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HYDROTHERMAL LIQUEFACTION PATHWAYS FOR LOW-NITROGEN BIOCRUDE FROM WET ALGAE

Final Report

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DISCLAIMER

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EXECUTIVE SUMMARY

Our SRI International (SRI) team has developed a new two-step hydrothermal liquefaction (HTL) process to convert wet algal biomass into biocrude oil. The first step in the process (low-temperature HTL or HTL1) yields crude oil but, most importantly, it selectively dissolves nitrogen-containing compounds in the aqueous phase. Once the oil and the aqueous phase are separated, the low-nitrogen soft solids left behind can be taken to the second step (high-temperature HTL or HTL2) for full conversion to biocrude. HTL2 will hence yield low-nitrogen biocrude, which can be hydro-processed to yield transportation fuels. The expected high carbon yield and low nitrogen content can lead to a transportation fuel from algae that avoids two problems common to existing algae-to-fuel processes: (1) poisoning of the hydro-processing catalyst; and (2) inefficient conversion of algae-to-liquid fuels. The process we studied would yield a new route to strategic energy production from domestic sources.

The following tasks were performed on this project:

- Established agreements with subcontractors, defined collaboration plan, and initiated new collaborations with entities outside the project team.
- Installed autoclaves and other laboratory systems.
- Developed analytical protocols.
- Performed parametric study on the high-temperature step of the process.
- Completed feasibility study of reusing N and C from the aqueous phase for algae growth.
- Conducted model compound study.
- Presented results to the technical community.
- Performed model compound studies (autoclave runs and separations).
- Wrote a draft paper on recycling of HTL1 and HTL2 aqueous phases as nutrient source for algae growth.
- Carried out multiple growth and HTL cycles to test long-term survivability of algae grown with nutrient recycling.
- Studied phase behavior of HTL products.
- Studied the effect of HTL1 conditions on algae morphology.
- Performed work on re-using HTL1 aqueous byproducts for algae growth
- Preliminary work on hydroprocessing of HTL1 biocrude oil.
- Performed the lab work a parametric study of HTL1 (autoclave runs, separations, and the chemical analyses).
- Conducted 2-step HTL runs using different types of algae.

In March of 2016, we stopped all work at SRI and our sub-contractors due to the lack of algae production caused by Algae Systems' non-operational status. We also stopped because no cost-share funds were available from Algae Systems (AS).

INTRODUCTION

Biocrude oil obtained from hydrothermal liquefaction (HTL) of algae can be a sustainable replacement for the fossil crude oil normally used in the production of fuels. HTL is a process in which biomass/water mixtures are heated to temperatures in the range of 200-400 °C. Under these conditions, similar to what might happen in the natural geological processes considered responsible for the production of fossil fuels, water becomes much more reactive, a series of chemical reactions takes place, and the biomolecules in the biomass feedstock are converted to biocrude oil.

HTL can be used to treat different feeds, such as cellulosic biomass, algae, and manure. Using cellulosic biomass poses two main problems: (1) a requirement for pretreatment with chemicals due to the recalcitrance of biomass; and (2) a high oxygen content in the biocrude due to the predominance of polysaccharides in the feed (with one atom of oxygen for each atom of carbon). Algae, which today is considered a biomass feedstock with high-impact potential, does not present recalcitrance and has a much lower fraction of polysaccharides as constituents than cellulosic biomass. Part of the mass includes lipids that have much lower O/C ratios than the remaining algal mass. However, a significant fraction of the mass consists of proteins high in nitrogen. As a result, best-in-class biocrudes from algal biomass have a C/N atomic ratio of 23 (with typical values around 15). Thus, in the best-case scenario, for every 23 atoms of carbon, the biocrude contains one atom of nitrogen. Unfortunately, the nitrogen atoms in the biocrude are typically part of an aromatic ring (i.e., aromatic heterocycles like pyridine). The formation of aromatic heterocycles takes place during the high-temperature HTL process if nitrogen is present. These molecules are extremely difficult to process in the steps necessary to upgrade biocrude to transportation fuels because they become a catalyst poison for the final fuel-upgrading steps. As a result, there is no single-step HTL process to date that can produce biocrude from algae that is an acceptable feed for refineries.

HTL is a convenient process for converting algae to biocrude because it requires water. Since algae grow in water, using HTL to process algae eliminates part of the energy expense associated with dewatering (a 10-20 wt% concentration of algae in water is an accepted range for HTL). SRI developed the two-step HTL process in collaboration with Algae Systems to solve existing limitations in state-of-the art HTL processes. This novel process separates the nitrogen-rich compounds that come from proteins. The nitrogen removal process reduces the amount of aromatic heterocycles in the biocrude oil, which can help meet environmental requirements and extend the lifetime of catalysts used during the upgrading to fuels.

The scope of this project was to study chemical pathways in the hydrothermal process, analyze products, evaluate upgrading potential of biocrude oils, study uses for compounds extracted in the aqueous phase, execute continuous operation tests, study scale-up, and carry out lifecycle analysis. After in-depth evaluation of the different scenarios, we intended to select operating conditions that optimized carbon and energy efficiencies while maintaining product composition within industrial specifications.

SECTION I. ACCOMPLISHMENTS AND MILESTONES UPDATE

1. Technical accomplishments

Over the extended term but significantly reduced funding of this project we performed much of the work originally planned to be done at SRI. These activities are presented in detail in the next sections.

1.1 Analytical protocols

In this section, we outline the process steps of the analytical techniques used. For a more detailed description of analytical protocols, the reader is referred to Appendix A of this document.

