy required/energy produced) of 0.37.

These studies are discussed in Part I and Part II, below, with Part I describing preliminary studies on solubilization of nutrients from algae biomass, anaerobic digestion and recycling of hydrothermal liquefaction by-products and Part II covering results from operation of integrated water and nutrient recycling systems.

Part I – Preparatory Studies on Nutrient Recycling and Anaerobic Digestion

The following sections summarize investigations on anaerobic digestion and hydrothermal liquefaction waste recycling.   Extensive details for the work described below are provided in Appendices A-C.

More specifically, experiments examined anaerobic digestion of algal biomass to produce biogas and solubilize nutrients for recycling to raceways for additional algal production. Hydrothermal liquefaction is an additional potential pathway of fuel production in the algal biorefinery model (Elliot et al 2015, Biller & Ross, 2012) and so using hydrothermal liquefaction aqueous phase byproducts as a source of recycled nutrients was investigated briefly. Bioflocculation and gravity settling/thickening are used in the envisioned process, but occasional poor settling necessitates the use of chemical coagulants. The effect of coagulant addition on the anaerobic digestion of produced algal biomass was examined at laboratory scale.

This Part I discusses small-scale experiments meant to isolate and evaluate some of the process variables important in the larger pilot-scale experiments discussed in Part II of this report. The results of these experiments allow extended interpretations of the integrated bench and pilot scale nutrient and water recycling studies described in Part II.

Anaerobic Digestion of Algal Biomass for Methane Production and Nutrient Solubilization

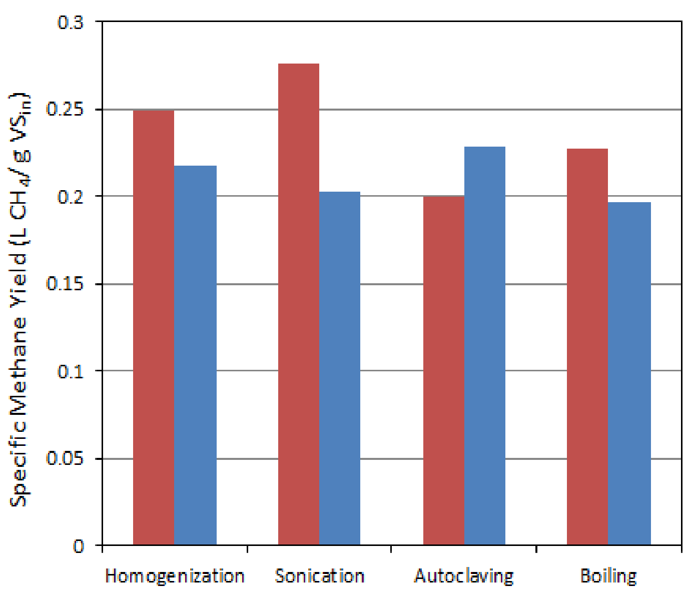

Figure 2. *Average specific methane yield from anaerobic digestion of algal biomass pre-treated by four methods. Blue bars = pre-treated biomass, red bars = non-treated controls.*

In the envisioned process with digestion, nutrient solubilization occurs initially anaerobically in the digesters, and then aerobically in the raceways receiving digestate. The following study examined nutrient recovery by this anaerobic-aerobic process using wastewater-grown algal polycultures.

In these studies, biomass was anaerobically digested for 39-43 days and then aerobically incubated in air-sparged beakers an additional 37-40 days, both in batch mode. Aerobic incubations were ended when biodegradation rates greatly declined, indicating near completion. In addition, four algae biomass pre-treatments were evaluated for their effects on methane yield and nutrient solubilization: high-pressure homogenization, sonication, autoclaving, and boiling. Autoclaving provided a short, high temperature treatment, whereas, boiling was a long, lower temperature treatment that might be practical using waste heat from methane-fired engines or imported from an offsite facility.

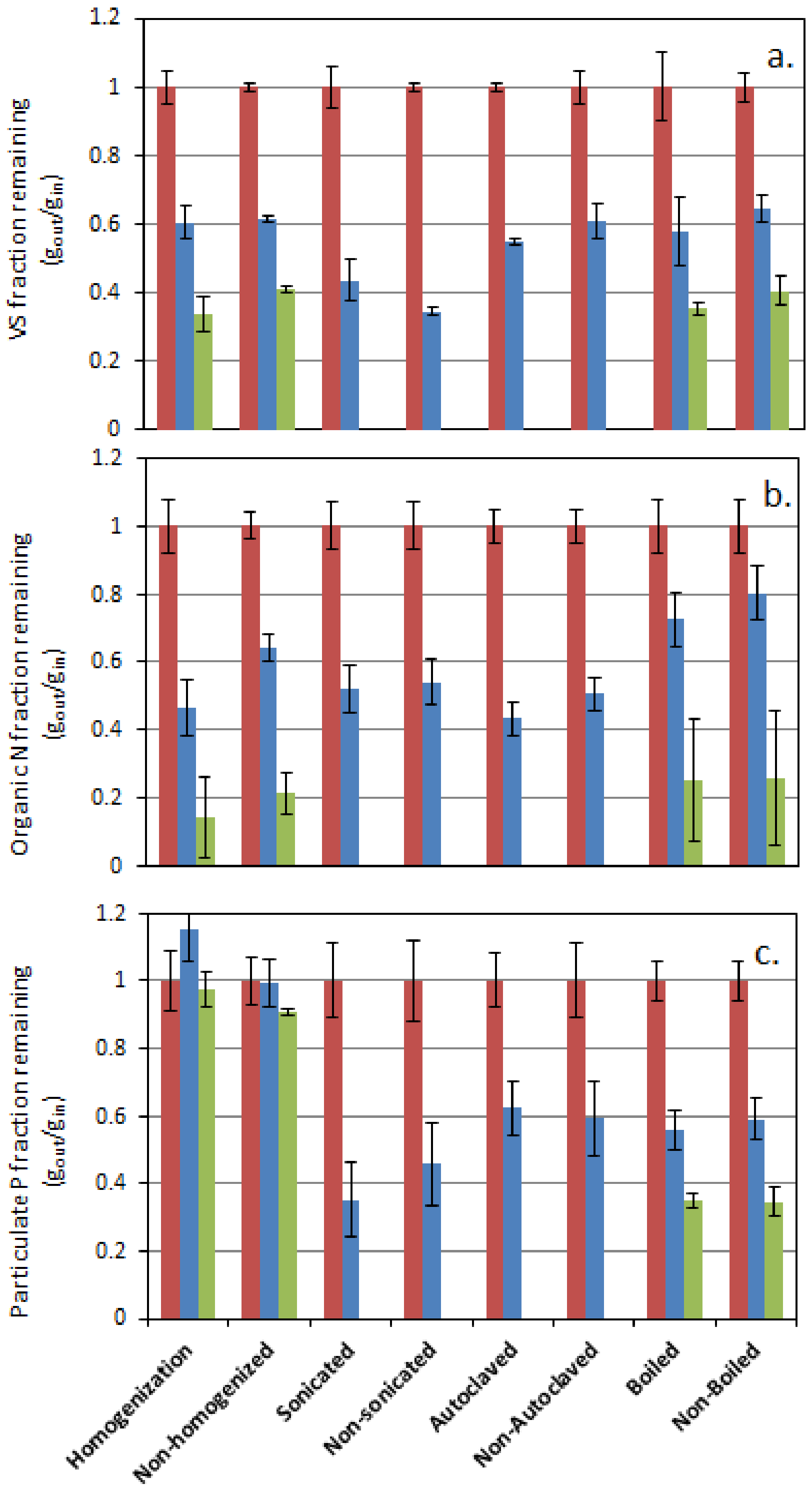

Figure 3. *Fraction remaining of (a) volatile solids, (b) organic N, and (c) particulate P. Red bars = initial fraction (100%); Blue = post-anaerobic digestion; Green = post-aerobic incubation. Initial and post-anaerobic error bars = maximum percent error between duplicate digesters. Post-aerobic error bars = maximum percent error between triplicate reactors.*

Methane Yield

Methane yield was quantified as liters of methane produced per gram of volatile solids introduced to the digesters (L_{CH4}/g VS_{in}). Volatile solids are a proxy for organic matter. Pre-treatment resulted in increased specific methane yields for homogenization, sonication, and boiling by 15%, 36% and 15%, respectively, whereas for autoclaving, the pre-treatment resulted in a 12% reduction in yield (Figure 2).

While homogenization and sonication are not practical to scale-up for biofuel applications due to their high energy intensity, they were meant in these studies to serve as the benchmark of cell disruption for comparison to other treatments. Even with the 36% specific methane yield increase for the sonicated samples, the estimated power use of full-scale sonication would more than consume the increase in fuel production. In fact, for each of the four pre-treatments, net energy estimates concluded that the non-treated biomass resulted in a more positive energy balance. These findings are consistent with a number of previous studies which investigated a variety pretreatments including mechanical, thermal, biological, ultrasound, microwave and chemical pretreatment (Santos, et al. 2014, Schwede et al. 2013, Ometto et al. 2014, Alzate et al. 2012, Passos et al. 2013, Cho et al. 2013, Gonzalez-Fernandez et al. 2012).

While many of the pretreatments in these studies resulted in an increase in methane production, the conclusion of most was that pretreatment either resulted in a net energy decrease due to the energy input requisite of pre-treatment or not enough information was available to determine the energy balance (Cho, 2013, Gonzalez-Fernandez et al. 2012, Passos et al. 2013).

Nutrient Solubilization

Biomass nutrients are solubilized during biodegradation of biomass particulate solids. The solubilization of volatile solids (VS) (Figure 3a) was examined to confirm measurements of nutrient solubilization. VS solubilization (i.e., VS loss) corresponded to soluble nitrogen (N) and phosphorus (P) increases in all cases except for P in the homogenized samples, likely due to P analytical error or P precipitate formation in the digesters.

The four pre-treatment experiments were not all conducted at the same time on the same biomass. Results showed larger differences in the extent of nutrient solubilization between experiments than between any pre-treatment and control. Solubilization of particulate N in the anaerobic phase ranged from 20% to nearly 60% (Figure 3b). Excluding the homogenization experiment in which P-rich precipitates formed (presumably struvite; Ohlinger, et al. 1998) giving negative solubilization, the P solubilization during anaerobic digestion ranged between 40-65% of the initial particulate P (Figure 3c). None of the pre-treatments resulted in substantially increased nutrient solubilization versus digestion without pre-treatment.

Aerobic degradation of the anaerobically digested biomass resulted in an additional 20-55% N solubilization for the homogenized or boiled biomass (Figure 3b) and an additional 20% P solubilization for the boiled biomass. As with the anaerobic digestion, the pre-treatments did not affect solubilization substantially.

The combined anaerobic-aerobic processes solubilized up to 85% of particulate N and 65% of particulate P, but more work is needed to understand the great differences is solubilization among the controls. Algae biomass grown in pilot raceways at different times appears to have largely different biodegradability. Previous studies have also found the effect of pretreatment to be species specific (S. Cho, 2013; F. Ometto, 2014; P. Bohutskyi, 2014; M.E. Alzate, 2012).

Kinetic Decay Rates

The maximum rates of biodegradation were also examined and no substantial difference in the rates of decay between treatment and control occurred. As expected, rates of decay were faster during anaerobic digestion than during the aerobic incubation in which only biomass resistant to anaerobic biodegradation remained to be solubilized. This was, however, not the case during anaerobic digestion of the boiled biomass. In that case, anaerobic rates of decay were similar to the aerobic decay rates and less than the anaerobic rates of the homogenization experiment. A possible explanation for this decrease is the formation inhibitory compounds when algal biomass is exposed to excessive heat.

Overall, it was concluded that none of the pre-treatments had enough of an effect on nutrient solubilization to merit incorporation of pre-treatment into the pilot scale process flow, especially given the evaluation of the net energy balances based on methane production. (For more detailed descriptions of the data discussed in this section, see Appendix A.)

Design, Installation and Operation of Pilot Scale Anaerobic Digesters

Knowing the pretreatment experimental results and extent of nutrient solubilization, the design of pilot digesters was completed. Two pilot digesters were assembled from 500-gallon HDPE vertical storage tanks, each made airtight by plastic-welding an HDPE plastic disk over the access port. The tanks were manufactured with threaded ports for biogas collection, temperature measurement, and feeding and draining at various elevations (Figure 4). Silicone caulk (Kwik Seal, DAP, Baltimore, Maryland) was applied to all seams and ports as a precaution against leaks. The gas-tightness of the digesters was confirmed by connecting the digester to an elevated tank of water, allowing the headspace gas to compress, while monitoring for a drop in water surface elevation over a period of 48 hours.

Digesters were placed directly on the underlying soil, and covered in thermal insulation (TekFoil Reflective/Bubble Insulation, Dyersville, Iowa) to reduce diel temperature fluctuations. Digesters operated at a liquid level of 300 gallons, leaving 200 gallons of headspace and requiring 7.5 gallons to be fed and drained every day to achieve a 40-day residence time. A gas volume buffer was needed to prevent negative pressure during nightly drops in headspace gas temperature. Equalization was provided by a truck tire inner tube (butyl rubber) connected to the gas effluent line (Tygon formulation E-3603) of each digester. Gas effluent lines included fittings for collecting biogas in Tedlar bags (Zeflon International, Ocala, Florida) for return to the laboratory for composition analysis.

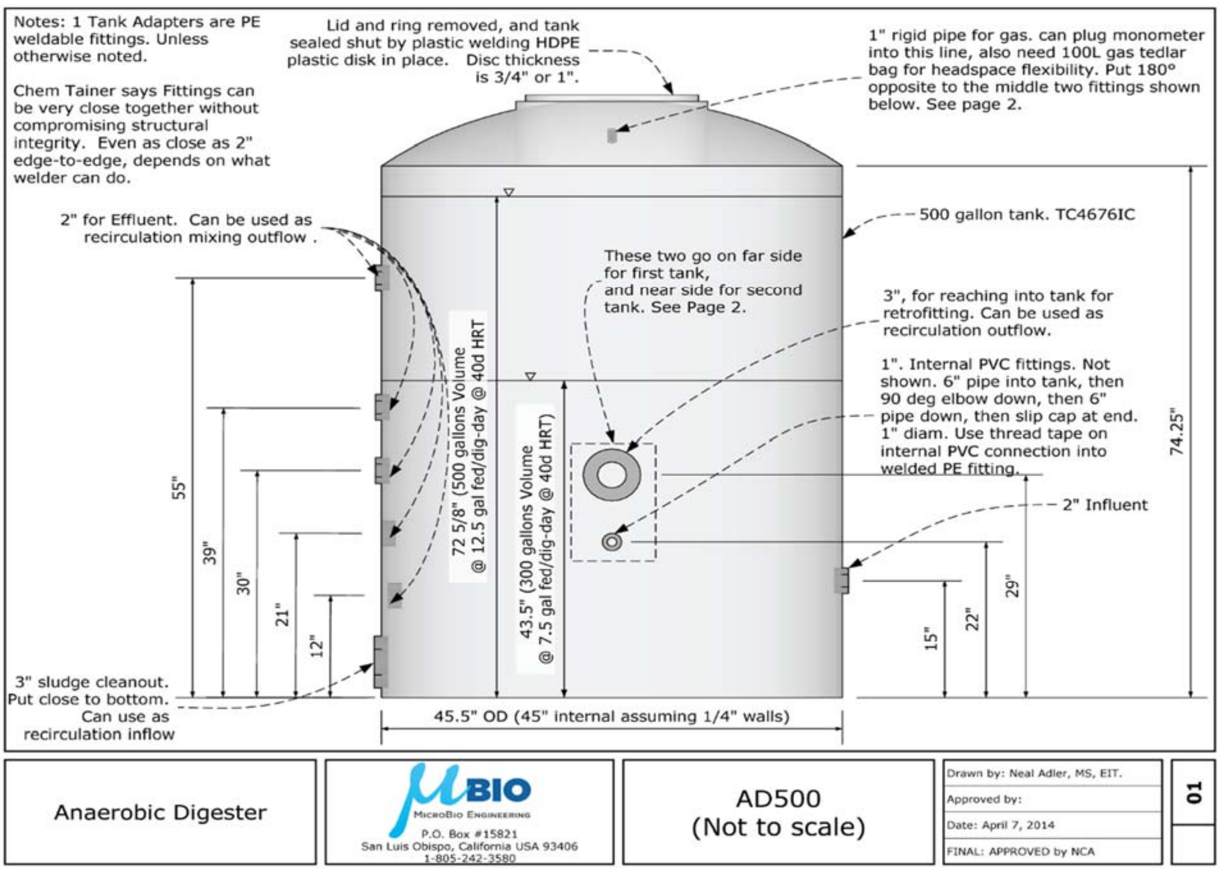

Figure 4. *Drawing of one of two identical pilot-scale anaerobic digesters. Influent was fed into the port 12" from the ground, and effluent was taken from the port 30" from the ground. Temperature probes were inserted into a capped, water-filled, 1"-diameter, dead-end PVC fitting located 22" from the ground that extended into the digester. Gas lines originated from a 1"-diameter port at the top of the digester and connected to a tipping gas meter in a shed 10' from the digesters, which included a connection to a tire inner tube for gas equalization.*

Comparison of Laboratory and Pilot Anaerobic Digesters

Anaerobic digester optimization experiments were conducted at laboratory and pilot scales with daily feeding, which is more realistic than the batch digestion used in the previously described pre-treatment experiments. Preliminary experiments were conducted to establish to what extent the laboratory digesters were a reasonable proxy for the pilot digesters. These studies compared methane production of the pilot digesters to lab digesters operated unmixed with varying feeding rate and varying temperature (simulating temperature fluctuations observed in the field). The volume-normalized methane production of the pilot and lab scale digesters differed by only 6%, allowing for expanded research to be done at a convenient lab scale with more confidence in their applicability to larger scale facilities.

Anaerobic Digester Evaluation and Optimization

The objective of the pilot digestion study was to assess biogas production and nutrient solubilization in a "lowest-cost" scenario, realistic for biofuel economics, consisting of unheated, unmixed digesters fed with gravity-thickened algae biomass. Simulating conditions at future full-scale facilities, the pilot digesters were operated under the conditions naturally imposed on them: daily and seasonal temperature fluctuations and varying feed composition. The biomass in the feed contained naturally-changing algal-bacterial species, and the organic loading on the digesters changed with raceway pond productivity and algae settling/thickening performance. However, the temperature fluctuations of the above-ground pilot digesters were more extreme than would be expected in full-scale lagoon digesters insulated by earthwork.

Simultaneous with the pilot operation, laboratory experiments were used to test the effect of variables on both biogas production and nutrient solubilization (Table 1). One set of lab digesters ("VFU") was operated in the same way as the pilot digesters—fed the same varied organic load depending on the daily algae harvest ("V"), field temperatures ("F"), and unmixed ("U"). As in the earlier batch digestion study, one set of digesters was fed sonicated biomass as a possible maximum methane yield case (SHU). The treatment codes can be understood from the first two columns of Table 1.

Table 1. *Operating conditions for the semi-continuous feed digester studies.*

Digester Name	Label	Average Organic Loading Rate (g VS/L-d)	Average Operating Temperature	Mixing status
Varied feed, Unheated, Unmixed	VUU	0.15 (range: 0.01-0.65)	20 °C	Unmixed
Varied feed, Heated, Mixed	VHM	0.15 (range: 0.01-0.65)	30 °C	Mixed
Constant feed, Heated, Unmixed	CHU	0.25	30 °C	Unmixed
Sonicated feed, Heated, Unmixed	SHU	0.25*	30 °C	Unmixed
Constant feed, Heated, Mixed	CHM	0.25	30 °C	Mixed
Lab Conditions: Varied feed, Field Temperature, Unmixed	VFU	0.15 (range: 0.01-0.65)	14 °C**	Unmixed
Field Conditions: Varied feed, Unheated, Unmixed	Pilot	0.15 (range: 0.01-0.65)	16° C**	Unmixed

*100 ml of 0.5%-2% VS thickened algal biomass sonicated daily for 10 minutes using Analog Branson sonifier 250 with 1/2" tapped tip.
**Average of variable daily temperatures from entire duration of experiment.

Effect of Varying System Conditions

The pilot digesters and the lab digesters that simulated pilot conditions (VFU) did have nearly identical methane yields (Figure 5), giving additional credibility to the results from the other lab digesters. The digesters with the conventional mode of operation with consistent feeding, heating to 30°C, and mixing (CHM) resulted in only middling methane yields. The highest yield came from the variable-feed, unheated, unmixed (VUU) digesters operated at 20°C. Lack of mixing allows thickening of settled solids in the digester providing a longer solids residence time for digestion than would be indicated by the imposed hydraulic residence time (i.e., volume divided by feed flow). Apparently this increased solids residence time allows sufficient additional digestion to outstrip the negative influence of lower temperature—a result that supports the notion that unmixed lagoon digesters might be practical for algae biofuel production. Thus, one of the key findings of the digestion studies was that, despite lower temperatures, unmixed VUU digesters had about 50% higher methane yield per gram VS introduced than mixed and heated digesters (CHM and VHM). This contrasts traditional thinking on digestion that mixing improves methane yield. However, in a few manure digestion studies, unmixed or minimally mixed digesters demonstrated increased methane yields in low organic loading scenarios compared to vigorously mixed digesters (Kaparaju, 2008, Khursheed, 2005). Therefore, for purposes of biofuel production where concentrating algal biomass can be costly, unmixed digestion may be beneficial in an integrated biofuel process by lowering energy input, providing resilience to variable organic loading, and by producing low-solids effluent for recycling into algae ponds. Implementation of a full-scale system using unmixed digesters could save mixing costs as well as result in increased methane yields.

The vastly better yield of the 20°C VUU digester compared to the 14-16°C VFU and pilot digesters might have been due to more than the average temperature difference. The VUU digester temperatures were a constant 20°C, whereas the VFU and pilot digesters experienced daily temperature variation, which is well-known to inhibit methanogenesis. In particular, nearly all instances of pilot effluent pH dropping below 6.70 (slightly lower than the optimum range) occurred during periods of prolonged low temperature.