We performed analyses at different points of the process:

- Incoming biomass: after drying the algae in a convection oven (at 55 °C), a fraction of the algae was ground with mortar and pestle and split into several samples to carry out three types of analysis:
  - Thermogravimetric analysis (TGA) to determine ash content
  - Elemental analysis to determine concentrations of C, H, N, and S in the biomass (O is determined by difference)
  - Lipid content by solvent extraction using a Soxhlet apparatus and a mixture of chloroform and methanol as solvent.
- Output of hydrothermal liquefaction: the different phases (organic, aqueous and solids) were separated in the laboratory by a combination of decanting in a separations funnel, filtration using paper or plastic filters, and centrifugation (usually at 4000 rpm for 10 minutes). The solids were dried in a convection oven at 55 °C. The biocrude was isolated by vacuum distillation of the solvent (often heptane, which simulates solvents that will be used industrially but is easy to distill out). The aqueous phase can be analyzed as is, but often we analyzed the residue left after evaporating water in a convection oven at 55 °C. The analyses included the following:
  - Elemental analysis was completed to determine concentrations of C, H, N, and S in the biocrude, solids, and aqueous phase residue.
  - Gas chromatography with mass spectrometry (GC-Ms) was used to determine the chemical composition of biocrude.
  - Biocrude oil was also analyzed at the University of Dayton Research Institute (UDRI) by means of GC x GC.
  - In addition, standard full characterization of selected biocrude samples was performed by external vendors.
  - LC-MS and GCxGC were also used to determine chemical composition of aqueous-phase samples.
- Biomass concentration of algae cultures: to determine the biomass concentration of algae cultures, a sample with a known volume (usually 50 mL) was taken. A small amount of an EDTA salt was used to ensure that bivalent cations were in solution and didn't contribute to artificially increasing the biomass. The sample was then centrifuged at 4000 rpm for 10 minutes. Afterward, the liquid was decanted. The biomass pellet was washed using deionized water,

placed into a pre-weighted glass dish, and dried in a convection oven (at 55 °C) to evaporate the water. The mass of dry algae left in the dish was then recorded.

1.2 Laboratory setup

Laboratory setup

A 500-mL autoclave (Parr Instruments Moline, IL) was used for these experiments. The model 4575A autoclave is part of Parr's high-temperature (500 °C), high-pressure (345 bar) series and comes with a jacketed heater, magnetic drive stirrer, liquid and gas sampling valves, inlet and outlet gas valves, and pressure and temperature sensors connected to an external controller. This autoclave was further modified for external heater position control with a low-pressure air bag lifting jack with high-temperature microporous insulation as protection. Lines were plumbed for inlet/outlet gas, liquid sampling, safety relief, gas sampling, water cooling, electrical mains, a cooling loop, an air supply for fluidization, and heater lifting/lowering and subsequent cooling.

For safety, the autoclave was installed in an isolated explosion-rated cell within the main lab. All controls for heating, cooling, and stirring were routed from the Parr 4848 controller module to a remote computer located outside of the cell. A separate mechanism was also installed to allow lowering of the heater via the air bag lift. The basic software package was also modified to improve operator usability via a more robust interface and allow for batch logging of experimental parameters. Separate areas were designated in the lab for setup and cleaning operations, including a fume hood.

A Techne SBS-4 sandbath was also refurbished and installed in the same explosion-rated cell. This fluidized sandbath is capable of heating media to 600 °C, allowing for rapid heat up of small reactors. An external proportional-integral-derivative (PID) controller was retrofitted to control this sandbath remotely. A conveyer track with chain was also installed in the cell to allow reactors to be inserted and removed from the sandbath externally. For use with the SBS-4 sandbath, several reactors were assembled from ½" Swagelok tube and fittings. These were comprised of a 6" length of ½" OD x 0.035" heavy-wall tubing terminated by ½" tubing caps. One side of these reactors also had an 1/8" reducer to allow for initial purging of the system and post-reaction evacuation.

HTL pump selection

Previous studies described in the literature[1,2,3] have detailed the various types of pumps suitable for operating at hydrothermal liquefaction conditions. Several non-standard requirements have made it difficult for pumps to be sourced at both the laboratory and plant scales; these include high temperature (100-350 °C), high pressure (>3500 psig), high solids loading (5-35% solids content), and high flow rate (>200 mL/min for the laboratory scale setup). Secondary concerns include the need for high-purity wetted surfaces such as 316SS or polytetrafluoroethylene (PTFE) to prevent any contamination or corrosion, having a domestic representative to allow for rapid troubleshooting, and being of a design conducive to scale to pilot studies and beyond.

[1] D.C. Elliott *et al*., Biores Technol 178 (2015) 147-156.
[2] C. Jazrawi *et al*., Algal Res 2 (2013) 268–277.
[3] D.C. Elliott, Algal Res. 2 (2013) 445–454.

Bergin *et al*[4] recommended three primary pumping technologies that met the primary requirements and were also conducive to scale up. The base choice included solids piston pumps, followed by diaphragm and rotary lobe pumps, which had unique characteristics that could be highly synergistic in HTL applications. Elliott *et al.* also cited significant experience with syringe pumps at the bench scale; though these did not effectively scale up, they were strong performers in the other requirements listed above.

Several pump manufacturers were researched based on the above requirements. Piston pumps were ruled out based on previous operational experience at SRI. The primary reasons for this were the need for multi-stage pumping and relatively low flow rates in the turnkey solutions proposed by manufacturers. The search was eventually narrowed down to two manufacturers: ProMinent GmbH for the diaphragm and rotary lobe type pumps, and Teledyne Isco for syringe pumps. This was based heavily on both companies having a current model in their lineup capable of meeting all of the required specifications, and extensive use in other HTL setups. SRI has also previously used a pressurized-gas injection system, but this was not used because it could not meet the pressure requirements.

Preliminary rheological testing of algae slurries prepared by Algae Systems gave us a baseline to compare against literature values for high-solids content slurries. Some of the slurries tested had viscosities 1-2 orders of magnitude higher than suggested by the literature.

We considered the ProMinent Orlita MfS35/12 hydraulic diaphragm pump priced as a pump and an accessory skid containing the mounting and all the hardware likely necessary for operation. It is reasonably priced for the pump-only system and was expected to meet our requirements. One concern we had was that the control system is not as straightforward (typical manual set, little variability) and there may be some dead volume in the pump. As of 7/1/15, ProMinent was re-evaluating for the higher viscosity. We suspect we would have higher solids loadings and, at their suggestion, modified to an Orlita DR rotary plunger pump.