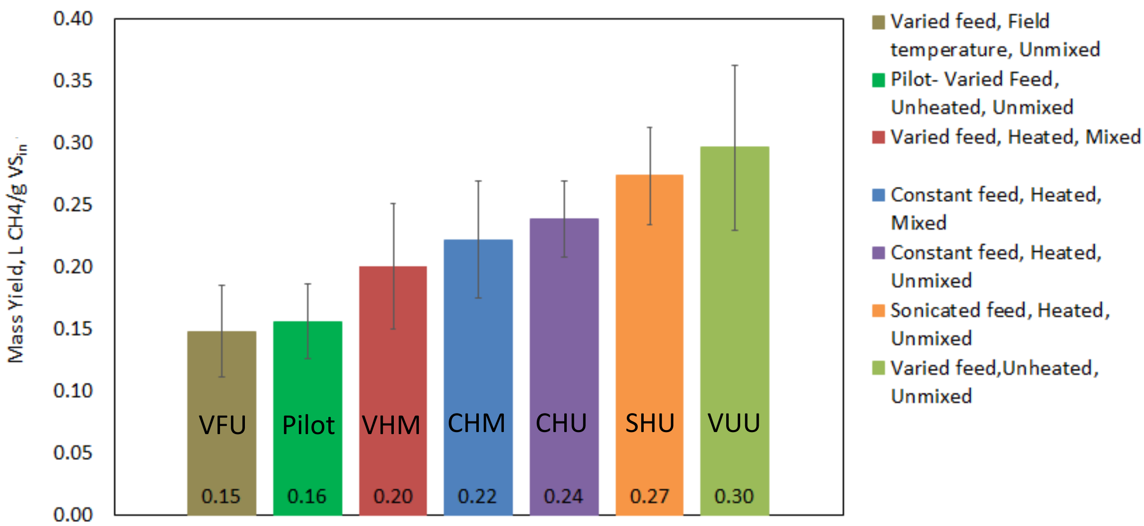

Figure 5. *Average specific methane yield of digesters for the operational conditions defined in the legend. Error bars represent the standard deviation of the means of duplicate digesters.*

Effect of Organic Load on Methane Yield

Optimizing methane production involves adjustments to the rate of organic loading to the digester. Studies were designed to determine whether load variations due to variable algal productivity would have detrimental effects on methane production. While constant feed digesters (CHM) showed greater yields than variable feed (VHM), the difference was ultimately more reflective of the greater overall organic load to CHM than the effect of variability in organic loading (average VHM organic load was approximately half of CHM organic load during the representative period). Methane yield per gram VS introduced and per gram VS destroyed were both higher for VHM digesters, even though total methane production was 65% higher for CHM digesters due to their higher loading. Assuming fluctuations in pilot scale productivity and settling performance were reflective of a typical system, feeding digesters such

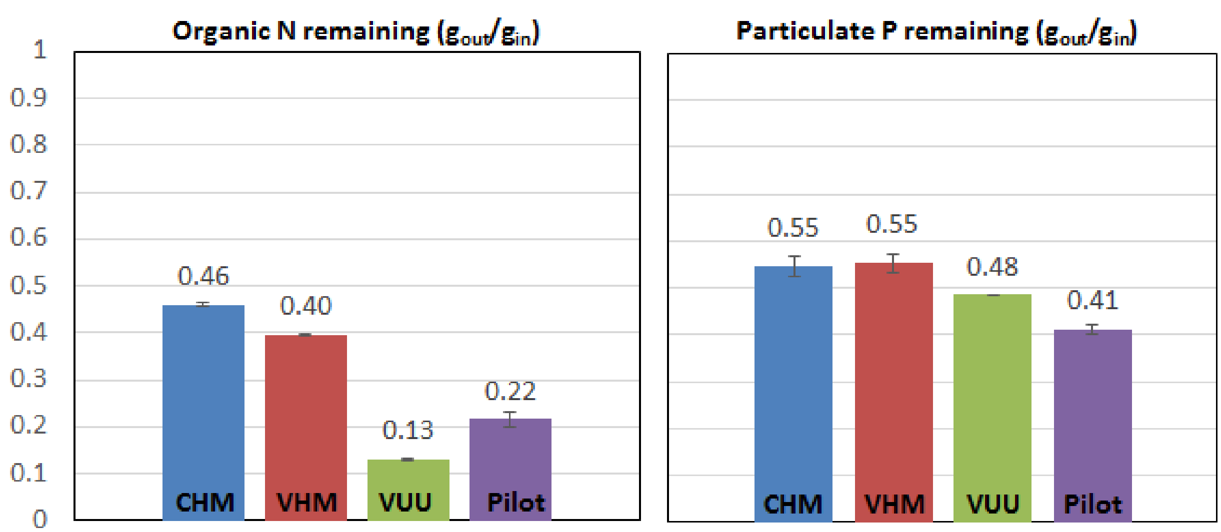

Figure 6. *Fraction of organic N and particulate P remaining in digester effluent as a function of digester operational parameters from 8/1/2014 - 4/1/2015. A shorter bar indicates a greater fraction of solubilization. Error bars represent one standard deviation of the means of duplicate digesters.*

varying organic loads should not be detrimental to methane production; thereby possibly eliminating the need for mechanical thickening prior to digestion.

Nutrient Solubilization as a Function of Operating Condition

Unmixed digesters appear to have higher nutrient solubilization than mixed. This is most likely to due to having a greater SRT than HRT - not mixing the digester allows for increased organic degradation and, as a result, increased nutrient release. Digester effluent from the unmixed digesters had more solubilized N and P than the mixed digesters. While this was more notable for N than P, it nevertheless suggested an unmixed set-up would be functional for nutrient recycling as well as biogas production (Figure 6).

These semi-continuous fed experiments showed slightly greater N solubilization and similar P solubilization compared to the previous batch experiments. In general, the experiments demonstrated that 50-75% of initial organic N and P could be solubilized during anaerobic digestion and available for subsequent rounds of algae cultivation.

Ultimately, studies suggest that unmixed digesters designed with effective insulation should have lower costs as well as improve nutrient solubilization and methane production compared to conventional digesters. The results also indicate that methane production should not be compromised as a result of variable organic loads within the range of typical algae raceway pond productivities and settling efficiencies.

Long-Term Operation of Pilot Scale Digesters

The two unheated and unmixed, 300-gallon (1,136-L) pilot digesters were operated at steady-state, 40-day residence time at the Cal Poly Algae Field Station (AFS) for 15 months.

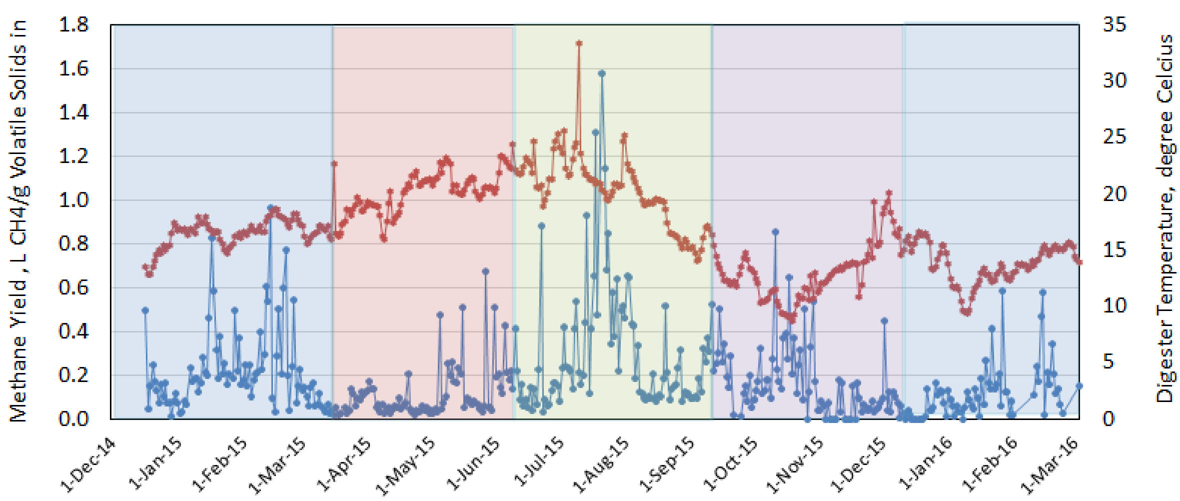

Figure 7. *Mean field digester methane yields (blue) are compared to digester internal fluid temperature (red) during 15 months of steady state operating conditions. Color blocks in the background represent seasonal delineations.*

Mean seasonal temperatures for the 15-month steady state digestion period were 14°C, 17°C, 21°C, 17°C, 13°C for Winter 2014/15, Spring 2015, Summer 2015, Fall 2015, and Winter 2015/16, respectively. Steady-state was defined as 120 days, or 3 residence times, from digester inoculation. The digester liquid temperature ranged from 9°C to 23°C with an average of 16°C (Figure 7).

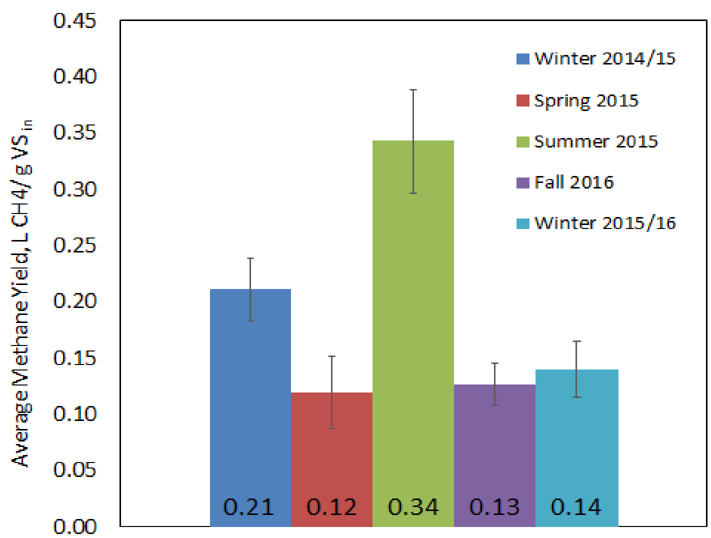

Figure 8. *Average methane yield for 15 months of steady state pilot digester operation. Error bars represent one standard deviation from the means of the two digesters.*

Summer months resulted in the greatest methane yields (Figure 8) and were comparable or better than yields achieved in other algal anaerobic digestion studies (Golueke et al. 1957, Mussgnug et al. 2010, Ramos-Suarez et al. 2014). These results suggest cost-effective means of stabilizing and potentially raising temperature (e.g., engine waste heat) will be important design considerations for a full-scale system. A cost analysis would be required to find the optimal amount of heating that would yield a net increase in energy.

For more detailed descriptions of the data discussed in this section, see Appendix B.

Recycling Nutrients from Hydrothermal Liquefaction Residuals

Hydrothermal liquefaction (HTL) uses high temperature and pressure to convert wet algae biomass into a biofuel intermediate suitable for upgrading to diesel and kerosene range hydrocarbons (Elliott et al. 2013, Elliott et al. 2015). It is especially promising because unlike other conversion technologies, wet biomass (~20% solids) can be fed into the reactor, thereby eliminating the time and expense associated with drying the biomass, as generally required for lipid extractions. HTL produces wastewater and waste solids that need to be disposed of or recycled. The aqueous phase wastewater (HTL-aqueous) is rich in essential algal nutrients (N, P, K, Fe, Mg, Ca) but also contains substances that can be inhibitory to algal growth (Jena et al. 2011; Pham et al., 2013). Toxic by-products such as heavy metals, remnants of catalysts, phenols, and heterocyclic nitrogen compounds can inhibit algal growth and nutrient recycling.

Lab scale experiments were conducted to determine procedures for treating HTL-aqueous and recycling it for algae growth. The following topics were explored:

- Supplementing HTL-aqueous into microalgae growth media as a means of recycling nutrients.
- Anaerobic co-digestion of HTL-aqueous with wastewater solids as a means of recovering carbon from HTL-aqueous as biogas.
- Supplementation of digested HTL-aqueous into media to identify whether anaerobic co-digestion detoxifies HTL-aqueous.

Recycling Nutrients via HTL-aqueous Supplementation

In the first study, algae were grown on either primary clarified wastewater (PE) or BG11 media supplemented with HTL-aqueous at different dilutions to provide sufficient N for replete growth conditions.

Results indicate that microalgal growth is inhibited by HTL-aqueous at dilutions between 40-200, with increasing inhibition at higher doses of HTL-aqueous. However, at dilutions of 200-1,000, this inhibition is no longer evident, and at 1,000 dilution factor (DF), the BG11 treatments dosed HTL-aqueous had slightly greater productivities than the control (Figure 9). Assuming 8 g N/L HTL-aqueous, a dilution factor of 400 should provide sufficient N for productivities of 20 g/m^2-day and a 4-day hydraulic residence time (HRT). Together these results suggest that dilutions of HTL-aqueous, in volume sufficient to provide adequate nitrogen, are not inhibitory. These results comport with previous work showing that dilution factors of 200-400 are sufficient to reduce toxins to non-inhibitory concentrations (Biller et al., 2012; Garcia Alba et al., 2013; Jena et al., 2011).

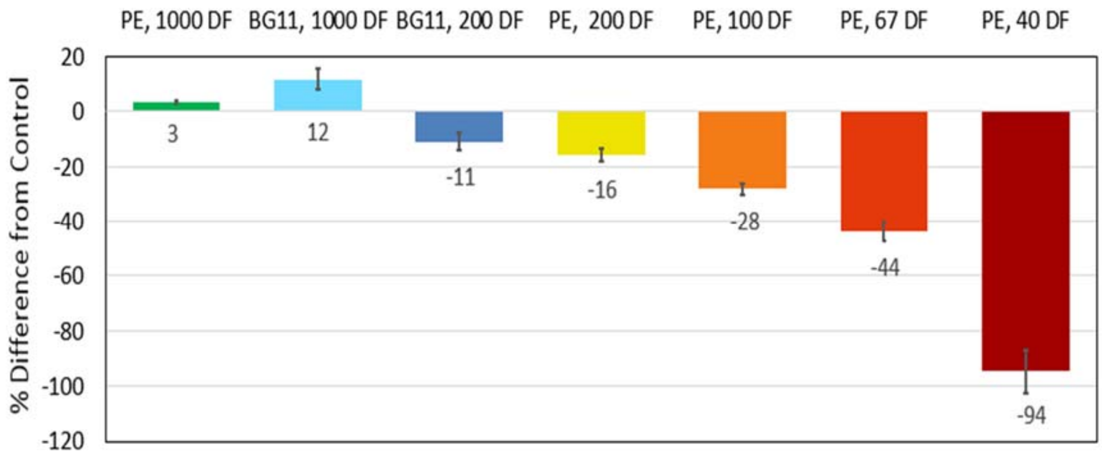

Figure 9. *Percent difference from primary effluent (PE) control productivity (mg/L-day). Error bars represent standard error of the mean of replicate reactors.*

Co-Digestion of HTL-aqueous to Recover Carbon

In an effort to recover carbon from the HTL-aqueous, the next study examined anaerobically co-digesting HTL-aqueous. The HTL-aqueous was digested with primary and secondary municipal wastewater solids to assure that the combined feed was balanced in terms of nutrients. HTL-aqueous from algae cultivated at two different sites (San Luis Obispo [S] and Delhi [D], California) was co-digested with wastewater solids in 1-L serum bottles for 76 days. Digesters supplemented with HTL-aqueous produced more methane (CH_4) than digesters with wastewater solids alone (Figure 10). Furthermore, the 30% dilution with San Luis Obispo algae HTL-aqueous (gray bar) produced ~12% more methane than the same dilution with Delhi algae HTL-aqueous, which is consistent with observations showing Delhi algal biomass to have higher ash content than biomass grown in San Luis Obispo (ash content data not shown).

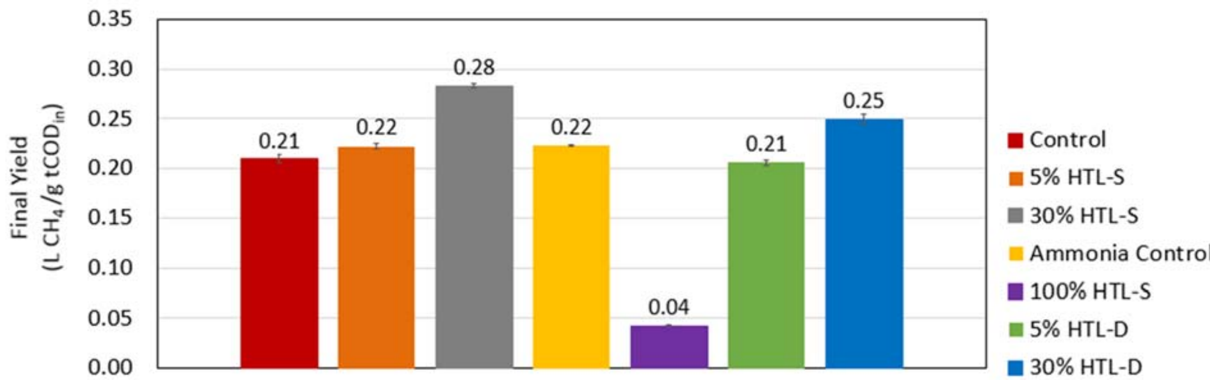

Figure 10. *Average cumulative methane (CH_4) production. Error bars represent one standard deviation from the mean of replicate digesters. Treatments appended with S indicate digestion of algae biomass from San Luis Obispo pilot raceways, D indicates digestion of algae biomass from Delhi pilot scale raceways (operated under a separate project).*

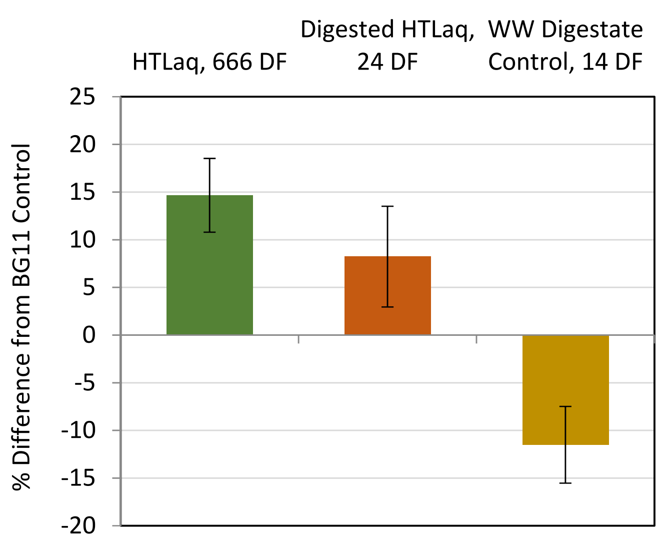

Figure 11. *Productivity percent difference from control as a function of HTL pretreatment. Note: Differences in dilution factors (DF) were calculated to provide requisite nitrogen for nutrient replete conditions.*

Depending on the source of the initial algae feedstock, the supplementation of 30% HTL-aqueous increased CH_4 yield between 20-33% whereas supplementation at 5% did not affect CH_4 yield and digestion of 100% HTL-aqueous resulted in an 81% decrease in CH_4 yield. These findings demonstrate that, although HTL-aqueous does not digest well alone, it may improve CH_4 yields when co-digested with wastewater solids and provide a means of converting residual waste carbon into fuel.

Anaerobic Digestion to Reduce HTL-aqueous Toxicity

The third set of experiments aimed at determining if anaerobic digestion of HTL-aqueous would reduce its toxicity was inconclusive. At first glance, results suggest that digesting HTL-aqueous might actually increase toxicity when recycled as a nutrient source (Figure 11). However, the reduction in available organic carbon as a result of digestion also reduces the potential for heterotrophic growth in photobioreactors supplemented with digested HTL-aqueous and may explain this decrease in productivity. The apparent reduction in productivity in the wastewater digestate controls at 14 DF is also probably due to the reduction in heterotrophic productivity, the additional decrease compared to the BG11 control may be a result of light limitation due to the dark color and light attenuation of the digestate.

In conclusion, the results of these three studies indicate HTL-aqueous is best anaerobically digested *or* used to supplement the nutrients in growth media in photobioreactors but not both. Undigested HTL-aqueous supplementation yields greater productivity due to its higher organic carbon content whereas the organic carbon in the digested HTL-aqueous is converted to methane before being fed into PBRs. A life cycle energy analysis may give insight for the most desirable approach. To our knowledge, these are the only studies to date to examine anaerobic digestion as a means of recovering carbon from the algal HTL-aqueous waste product and as means of reducing its toxicity for use as a nutrient source. It is significant to note that results of both of these studies show promise in their ability to help streamline an algae biofuel production system into a desirable route for producing biofuels.

For more detailed descriptions of the data discussed in this section, see Appendix C.

Effect of Coagulant Dosing and Nutrient Recycling on Methane Production

Anaerobic digestion of algae biomass is important in the process model for producing biofuel and solubilizing nutrients for reuse, but these functions might be affected by coagulants used in algae harvesting. Additionally, when water and/or algae biomass exposed to coagulants are recycled, coagulant residual build-up might affect digestion of the resulting biomass. In this study, the effects of chemical coagulants, influent water source, and recycled nutrients on methane yield were tested in 1-L batch anaerobic digesters operated for 70 days.

In the first experiment, a cationic starch coagulant and an inorganic aluminum chlorohydrate coagulant were tested at high (7200 ppm) and low (3500 ppm) doses added to algae grown on reclaimed municipal wastewater. Compared to the reclaimed water control, both the high and low dose reduced the methane yield. Starch coagulant reduced yield by 14% (high dose) and 10% (low dose), and the inorganic coagulant reduced yield by 26% (high dose) and 14% (low dose) (Figure 12). Consistent with the findings of Anthony et al. (2013), the inorganic coagulant had more of a detrimental effect than did the organic coagulant.