The Teledyne Isco 500D high-pressure syringe pump contains the controller as well and has low dead volume by design. We expected this might make it better for delivering single charges to the autoclave system. An additional modification to the stock setup was available to improve the syringe pump for pumping high solids slurries via enlargement of the valve bore and used a shallow angle to reduce impingement. The simplex 507 mL model was expected to serve our needs for single charge delivery of algal slurry, and it was well-matched to our 500-mL autoclave. As an option, we considered using a smaller 266-mL system instead filled to capacity, but we were concerned this would halve flow rate while doubling outlet pressure. We obtained quotes for several different variations on the requested system.

We waited for the engineering team at ProMinent to finish their assessment of the higher viscosity requirement before ordering the pump so that we could assess the suitability of the pump for our system.

[4] E.J. Berglin *et al.*, Review and Assessment of Commercial Vendors/Options for Feeding and Pumping Biomass Slurries for Hydrothermal Liquefaction. PNNL-21981. Prepared for the U.S. Department of Energy under Contract DE-AC05-76RL01830. November 2012.

1.3 Parametric study of HTL2

Initial studies of HTL2 conditions

We carried out a parametric study of HTL2 changing reaction temperature, dwell time (time that the system is kept at the target temperature), and adding solvents in some of the runs. The objective was to determine the effect of these parameters in the yield and quality of oil, and study the mass distribution between the several phases.

The experimental design was as follows: the experiment consists of 24 runs (full factorial design with one factor at three levels and two factors at two levels). Each run had two steps:

- Step 1 (HTL 1). The first step was common for all 24 experiments. The procedure was:
  - Load 30 g of dry algae into autoclave.
  - Add 270 mL of DI water.
  - Add 150 mL of heptane.
  - Purge with Ar (but don't build up pressure) and close valves.
  - Start stirring at 1000 rpm and heat up to 200 °C (once temperature reaches 195 °C, ramp time is considered finished).
  - Maintain target temperature for 30 minutes (dwell time).
  - Cool down to room temperature.
  - Sample gas using a sample bag.
  - Purge reactor with Ar.
  - Retrieve all material from autoclave. If needed, use water to recover material left on walls.
  - Separate solids, water phase, and organic phase.
  - Wash solids from with DI water (2 x 100 ml).
  - Store or analyze water and organic phases as planned.

Naming of phases: CH01-X A1 (organic), CH01-x A2 (emulsion, if any), CH01-x A3 (water) and CH01-x A4 (solids). X = run number (from 1 to 24, see table below)

- Step 2 (HTL 2). In the second step, we changed three factors: temperature, dwell time, and the presence/absence of solvent (dodecane in six runs, heptane in two runs). The procedure was:
  - Load wet solids from HTL 1 into autoclave
  - Add 200 mL of DI water if solvent will be used, or 250 mL of water for runs with no solvent.
  - If the run requires addition of solvent, add 50 mL of dodecane.
  - Purge with Ar without pressurizing and seal the reactor.
  - Start stirring at 1000 rpm and heat up to target temperature (once temperature is within 5 °C of target temperature, ramp time is considered finished).
  - Maintain target temperature for 15 or 90 minutes, depending on run conditions (dwell time)
  - Cool down to room temperature.
  - Sample gas using a sample bag.
  - Purge reactor with Ar.
  - Retrieve all material from autoclave. Use solvent to recover material left on walls (then rotovap it and mix resulting solids with rest of sample).
  - Separate solids, water phase, organic phase and possible emulsions.
  - Store or analyze each of the phases as planned.

Naming of phases: CH01-X B1 (organic), CH01-x B2 (emulsion, if any), CH01-x B3 (water), and CH01-x B4 (solids). X = run number (from 1 to 24).

The factors and levels were:

- Factor 1: temperature. Three levels: 300 °C (-), 325 °C (=) and 350 °C (+)
- Factor 2: dwell time. Two levels: 15 min (-) and 90 min (+)
- Factor 3: solvent (dodecane). Two levels: no solvent (-) and solvent (+)

We did one repetition for each time and temperature on non-solvent runs and only a single experiment for each time and temperature on runs with solvent. In addition, we did two experiments at 300 °C with a dwell time of 15 minutes and using heptane as solvent.

After each run, all phases were separated. In runs in which no solvent was added in HTL2, we retrieved the sample and added dichloromethane to aid in the separation of phases. Dichloromethane was then distilled under vacuum to isolate the oil fraction (B1). Aqueous phases (A3 and B3) were dried out in a convection oven to record the weight of solid residue. Interlayers between aqueous and organic phase were separated and characterized as independent phases (A2 and B2).

Figure 1 shows the distribution of mass, C, O, N, and the higher heating value (HHV) among the phases. The HHV was calculated using the well-known Dulong formula. The plot shows the percentage of the original content in the algae that went to each phase. The values were computed as an average between the 12 runs with no solvent added in HTL2, and they were independent of the individual conditions in each experiment. A large fraction of the original mass (47%) ended up in the water phase after HTL1 (A3). When looking at individual elements, the percentage of C that ended up in this phase was similar, but the percentage of N was significantly higher: 72%. This is a desired result, because the main objective of doing HTL1 is to extract as much N as possible, so it does not end up in the biocrude oil when doing HTL2. The percentage of original mass that ends up as oil (A1+B1) is 21%. However, due to the high C content of oil, the percentage of C that ends up in the oil is 35%. The C yield of the process is higher than this value because it is possible to recycle some of the C that ends up in the aqueous phase in HTL1 (A3) for algae growth. Results regarding the C yield are discussed below. The numbers in terms of heating value get even higher when accounting for the effect of C recycling for algae growth. Interestingly, only around 3% of the original mass ends up in the aqueous phase from HTL2, probably because the water-soluble compounds are mostly extracted in HTL1. The solids after HTL2 (B4) collect 11% of the original mass of the algae, but they have only 7% of the original C. Due to their highly oxygenated nature, they contain only a 2% of the original heating value. Finally, the amount of mass in the interphases is less than 2% of the original mass.