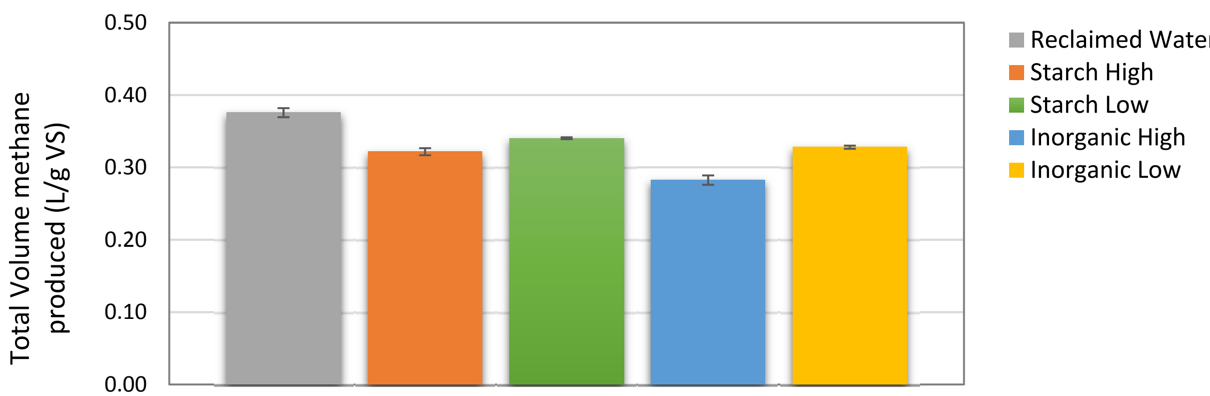

Figure 12. *Cumulative methane yield per g VS introduced to the digesters, as a function coagulant type and dose. The reclaimed water was highly treated, nitrified municipal wastewater.*

In the second digestion experiment, algae from raceways undergoing both water-and-nutrient recycling were compared to two control biomasses: algae grown on recycled water with fertilizer for nutrient make-up and algae grown on primary clarifier effluent wastewater. The recycled nutrients were in the form of whole algae digestate.

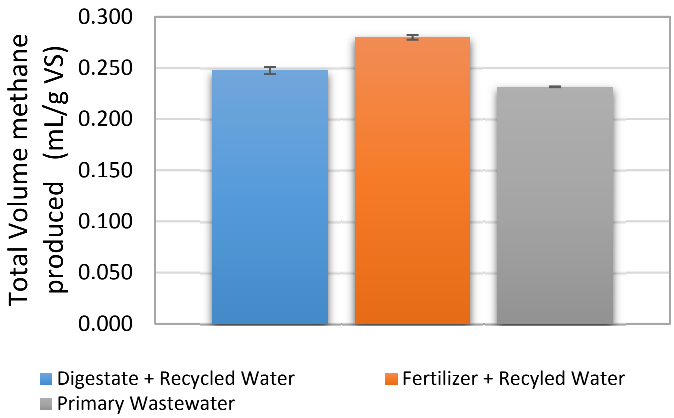

Figure 13. *Cumulative methane production as a function of water and nutrient source.*

The algae grown with both recycled water and nutrients had a 13% lower methane yield than algae grown with recycled water and fertilizer (Figure 13). This result was expected because some of the biomass harvested was previously digested. Interestingly, the yields with biomass from recycled nutrients and/or water were slightly higher than the yield for biomass grown on primary clarifier effluent. To better judge the reasons for such differences, future studies of this type should include biomass biochemical composition analysis. However, the main empirical finding of a minor decrease in yield due to whole digestate recycling has value and should be confirmed in future experiments.

Part II – Integration of Nutrient and Water Recycling at Bench- and Pilot-Scales

The next sections summarize the major experiments and studies conducted for the project. The culminating experiments of this project were pilot-scale algae cultivation, anaerobic digestion (described in Part I), with recycling of both water and nutrients from digestate and with makeup of water and nutrients using wastewater and chemical fertilizers. However, prior to the final pilot-scale experiments, the unit operations of the eventual pilot system were first tested at bench-scale to establish feasibility, needs, and operating variable levels. In addition, the potential accumulation of compounds inhibitory to algae growth was investigated at bench-scale.

Pilot-scale experiments were conducted on the central coast of California in San Luis Obispo at the municipal Water Resource Recovery Facility (SLOWRRF). The site included paddle wheel mixed algae raceway ponds, settling tanks, and anaerobic digesters. In order to operate with realistic cost projections for full-scale biofuel applications, algae biomass harvesting was accomplished in this system by means of bioflocculation followed by gravity settling. The initial pilot plant configuration used tube settler harvesters, which worked adequately in early experiments. However, as algae solids loading increased during productive summer months, these settlers were overloaded and were often seen to allow carryover of suspended solids. In the ultimate configuration, additional cone bottom settling tanks were added following the tube settlers to capture carryover. During the final set of recycling experiments, polymer coagulation was added in order to eliminate the confounding variable of different suspended solids concentrations in the media recycle flows into the water recycle treatment raceways. These systems and procedures provided sufficient harvesting efficiency that further clarification equipment, such as filters or centrifuges, was not deemed necessary. The clarified supernatant was then split evenly and recycled back into the duplicate raceways. A fixed volume of the settled biomass from the tube settlers was injected daily into the duplicate 500-gallon anaerobic digester tanks previously described. The digesters were operating in steady state for 4 months before digestate was collected for the pilot nutrient recycling experiment.

Extensive details for the work described below are provided in Appendices D-H.

Inhibition Studies of DOE1412 during Media Recycling

Multiple pathways can lead to an accumulation of inhibitory compounds in recycled water. Allelopathy - the direct or indirect harmful effect of one species on another by the secretion of chemically active compounds - is one such pathway. Evidence of allelopathy between competing organisms has been demonstrated in multiple algal species including *Chlorella vulgaris* (Fergola et al. 2007), *Cladosiphon okamuranus* (Kakisawa et al. 1988), and *Ishige sinicola* (Hirao et al. 2012). A second potential pathway of growth inhibition is the production of auto-inhibitory substances. Auto-inhibition is distinct from allelopathy in that the substances produced act on the species that produce them and was demonstrated in *Chlorella pyrenoidosa* and in green algae species within the family *Volvocaceae* (Harris et al. 1971, Ikawa et al. 1997). Past research has isolated and identified auto-inhibitory substances produced by *Chlorella* during cultivation (Ikawa et al. 1997). Additionally, products of organic degradation may accumulate, limiting light available for algal photosynthesis. The reuse of water also makes possible the buildup of salts or metals in the system that could have a deleterious impact.

In order to further understand the effects of water recycling on algae growth and identify the presence of potential inhibitors, a single batch of media was recycled through four rounds of regrowth. Control and experimental variable conditions were inoculated from a single stock culture of *Chlorella sorokiniana* (DOE1412) to a density of 0.06 OD_{750nm} at the beginning of each round of regrowth. Prior to each round of regrowth nutrients were replenished to match initial nutrient concentrations and ensure any observed inhibition could not be attributed to nutrient limitation. Media was recycled a total of four times throughout the course of this research, completing a total of 5 cycles of cultivation and harvesting on a single batch of water. Experimental cultures were only compared to control cultures grown within the same round of regrowth. Evaporative losses throughout multiple rounds of media recycling led to an accumulation of salts and could potentially cause inhibition due to excessive salt build up. Thus, in addition to the fresh BG11 controls, an additional set of replicate bottles were cultivated in parallel, amended with sodium chloride to match the salinity measured in the Round-5 recycled media. Results of these cycles of regrowth showed increasing inhibition with each successive round of regrowth (Figure

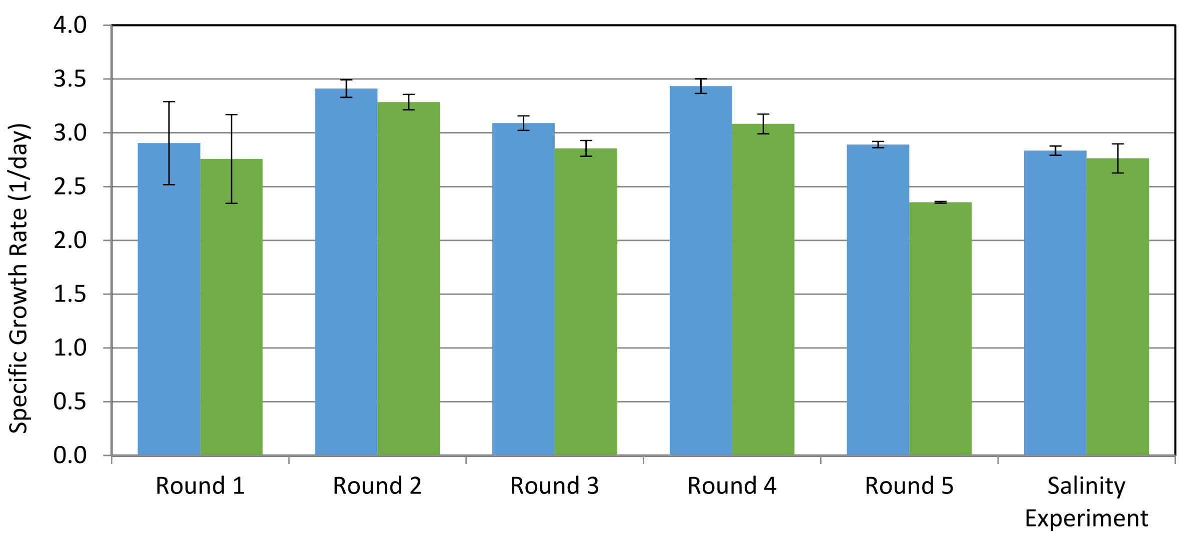

Figure 14*. Log-phase specific growth rate as a function of round of regrowth. Blue bars = control cultures grown on freshly prepared BG11 media, green bars represent treatment cultures grown on recycled media with replenished nutrients. During salinity experiment, treatment cultures had media with salt concentration equal to round 5. Error bars represent +/- standard deviation of specific growth rate of duplicates.*

14).

More specifically, during log-phase growth the initial growth rate decreased steadily by 4±3% for each successive round; however, linear-phase growth inhibition was only observed in the last round of regrowth. Salinity experiment results confirmed that the observed inhibition was not attributed to salt accumulation. This research demonstrated that cultivation of DOE 1412 on recycled media can lead to the build-up of measurable amounts of organic material during cultivation, some of which may be inhibitory in nature. It is possible that the observed inhibition was caused by an accumulation of some inhibitory compound in recycled media exuded by the algae or associated bacteria during cultivation. Furthermore, polyunsaturated fatty acids, which have been previously shown to be auto-inhibitory in nature, were identified in the Round 5 media. Another possible cause of inhibition during log-phase growth could have been the accumulation of zinc, manganese, and molybdenum observed in recycled media.

This accumulation of metals could be avoided in future experiments by not adding additional zinc after each round of regrowth. Given that open-pond systems commonly employ the use of polycultures maintained continuously in linear-phase of growth the following section discusses results of studies investigating inhibition in a wastewater derived algae polyculture. For more detailed descriptions of the data discussed in this section, see Appendix D.

Inhibition Studies of Wastewater Derived Algal Poly-Culture during Media Recycling

This lab study examined the potential for inhibition of algae growth due to water and nutrient recycling without the weather variability of the pilot studies, described in later sections. Wastewater algal polycultures were used with whole digestate at the recycled nutrient source. Table 2 describes the control and experimental treatments, including the source of water and nutrients and the volume of fresh and recycled constituents. Fifteen rounds of recycling were achieved in 1-L photobioreactors over the course of six weeks between August 17, 2015 and September 21, 2015. Although productivity fluctuated, no inhibition was observed as a result of these successive rounds of recycling.

Table 2. Water and nutrient source and volume for the control and three experimental treatments designed to identify potential inhibition as a result of recycling.