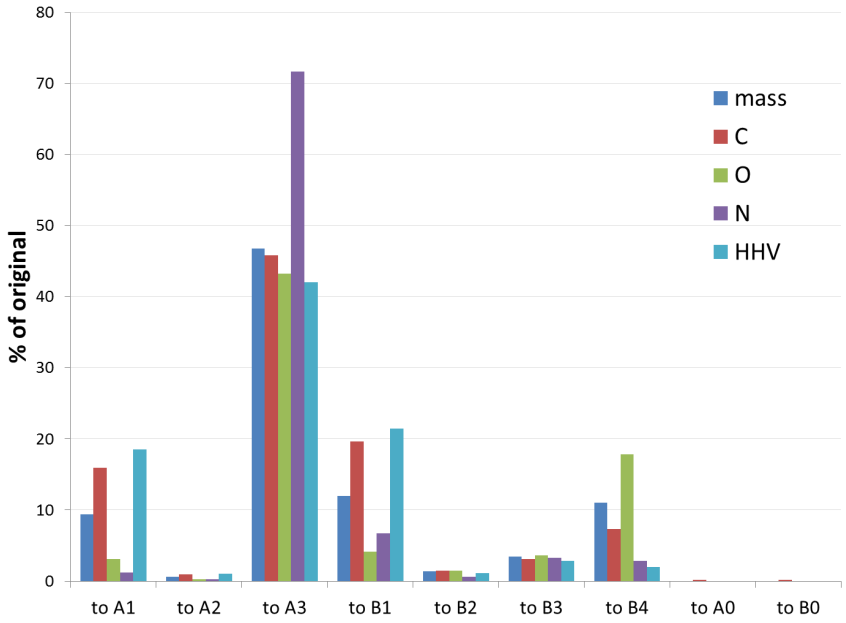

Figure 1. Distribution of mass, C, O, N and higher heating value (HHV) among the phases (average of 12 runs done at different dwell times and temperatures). The plot shows the percentage of the original content in the algae that went to each phase.

Figure 2 shows the composition of the most relevant phases. The O content for algae was measured by combustion analysis, but for the other samples it was determined by difference (taking into account C, H, N and S). For oil phases this is likely to be a good assumption, but it could lead to significant errors in the residues of the aqueous phase due to the presence of inorganic salts, and possibly also errors in the case of solids. Nevertheless, it is interesting to see that oils are richer in C and H than the starting algae, and they contain fewer heteroatoms.

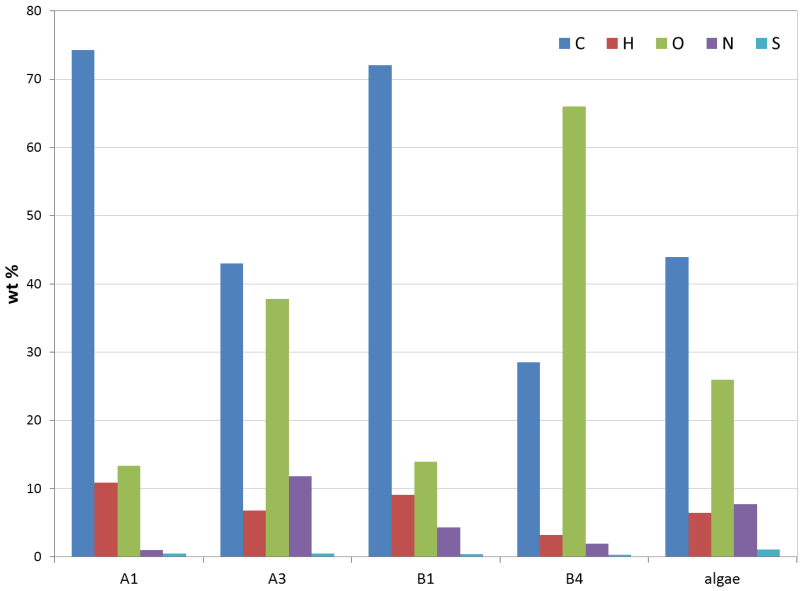

Figure 2. Composition (in wt%) of some phases: oil from HTL1 (A1), residue from HTL1 aqueous phase (A3), oil from HTL2 (B1), and solids from HTL2 (B4) and starting algae.

While the results discussed above are based on average values for runs done over a range of experimental conditions, Figure 3 shows the C yield for each of these conditions (C yield is defined as the percentage of C fed to the process that enters and ends up as oil from HTL1 or HTL2. Each point is the average of two values, and the error bars indicate the dispersion of these two values. For the yield calculations, we averaged the mass of oil and the wt% of C obtained in four different HTL1 experiments and used the average values for all conditions. We used a 65% yield of recycling C from HTL1 for algae growth (see results in section 1.4). We did not account for any CO_2 recycling. Blue diamonds and red squares indicate runs in which there was no added solvent in HTL2, and the blue circle is for the two runs with heptane in HTL2. The target value of 65% specified in Milestone 1.4.1 is marked by the green line. In some cases, the C yields we obtained reached the milestone target value. It is difficult to draw conclusions about the effect of dwell time and temperatures, because in most cases the differences observed are not significant. Nevertheless, one can conclude that there is no apparent benefit from operating at the highest temperature explored; if anything, it seems that in the short runs (blue diamonds) there is a trend for the overall C yield to decrease with increasing temperature. In further tests, we may expand the range of temperatures to values lower than 300 °C. There is no clear evidence that increasing the dwell time provides a benefit in terms of C yield. The chemical pathways are complicated and involve hydrolysis of biomolecules, formation of oil by reactions between the resulting monomers, but also formation of solids by re-polymerization of small organic molecules. While increasing dwell time will ensure further conversion of monomers to biocrude oil, it has also the potential to increase the formation of solids.