<table>
<tr><th>Condition</th><th>Fresh influent</th><th>Recycle</th><th>Total influent</th><th>Waste and samples</th></tr>
<tr><td>Recirculated water and digestate</td><td>10 mL/d digestate</td><td>90 mL/d recycle</td><td rowspan="4">100 mL/d</td><td>10 mL/d</td></tr>
<tr><td>Recirculated water and clarified wastewater</td><td>10 mL/d clarified wastewater</td><td>90 mL/d recycle</td><td>10 mL/d</td></tr>
<tr><td>Recirculated water and BG11</td><td>10 mL/d BG11</td><td>90 mL/d recycle</td><td>10 mL/d</td></tr>
<tr><td>Fresh water and BG11</td><td>100 mL/d BG11</td><td>0 mL/d</td><td>100 mL/d</td></tr>
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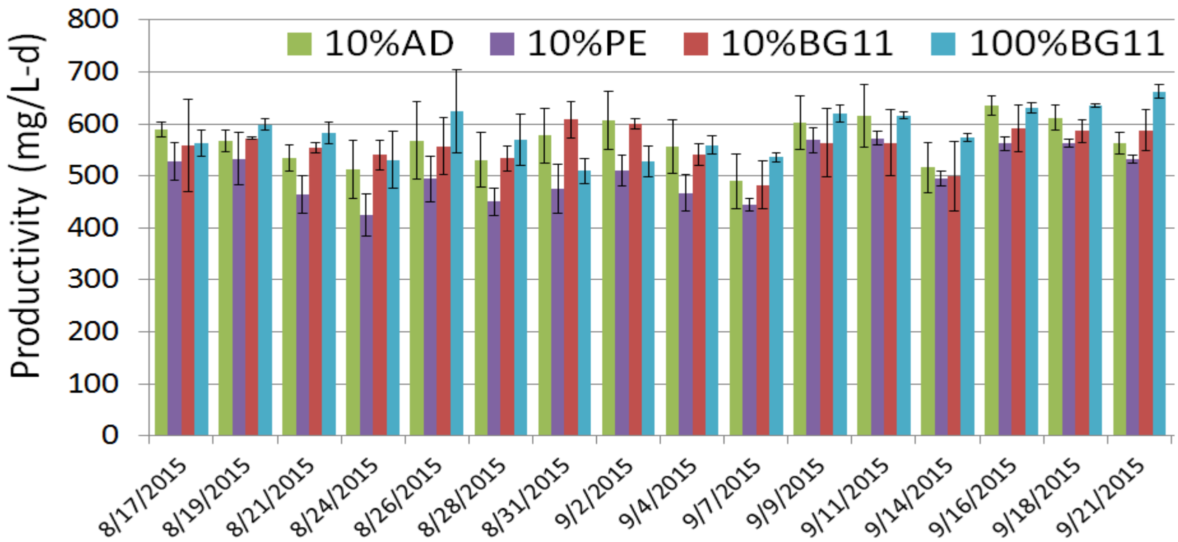

Figure 15 *Mean productivity over time for three experimental treatments and the control. Treatment conditions are described in more detail in Table 2. Error bars represent standard deviation of triplicate photobioreactors.*

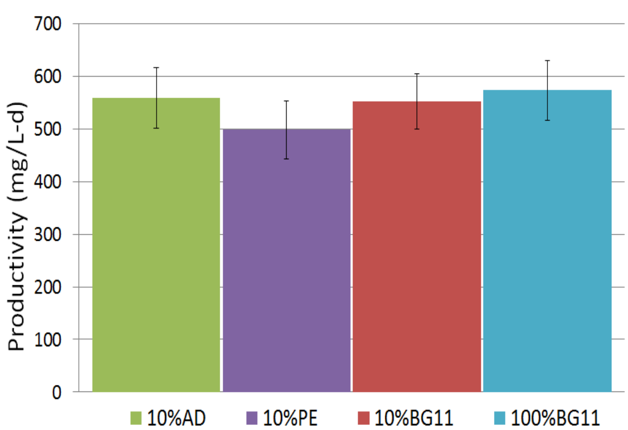

Figure 16. *Average productivity as a function of water and nutrient source over 15 successive rounds of recycling.*

Conductivity and absorbance (data not shown) over time show accumulation of salts and possibly humic matter within the recycled media. However, neither of these had any discernable effect on productivity (Figures 15 and 16).

In addition to absence of inhibition associated with successive rounds of recycling, there was also no significant difference in the average productivities between any of any of the treatment conditions (Figure 16). For more detailed descriptions of the data discussed in this section, see Appendix D.

Raceways in Series to Supply 100% Recycled Water and Nutrients

Long term pilot water recycling trials were conducted in triplicate 10,000-L raceways during March 2013 to September 2014. Water recycling was achieved by operating triplicate raceway sets in series with intermediate algae harvesting, creating “Round-1” and “Round-2” raceway sets. The Round-1 ponds were fed effluent from the City of San Luis Obispo wastewater treatment plant primary clarifier. Round-1 effluent was clarified by gravity settling, with the supernatant fed to the Round-2 ponds, thereby providing 100% recycling of water. These raceways in series were run with a 3-day HRT. An additional triplicate set of raceways was fed primary clarifier effluent for a 2-day HRT, to compare productivity. During these trials, nutrients were not recycled. Instead, the Round-2 ponds received sufficient residual soluble nutrients from the Round-1 ponds.

Productivity was assessed on a gross basis (effluent biomass production) and a net basis (effluent minus

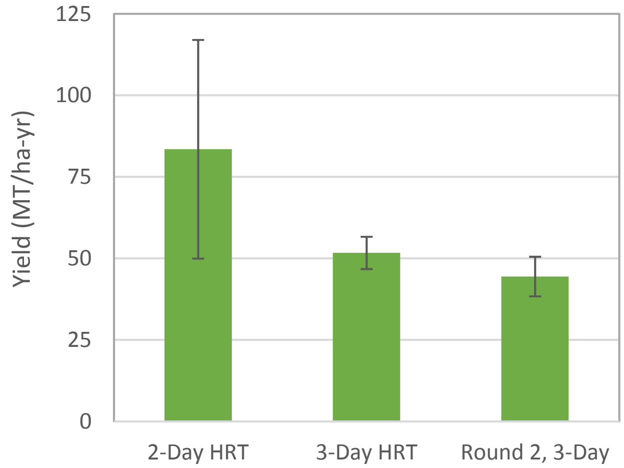

Figure 17. *Averaged annual net areal productivity from 9/2013 – 9/2014. Error bars represent average of the standard deviation of triplicate raceways over time.*

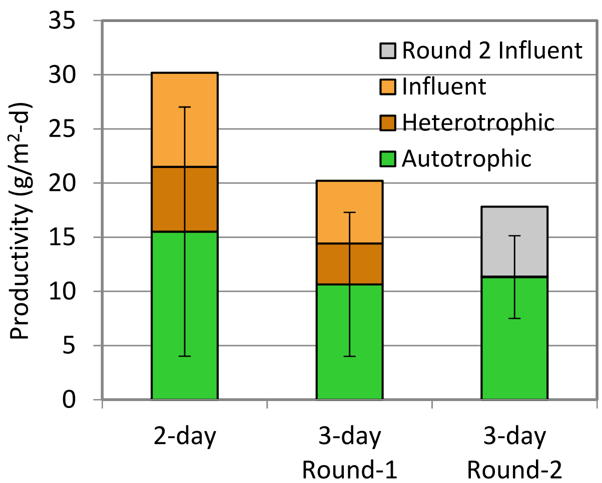

Figure 18. *Type of growth comprising mean gross productivity during March 2013 – August 2014. "Influent" refers to biomass in the pond's influent. Error bars represent average of the standard deviation of triplicate raceways over time.*

influent biomass production). Average annual gross productivities for the 2-day, 3-day Round-1, and 3-day Round-2 raceways were 32, 23 and 19 g/m^2-day, respectively. On a net basis, the 2-day HRT raceways produced an average of 23 g/m^2-d (83 metric tons per hectare-year), compared to 14 and 11 g/m^2-d (52 and 41 mt/ha-yr) produced in the Round-1 and Round-2 raceways, respectively (Figure 17 upper). The 24% higher net productivity in the Round-1 ponds was likely caused by heterotrophic (and/or mixotrophic) growth on wastewater organic matter in the Round-1 ponds influent. While biomass produced from organic matter consumption can be used as biofuel feedstock, net productivity it relevant to biofuel production by systems recycling media or not using wastewater.

Net heterotrophic growth was estimated from organic matter consumption multiplied by a typical heterotrophic yield coefficient. In the present case, soluble carbonaceous BOD_5 consumption and a typical wastewater engineering yield coefficient were used (Metcalf and Eddy, 2003). Autotrophic growth was then estimated by subtracting the calculated heterotrophic VSS concentration from total net VSS concentration. Results of these analyses suggest heterotrophic growth was ~27% of the total growth in both the 2-day raceways and Round-1 raceways (Figure 18). Heterotrophic growth was virtually absent from the Round-2 raceways due to the removal of BOD_5 in the Round-1 ponds. Round-2 influent biomass (gray bar in Figure 17) was primarily the result of incomplete algae harvest and incomplete removal of organic material in Round-1 raceways. Overall, the ponds-in-series configuration increased the algae yield per unit water and nutrients by 1.8 times. For more detailed descriptions of the data discussed in this section, see Appendix E.

Nitrogen Removal and Mass Balances

Comparison of winter and summer nitrogen mass balances showed that during the summer, Round-1 and Round-2 raceways experienced greater nitrification and assimilation of soluble nitrogen into organic nitrogen than during winter (Figure 19). During the summer, Round-1 raceways converted 55% of the influent soluble nitrogen into organic nitrogen and oxidized 30% of the influent total ammonia nitrogen (TAN) into oxidized nitrogen. The Round-2 raceways converted an additional 26% of the influent soluble nitrogen to organic nitrogen by assimilation. In total, 81% of the influent soluble nitrogen was

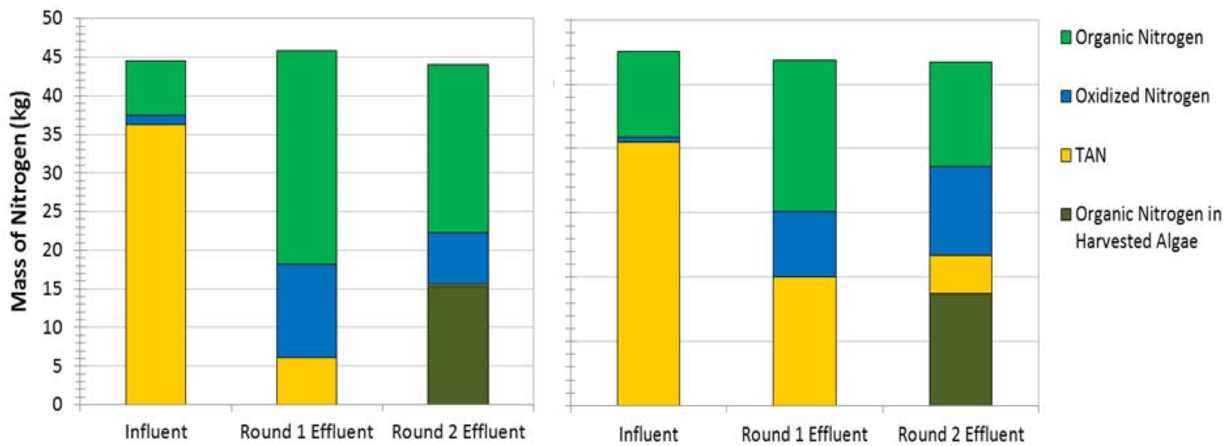

***Figure 19**. Nitrogen mass balances for Influent, Round-1 and Round-2 3 day HRT raceways in Summer 2013 (left) and Winter 2013/14 (right). The Influent bar refers to the primary clarifier effluent, which served as the influent to Round 1. Oxidized nitrogen is the sum of the nitrate and nitrite concentrations. Round-2 received Round-1 effluent after solids separation (algae harvesting).*

assimilated to create organic nitrogen in the biomass (Figure 19 left). High assimilation efficiency increases the feasibility of algal biofuels. The mass balances also show that nitrogen was conserved within the system and little to no nitrogen was lost via ammonia volatilization or denitrification during both summer and winter. For more detailed descriptions of the data discussed in this section, see Appendix F.

Simultaneous Water and Nutrient Recycling at Pilot Scale

The culminating experiment of this project was to measure the effects of simultaneous water and nutrient recycling at pilot-scale. Four different treatments, operated in duplicate, were compared (Figure 20). The heterotrophic/mixotrophic control raceways received both fresh water and fresh nutrients in the form of municipal wastewater primary clarifier effluent. In Treatment-1, algae settling tank supernatants were returned to the raceways, and nutrients were provided as whole digestate from algae-fed anaerobic digesters. In Treatment-2, water was recycled as in Treatment-1, but nutrients were provided as chemical fertilizer. The autotrophic control raceways were operated on reclaimed municipal water (biologically oxidized, filtered, chlorinated, and dechlorinated), which is high in nitrate and phosphate. All ponds operated with a 4-day hydraulic residence time.

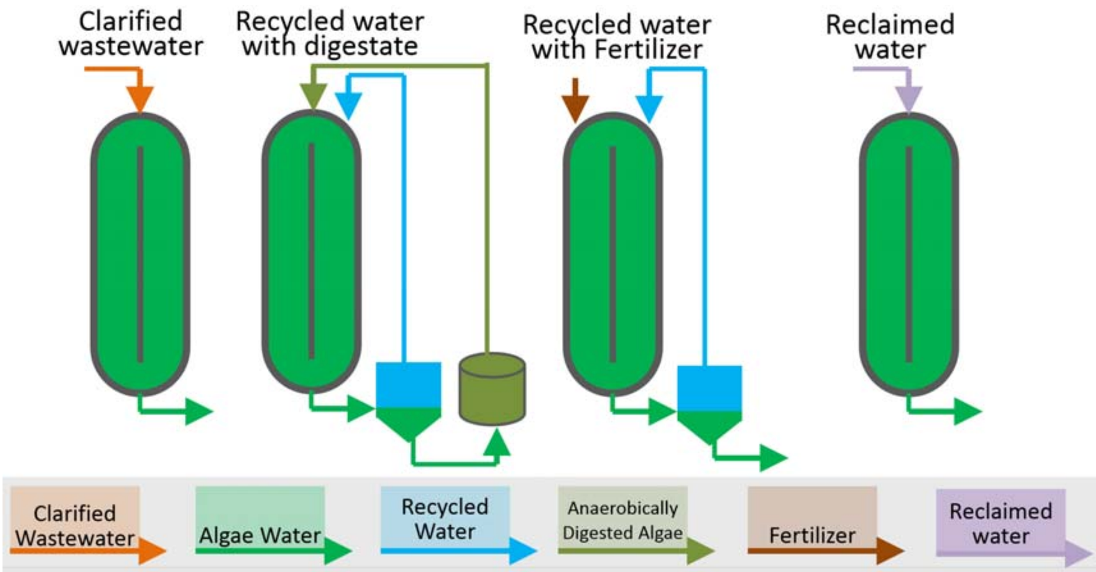

Figure 20. *Pond configuration defining water and nutrient source for control and treatments.*

For Treatments 1 and 2, an aluminum chlorohydrate coagulant was used, as needed, to ensure thorough removal of biomass before recirculation to the raceways. The systems were operated during April 27 to December 3, 2015 in three phases according to the amount of digestate fed the Treatment-1 raceways. During Phase I (April 27-June 18, 2015), depending on soluble nutrient measurements, raceways were dosed with 15-100 gallons of digestate every two to three days to ensure replete soluble nutrient concentrations. During Phase II (June 19 – September 3, 2015), digestate dosing was scaled back to 7.5 gallons to each pond daily, which was thought to be sufficient to provide replete nutrients. During Phase III, daily dosings of 7.5 gallons were continued as in Phase II, but these nutrients were supplemented with fertilizer, the amount determined by weekly soluble nitrogen

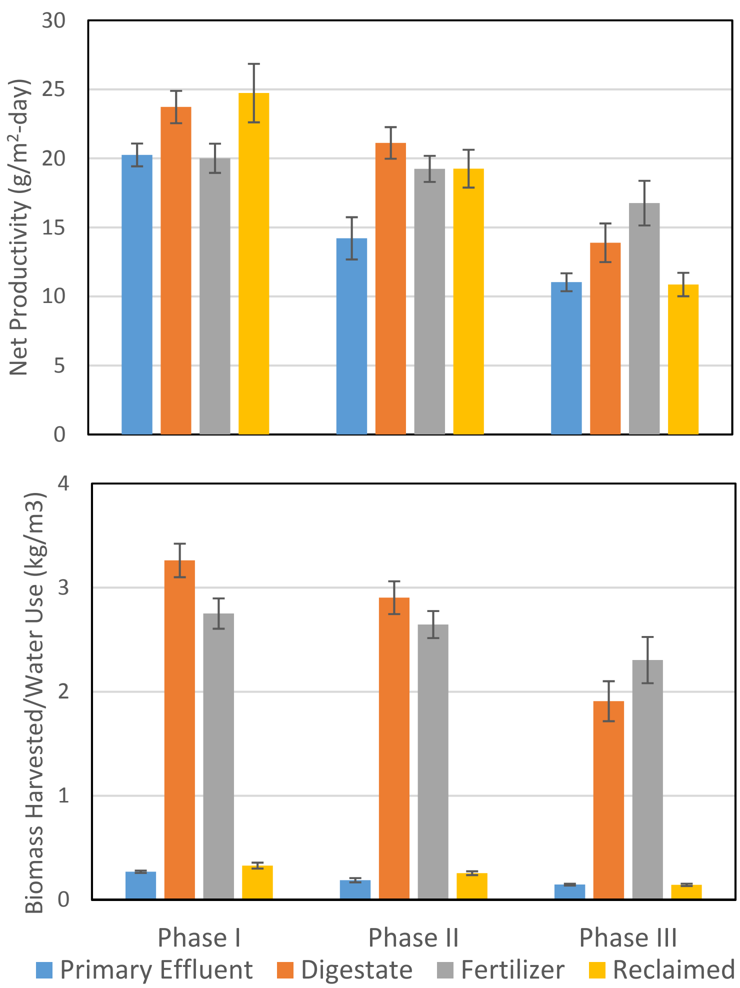

Figure 21. *Average net productivity (upper) and biomass harvested per m^3 of water used (lower) during each phase of the experiment as a function of water and nutrient sources. Error bars represent the standard error of duplicate ponds for the given time period.*

measurements. The larger dosings of Phase I were not sustained in the later phases due to limited digestate supply. For Treatment-1 and Treatment-2, water lost through evaporation and biomass harvesting was made-up with reclaimed water. The added reclaimed water amounted to 7% of the Treatment-1 and Treatment-2 raceway volumes, which is equivalent to 14 rounds of water reuse.

The greatest risk to productivity of water and nutrient recycling was probably accumulation of digestate organic matter, which might have contained inhibitory, or at least inert light-blocking, compounds or particles. However, recycling water and nutrients did not negatively affect productivity (Figure 21 top). The net productivity in the recycled water + recycled nutrient raceways (Treatment-1) was comparable to both the reclaimed water and fertilizer raceways and was consistently greater than the raceways fed primary clarified effluent. The greater net productivity of the recycled water + recycled nutrient raceways compared to the primary effluent-fed raceways is surprising given that the primary effluent would have fueled heterotrophic growth in addition to the photoautotrophic growth. The results also illustrate the seasonality of the productivities, with all treatments exhibiting lower productivity in fall (Phase III, September 3-December 3). Figure 21 (lower) emphasizes the dramatic increase in biomass harvested per unit of water consumed when water is recycled. Raceways using recycled water produced approximately ten-times more biomass per m^3 of water consumed than did the non-recycled raceways.

This experiment demonstrated at pilot scale, over nearly 8 months of operation, that water and digestate nutrients can be recycled without reduction in algal productivity. In addition, 100% of the necessary nutrients were supplied through recycling biomass after it had been anaerobically digested for a minimum of 4 months (Phase III not included because of fertilizer supplementation). Biochemical analysis of the biomass produced during these experiments show that there is also not a significant difference in the percent fatty acid methyl esters (FAMEs) between the treatments and in each case FAMEs made-up approximately 5% of the total biomass. Protein was the most abundant constituent in every treatment and typically made up between 30-40% of the biomass. For more detailed biochemical data, see Appendix G.

Techno-Economic Analysis

Results of the techno-economic analysis give three different minimum fuel selling prices (MFSPs). The first is with no additional revenue sources, second is the MFSP with wastewater treatment revenue, and finally the MFSP with wastewater treatment revenue and LCFS/RIN credits. The value was calculated by subtracting revenue sources, if any, from the Total Cash Inflow Required value calculated previously in the cash flow analysis section. This value is divided by the gallon gasoline equivalent (GGE) value of the upgraded biogas produced. Table 3 summarizes the results of the TEA and Figure 22 shows the MFSP for the three scenarios.

Table 3. Techno-economic analysis summary with three different revenue scenarios

TEA Financial Summary	
Total Capital Expense	$118,098,310
Bond Repayment (annual)	$7,581,211
Total Operating Expense (annual)	$12,716,793
Net Cash Inflow Required @ 20% IRR	$5,177,173
WWT Revenue (annual)	$5,410,217
Potential LCFS/RIN Credits (annual)	$6,647,735
Total Cash Inflow Required, no revenue (annual)	$25,475,358
Total Cash Inflow Required, WWT revenue (annual)	$20,065,141
Total Cash Inflow Required, WWT revenue and LCFS/RIN credits (annual)	$13,417,406

One thing to note is that this TEA is based on an annual average gross productivity of 33 g/m^2-day based on Delhi and San Luis Obispo, California, pilot raceway results in a separate project. During the water recycling and nutrient recycling portion of the project, net productivities were estimated rather than gross and averaged 20 g/m^2-day, falling short of the value assumed in the TEA. However, a better comparison is made with data collected earlier in the project during the raceways in series experiment wherein gross productivities annually averaged 33 $\pm$ 4 g/m^2-day in the 2-day HRT raceways. The TEA also assumes make-up water will be provided via primary effluent, a media rich in in organic carbon and conducive to heterotrophic productivity (Metcalf & Eddy, 2003) in addition to the photoautotrophic growth achieved in the water and nutrient recycling experiment.