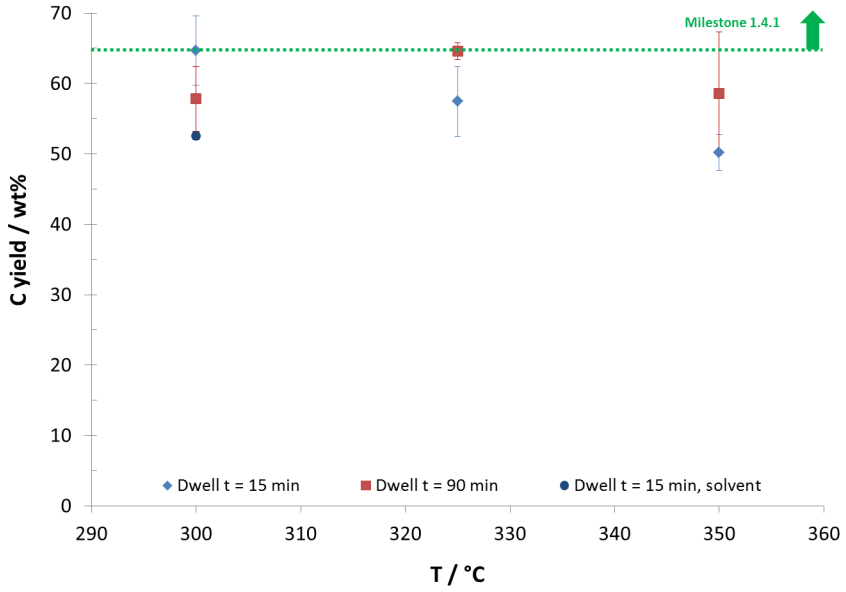

Figure 3. Carbon yield as a function of temperature and dwell time in HTL2 for the 12 runs with no solvent the two runs with heptane. A 65% C recovery from HTL1 aqueous phase for algae growth was used.

A key feature of the technology is the capability to obtain oils with a low N content, so they can be easily upgraded to liquid fuels using existing infrastructure and maximizing lifetime of catalysts. In the two-step HTL process, this is accomplished by extracting N compounds in the

aqueous phase in HTL1. The results discussed above show that over 70% of the N in the algae ends up in this phase, and it is not exposed to the high-temperature step in which it is more likely that N ends up forming aromatic heterocycles. Elemental analysis of the starting algae showed that the N content (dry basis) is 7.7 wt% and the C/N mass ratio is 5.7. On the other hand, the soft solids that are moved from HTL1 to HTL2 have only a 5.0 wt% N, and the C/N mass ratio is 8.9. This selective removal of N is the basis to achieve oils with low N content, and it is the result of a non-optimized HTL1 step. Future efforts will be dedicated to improve the extraction selectivity. As shown in Figure 4, the oils obtained in the two-step process have an N concentration in the range of 2.5-3.0 wt%, which is lower than the values for oils obtained in a single-step HTL (typically in the range 4-6 wt%). Blue diamonds and red squares are for runs without adding solvent in HTL2, and the blue circle is for the two runs with heptane in HTL2. Although there are no significant differences between samples, it seems that when operating at 350 °C the resulting oils have a slightly lower N content. The values are nevertheless higher than the 1.3 wt% target for Milestone 1.4.1. We carried out two experiments in which we added heptane, and the resulting oil has an N content close to the milestone target. This is a promising result.

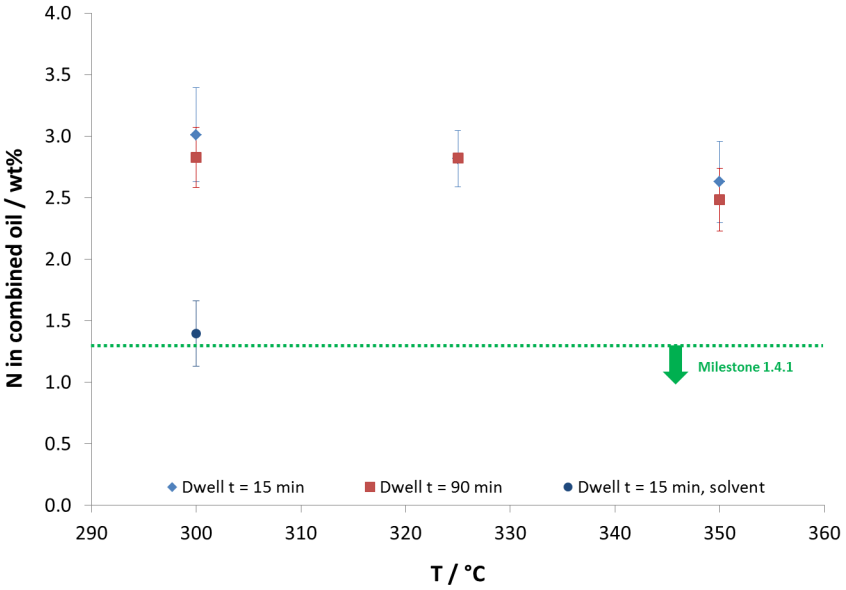

Figure 4. N content in oil (combining HTL1 and HTL2 oil) as a function of temperature and dwell time in HTL2 for the 12 runs with no solvent the two runs with heptane.

Figures 5 and 6 show O and H content in oils. As before, blue diamonds and red squares are for runs in which no solvent was added in HTL2, and the blue circle is for the two runs with heptane in HTL2. Almost all of the oils are within the project target for O content. The exception was the oil in which HTL2 was done at 350 °C for 90 minutes, the most extreme condition; it was unlikely this temperature and time would be selected for regular operation. Interestingly, it seems that with a short dwell time, O content decreases with temperature, and at long dwell times it increases with temperature. The chemical pathways may explain this observation. The H content was above the minimum that was set as target in the project in all samples.