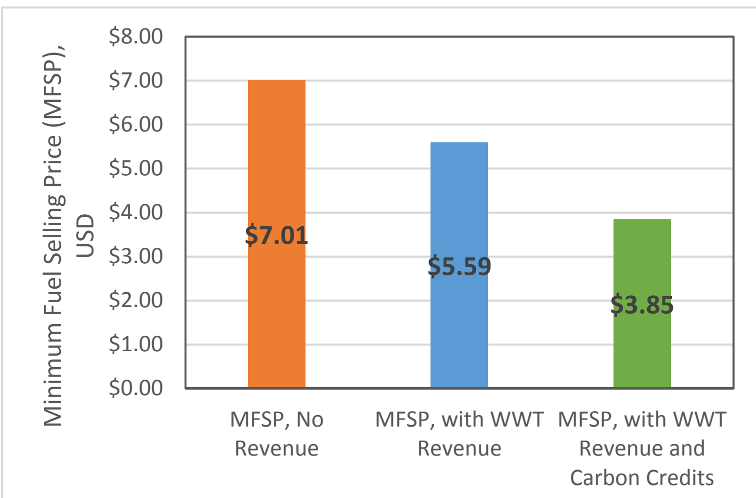

Figure 22. *Minimum fuel selling price based on three different revenue scenarios.*

Life Cycle Assessment

The energy requirements and GHG impacts of the facility were summed together with the end use energy and GHG emissions to provide a “Well to Tank” analysis of the system. The total well to wheel emissions of the biogas product was found to be 40.7 g CO_{2eq}/MJ (Table 4).

Table 4. Summary of life cycle assessment results

Source	Energy Required (Btu/mmBtu)	GHG Emissions (gCO_{2e}/MJ)
Processing Parasitic Energy	255,436	26.9
Biogas Upgrading	275,984	15.31
Transport and Distribution	1,350	0.45
Compression at Refueling Station	40,748	2.15
Anthropogenic CO2 Credit	0	-47
Wastewater Treatment Credit	-200,515	-3.68
Avoided Flaring Credit	0	-11.17
Total Well to Tank	**372,525**	**-17.09**
Carbon in Fuel	1,000,000	55.20
Vehicle CH4 and N2O	0.00	2.53
Total Tank to Wheel	**1,000,000**	**57.73**
Total Well to Wheel	**1,372,525**	**40.7**

For comparison, North American compressed natural gas (CNG) and California Reformulated Gasoline Blendstock for Oxygenate Blending (CARBOB) were found to have GHG impacts of 78.36 g CO_{2eq}/MJ and 99.78 g CO_{2eq}/MJ respectively. The energy required to produce 1 mmBtu of fuel, based on the lower heating value, was found to be 1,372,525 Btu, or a net energy ratio (energy required/energy produced) of 0.37 which is an improvement over previous LCAs with net energy ratios ranging from 0.93-0.50 (Lardon et al. 2009, Stephenson et al. 2010, Slade & Bauen, 2013).

For more detailed descriptions of the TEA/LCA summarized in this section, see Appendix H.

Conclusions

Together lab- and pilot-scale experiments indicate the feasibility of repeatedly reusing water and nutrients to grow algal biomass. These resources are critical to cost and sustainability and, therefore, the practicality of algal biofuels.

Anaerobic digestion of algae biomass was the main method of biofuel production and nutrient solubilization considered in this project, with whole digestate, including solids, being recycled into algae growth reactors. Another biofuel pathway option is hydrothermal liquefaction of algae biomass, and the reuse of residual nutrients from that process was also studied.

In laboratory experiments, the release of nutrients during the biodegradation of the biomass was quantified during anaerobic digestion and then during subsequent aerobic treatment meant to simulate the raceway ponds that would receive the digestate. Experiments also investigated the potential for accumulation of inhibitory compounds as a result of multiple rounds of water recycling. These lab results help in determining the sizing and operational parameters of the pilot systems.

The culminating experiments of the project were conducted in the pilot facility, with extended demonstration of water and nutrient recycling. These demonstrations included two major experimental periods. In one, operated for 18 months continuously, two sets of raceways were operated in series, with intermediate algae harvesting. In this way, the second-in-series raceways were receiving recycled water and residual nutrients from the first raceway set. In the second experimental period, lasting 8 months, clarified effluents following algae harvesting were continuously recycled into their source raceways while nutrient needs were provided by recycled nutrients in anaerobic digetate.

The second-in-series raceways had lower net productivity than the first-in-series, but this was likely due to extra growth fueled by wastewater organic matter in the first-in-series pond. In the experiment with continuous water and nutrient recycling, harvesting was complete and productivity was not noticeably affected, even when the water was recycled the equivalent of 14 times and 100% of the nutrients were derived from whole algae digestate.

Quantitative conclusions are summarized in the Executive Summary.

Broader Impacts

The impact of this funding extends well beyond the research findings and successful execution of tasks and milestones. From hosting visiting international scholars (funded by their respective institutions), to mentoring Cal Poly M.S. and senior project students and creating a campus wide interdisciplinary research program for undergraduate and graduate training, those involved have not only contributed to the project but also take with them the lessons learned and knowledge gained to a diverse array of research settings and career paths in the water and energy nexus.

International Research Scholars Hosted

2016: Alessandro Solimeno, Ph.D Student, Environmental Engineering and Microbiology Research Group, Universitat Politècnica de Catalunya, Barcelona, Spain.

2015: Thomas Chene, M.S. student, Centre d'Etudes Superieures Industrielles (CESI), School of Engineering, Angouleme, France and Gustavo Henrique Oliveira, B.S student, Brazil Scientific Mobility Program. (Both at Cal Poly for the summer.)

California Polytechnic State University M.S. Theses Describing This Research

- Hill, Alex. 2014. The Effect of Pretreatment Methods on Methane Yield and Nutrient Solubilization During Anaerobic Digestion of Microalgae.
- Bogess, Chad. 2014. Optimization of Growth Parameters for Algal Regrowth Potential Experiments.
- Nicolai, Eric. 2014. Media Recycling, Growth media Comparison, and Lipid Determination Comparison in High Rate Algae Ponds.
- Chang, Michael. 2014. Water and Nutrient Recycling in Wastewater Fed High Rate Algae Ponds.
- Fresco, Elai. 2015. Digestion of Microalgae for Methane Production and Nutrient Recycling
- Kraetsch, Justin. 2015. Nutrient Removal from Clarified Municipal Wastewater Using Microalgae Raceway Ponds.
- Roberts, Alec. 2015. Production and Harvest of Microalgae in Wastewater Raceways with Resource Recycling.
- Reiff, Carter. 2015. Nutrient Transformations in Algae Raceway Ponds Fed Municipal Wastewater.
- Spence, H. 2017. Algal Regrowth Potential on Recycled Water and Nutrients.

In Preparation:

- Zardouzian, E. 2017. Water and Nutrient Recycling in Algae Raceway Ponds for Biofuel Feedstock Production and Wastewater Treatment.
- Racz, T. 2017. Optimization of Methane Production and Nutrient Solubilization from the Anaerobic Digestion of Microalgae Grown on Clarified Wastewater.
- Bowen, C. 2017. Microalgae Production, Harvest and Inhibition in Resource Recycling Raceway Ponds.

NSF Research Experiences for Undergraduates (REU) Students Involved
Meggie Gidula, Jenny Salgado, Elai Fresco, Kimmy Pugel, Lili Gevorkian, Noelle Patterson

Involvement of Cal Poly Undergraduates
The Water and Energy Sustainability Training Team (WESTT) was created by Dr. Lundquist as an interdisciplinary research program to provide students hands-on exposure to research. Through this program, students have the opportunity to participate in hands-on research, experience a safety culture, participate in a large task/goal-driven organization with complicated scheduling, have a foretaste of graduate school, learn the importance of precision, analyze data of their own making, and contribute to innovative solutions in the water-energy environmental space. During the course of the Water and Nutrient recycling project almost 300 undergraduates were directly involved in the research through involvement in this program.

Selected Presentations
R. Spierling. *Nutrient and Water Recycling in Wastewater-Based Algae Biofuel Production.* Algae Biomass Summit - San Diego CA. 2014.

T. Lundquist, Hutton, M., Hill, A., Boggess, C., Chang, M., Kraetsch, J. *Towards Scale-Up of Wastewater-Based Algae Biofuels.* Algae Biomass Summit - San Diego, California. 2014.

J. Benemann, Hutton, M., Speirling, R., Chang, M., Kraetsch, J., Hill, A., Boggess, C., Crowe, B., Lundquist, T. Recycling Nutrients and Water in Microalgae Biofuels Production. 4th International Conference on Algae Biomass, Biofuels & Bioproducts – Santa Fe, New Mexico. 2014.

J. Benemann, Woertz, I., Hutton, M., Spierling, R., Crowe, B., Lundquist, T. *Algal Biofuels Production Combined with Wastewater Treatment – Benefits and Challenges.* 5th Congress of the International Society for Applied Phycology – Sydney, Australia. 2014.

T. Lundquist. *Microalgae Cultivation for Wastewater Treatment and Biofuels Production*. NET 3rd International Symposium. Watanabe group and Tsukuba University, Japan. 2014.

J. Benemann. *Roadmap to Commercialization of Algal Bioproducts – From Specialties to Commodities.* Presentation to the European Algae Biomass Association – Spain. 2014.

T. Lundquist, Kraetsch, J., Chang, M., Reiff, C., Roberts, A., Hutton, M., Spierling, R., Murrawsky, G., Blackwell, S. *Ponds-in-Series for Algae Production and Wastewater Treatment.* 5th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2015

R. Spierling. *Nutrient and Water Recycling in Wastwater-Based Algae Biofuel Producation.* 5th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2015

I. Woertz, Lundquist, T., Benemann, J. *Techno-Economic Analysis and Life Cycle Assessment of Algae-Based Wastewater Treatment and Biofuel Production Compared to Conventional Activated Sludge Process.* 5th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California.

B. Crowe, Nicolai, E., Spence, H., Lundquist, L. *Comparison of Wastewater and Defined Medium in Outdoor Raceways.* 5th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2015

H. Spence, Crowe, B., Lundquist, T., Lehi, C. *Algal Regrowth Potential on Recycled Water with Replenished Nutrients.* Poster presentation at 5th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2015*

L. Gevorkian, Spierling, R., Lundquist, T. *Anaerobic Co-Digestion of Hydrothermal Liquefaction Process Water with Wastewater Solids.* Poster presentation at Algae Biomass Summit – Washington D.C. 2015. *

M. Hutton, Spierling, R., Crowe, B., Lundquist, T. *Algae Cultivation on Wastewater with Media Nutrient Recycling.* Algae Biomass Summit – Washington D.C. 2015.

R. Spierling, Lundquist, T., Hutton, M., Racz,T., Fresco, E., Gevorkian, L., Pugel, K. *Nutrient Recycling Through Anaerobic Digestion in Wastewater Based Algae Biofuel Production*. Algae Biomass Summit – Washington D.C. 2015.

T. Lundquist, Spierling, R., Pittner, C., Adler, N., Elliot, D. *Integration of Algae Cultivation and Hydrothermal Liquefaction Based on Pilot Studies Using Wastewater Media*. 6th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2016.

R. Spierling, Lundquist, T., Hutton, M. *Water, Nutrient and Carbon Recycling in the Biorefinery System-Yield at the N^{th} Pond.* 6th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2016.

E. Zardouzian, Hutton, M., Spierling, R., Lundquist, T. *Water and Nutrient Recycling in Algae Raceway Ponds for Biofuel Feedstock Production and Wastewater Treatment.* 6th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2016.*

T. Racz, Fresco, E., Zardouzian, E., Bowen, C., Spierling, R., Hutton, M., Adler, N., Lundquist, T. *Optimization of methane Production and Nutrient Solubilization from the Anaerobic Digestion of Microalgae Grown on Clarified Primary Wastewater.* Poster presentation at 6th International Conference on Algae Biomass, Biofuels & Bioproducts – San Diego, California. 2016.*

*Indicates Student Presentation

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