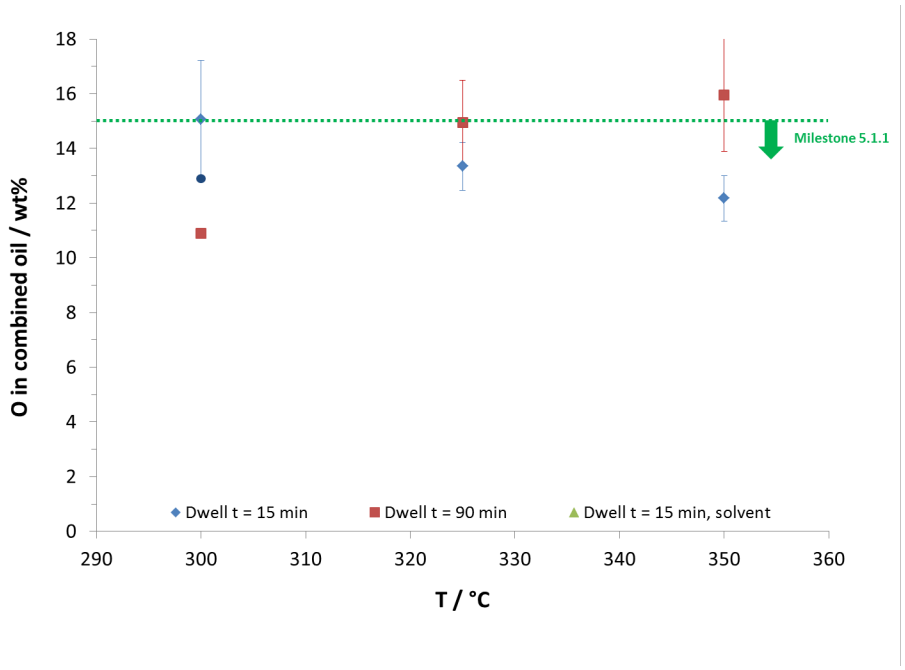

Figure 5. O content in oil (combining HTL1 and HTL2 oil) as a function of temperature and dwell time in HTL2 for the 12 runs with no solvent the two runs with heptane.

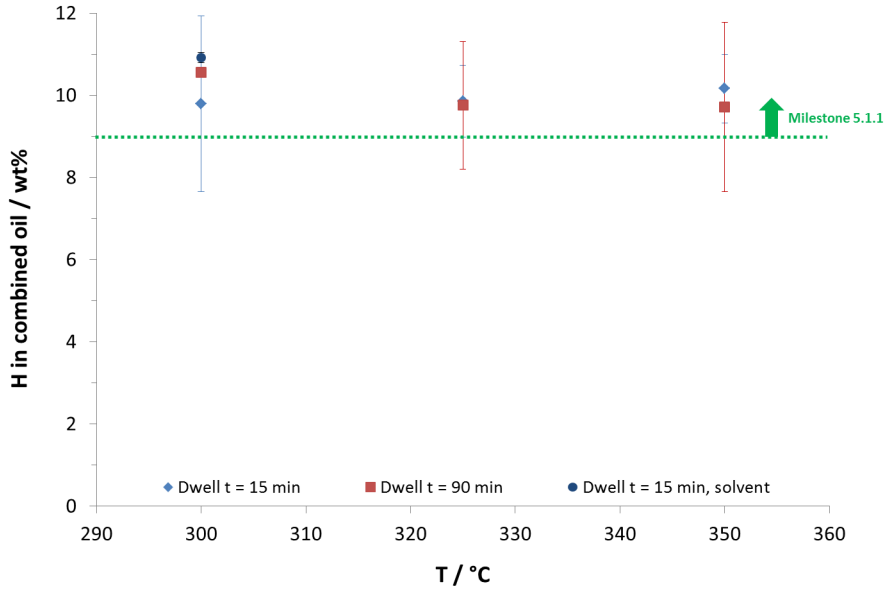

Figure 6. N content in oil (combining HTL1 and HTL2 oil) as a function of temperature and dwell time in HTL2 for the 12 runs with no solvent the two runs with heptane.

HTL2 runs on different algae strains

For two-step HTL1+HTL2 experiments, we used three types of algae: aqueous slurries of *Scenedesmus dimorphous*; aqueous slurries of a polyculture grown in wastewater; and dry

Chlorella (Cellusyn Labs, broken cell wall, dry powder). Elemental analysis of the feedstock is presented in Table 1. The first HTL step was done as described above, with a temperature of 220 °C, dwell time of 30 minutes, and a low-solvent mixture. After separation of phases, the soft solids were then treated in a HTL2 step. They were mixed with 168 ml of water and 42 ml of heptane (or with 210 mL of H_2O if no solvent was use in HTL2). The reactor was torqued, purged twice with argon gas, and then heated up at 7 °C/min to either 300 or 325°C, maintained at that temperature for 15 min, and cooled down to room temperature (cooling rates for the first 100 °C were higher than 15 °C/min). Biocrude and solid residues from HTL2 were collected following a similar protocol described for HTL1. Following a comparable approach described in a paper by Savage's group[5], we added an additional phase referred as heavy bio-oil, which is oil soluble in dichloromethane but insoluble in heptane.

Table 1. Elemental analysis results for the strains of algae used and the polyculture.

algae	wt% N	wt% C	wt% H	wt% S
Chlorella	8.5	48.8	7.4	0.8
WW polyculture	9.2	52.0	8.0	0.7
Scenedesmus	7.9	48.7	7.3	0.2

Figure 7 shows the distribution of C among several phases obtained in the 2-step HTL process. The largest fraction of the C ended up in the aqueous phase in HTL1. As discussed above, this is unavoidable if one wants to obtain bio-oils with low N content. Amino acids need to be extracted before the biomass is exposed to the high temperatures typical of HTL processes, and amino acids contain C atoms. In the HTL1 step, we extracted organic matter with an N/C ratio close to the ratio in proteins, so the co-extraction of C was difficult to reduce. However, our results on recycling nutrients showed that almost 70% of the aqueous C from HTL1 can be recycled for algae growth. The amount of C found in the aqueous by-products from HTL1 was similar for the two types of algae studies in this work. Interestingly, the amount of C found in HTL2 aqueous byproducts is much smaller than in HTL1. There are two reasons for this: first, if HTL1 extraction is effective, most of the aqueous-soluble compounds may have been already extracted in HTL1; second, some of the water-soluble organic molecules may react at the high temperatures of HTL2 to form oligomers or polymers that are large and insoluble in water.

[5] P. E. Savage *et al.*, Energy and Fuels, 27 (2013) 1391-1398.

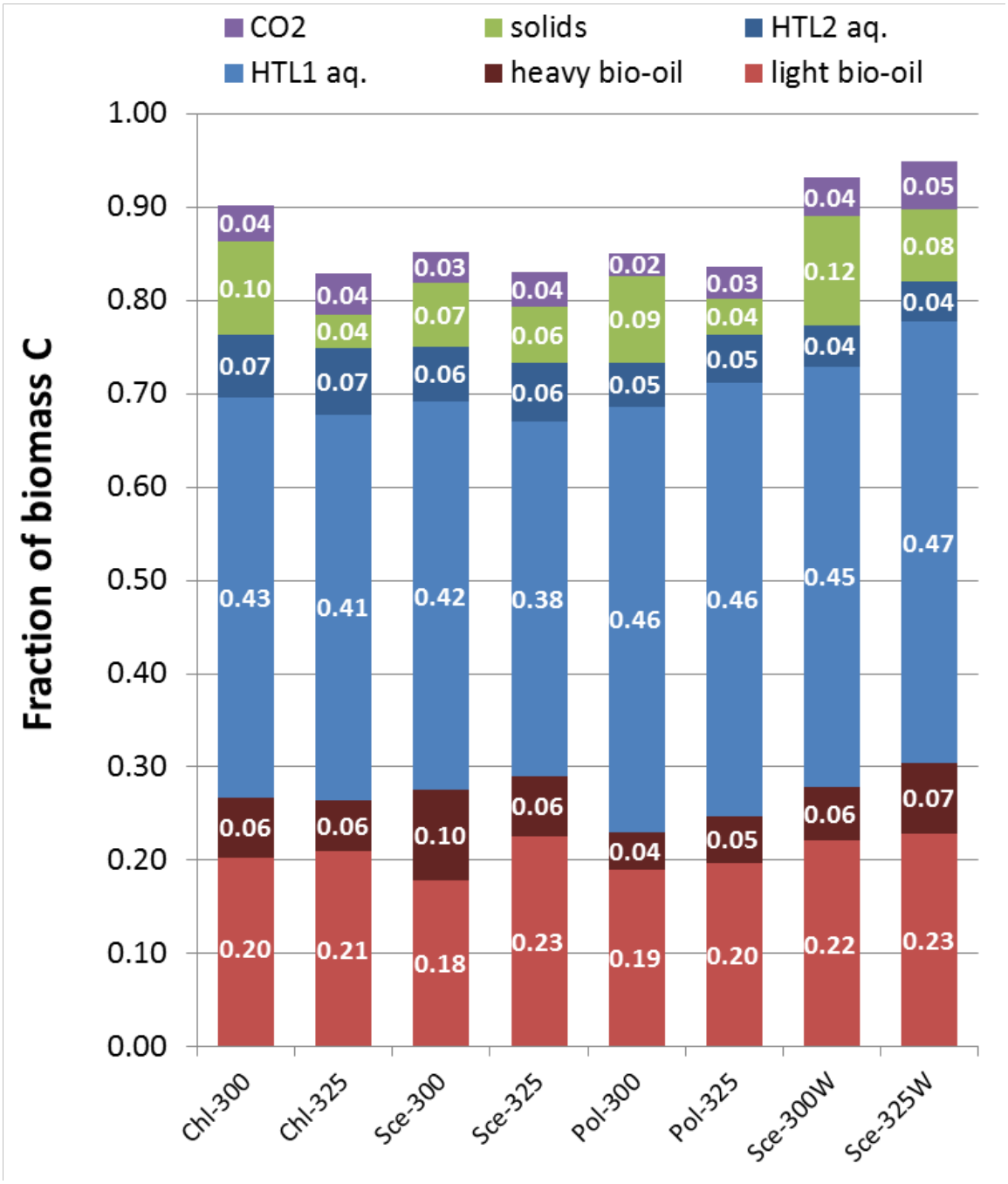

Figure 7. Carbon distributions between phases in the two-step HTL process for three types of algae, *Chlorella* (Chl), *Scenedesmus Dimorphous* (Sce), and polyculture grown in wastewater (Pol). HTL2 runs were done either at 300 °C or 325 °C. Dwell time for HTL2 was 15 minutes in all cases. The runs labeled Sce-300W and Sce-325W were done without solvent in HTL2.

The light bio-oil is a combination of bio-oil extracted in HTL1 and bio-oil found in the organic phase in HTL2. In both cases, the bio-oil was recovered after vacuum distillation of the heptane solvent in a rotary evaporator, so small volatile organic molecules were lost. The phase labeled as heavy bio-oil is the dichloromethane-soluble fraction of the resulting solids after HTL2, and it

was obtained by extraction with dichloromethane at room temperature in a round-bottom flask. This was performed to provide a better comparison with HTL studies reported by other authors, in which common practice is to recover bio-oils by extraction with dichloromethane. From an industrial point of view, characterization of oils extracted with dichloromethane is not representative, because they include molecules that won't be part of the main algae-to-fuel stream in an industrial production. In the most common scenario, bio-oil is to be further upgraded. The first step in the downstream upgrading is dehydrogenation to remove heteroatoms, which is done in blends with hydrocarbons. Thus, it is better to use a hydrocarbon for lab separation of oil, or to distinguish between light (alkane soluble) and heavy (alkane insoluble) bio-oil, as proposed by Savage's group[6]. In this study, the C yield of light bio-oil was in the range of 18% to 23% for all conditions, and the C yield in the total bio-oil was around 26% to 29%. Although each experimental condition was tested only once, the results suggest that yield differences caused by type of algae and temperature are small in the explored range. At a first glance, these yields appear to be low compared to yields reported by other groups. For example, in two key papers from the universities of Twente[7] and Michigan[8], authors reported the best C-to-bio-oil yields were 65% and 75%, respectively, for direct HTL in the temperature range of 300-350 °C. However, the separation of bio-oil from the HTL mixture was done using dichloromethane. The Michigan group reported in a later study that total bio-oil and light bio-oil yields for HTL of algae in the same temperature range had C yields of 51-69% for total bio-oil, but a C yield of only 29-36% for light bio-oil, defined as the fraction soluble in hexane (note that for this calculation, the C content of 75 wt% was assumed for the light bio-oil because the only elemental analyses reported in the study were for total bio-oil).

The rest of the C is distributed among several phases: the remaining solids after the two-step HTL process (insoluble in dichloromethane) contain less than 10% of the original C. These solids have high oxygen content, typically higher than 25%, and are possibly formed by re-polymerization reactions of small molecules. This material can be considered a waste stream that could possibly be incinerated on site in a production facility. Finally, about 4% of the C ends up as CO_2; this is potentially recoverable for algae growth.

Tables 2 and 3 show the elemental analyses results for light and total (light + heavy) biocrude oils obtained using the two-step HTL process. Light bio-oils have a desirable elemental composition, with C contents of 74 wt% or higher, H contents around 11 wt%, and N contents in the range 1.2 wt% to 1.5 wt%, which are much smaller than those typically found in HTL oils. By contrast, heavy bio-oils have smaller C and H contents and higher N contents. If both fractions were combined, the resulting bio-oils would still have N contents in the range 1.6 wt% to 2.2 wt%, which is smaller than state-of-the-art algae HTL bio-oils. However, a main drawback of the heavy bio-oil is its high O content, which translates to higher upgrading costs due to the need for more hydrogen to carry out the hydrogenation step. If O is assumed to be the rest of the oil, light bio-oils have O contents under 14 wt%. However, if heavy bio-oil is also included as a product, most of the resulting oils have O contents around 20 wt% to 22 wt%.

[6] J. Faeth *et al.*, Energy Fuels 27 (2013) 1391.
[7] L. Garcia-Alba *et al*., Energy Fuels 26 (2012) 642.
[8] A.B. Ross *et al.*, Fuel 89 (2010) 2234.

Table 2. Elemental analysis results for light bio-oils produced under different experimental conditions using the two-step HTL process. Light bio-oil is the combination of oil extracted in HTL1 and the heptane-soluble oil from HTL2.

	wt% C	wt% H	wt% N	wt% S
Chl-300	74.7	10.9	1.3	0.1
Chl-325	73.9	10.8	1.2	0.2
Sce-300	77.8	11.4	1.3	0.0
Sce-325	76.4	11.0	1.5	0.0
Pol-300	78.5	11.5	1.3	0.0
Pol-325	69.9	10.5	1.5	0.4
Sce-300W	76.2	11.3	1.3	N/A
Sce-325W	75.7	11.0	1.4	N/A

Table 3. Calculated elemental composition for the combination of light+heavy bio-oils, which would be the bio-oil obtained if dichloromethane was used to recover oil after the HTL process.

	wt% C	wt% H	wt% N	wt% S
Chl-300	65.7	10.1	1.6	0.0
Chl-325	72.8	10.1	2.2	0.3
Sce-300	68.7	10.3	1.7	0.0
Sce-325	67.1	10.3	1.6	0.0
Pol-300	76.6	11.0	1.8	0.0
Pol-325	66.7	10.0	1.8	0.3
Sce-300W	74.9	10.4	2.3	N/A
Sce-325W	74.9	10.2	2.6	N/A

The C yields in oil in these series of experiments were lower than the yields for the same type of algae in the experiments reported in our Y15Q2 report. The previous results were obtained using a different autoclave. Some of the differences between both autoclaves were:

- Different surface-to-volume ratio: In earlier runs, we were using a larger autoclave (1–L capacity) than in recent runs (0.5-L capacity). Surface-to-volume ratio decreased with vessel size.
- The 0.5-L autoclave has an internal cooling coil; the 1-L autoclave doesn't.

- Heating and cooling rates were faster in the 0.5-L autoclave.
- Stirring speeds were different. In the 1-L autoclave, the speed was 1000 rpm, in the 0.5-L autoclave, the stirring speed was 500 rpm.

Figure 8 shows the fraction of biomass C in each phase for an average of two runs done in the 0.5-L autoclave and 10 runs in the 1-L autoclave. We used the same algae type and similar conditions in both cases. For most phases, values were very similar. However, there are a few differences:

- The amount of HTL 2 oil recovered was larger in the 1-L autoclave. This may be due to losses of oil that stuck to the internal surfaces of the autoclave. Although a wash with acetone was done after retrieving the products, some of the oil was not recovered.
- The total amount of C accounted for was 97% in the 1-L autoclave runs, but only 93% was recovered in the 0.5-L autoclave runs. This is likely related to the oil losses just described.
- The amount of solids is slightly lower in the 1-L autoclave. The reasons for this difference are unclear. A possible reason is the increased internal surface area, which would be important if re-polymerization takes place preferentially at the walls. Alternatively, it could be caused by entrapment of solids at the internal cooling coil (or between coil and dwell tubes) in the 0.5-L autoclave; entrapped solids are at least partially in the boundary layer and subject to a different process environment than that of materials circulating around the vessel.

This is an area of interest, and the reasons of differences could be of value in scale-up considerations. Some of the potential tests might be: direct comparison between the two autoclaves with improved solvent wash of the internal surfaces after retrieving products; comparison between runs in the 0.5-L autoclave with and without cooling water in the coil; comparison between runs in the 0.5-L autoclave with and without the internal coil; and the addition of non-metal liners (i.e., quartz) inside the vessel.

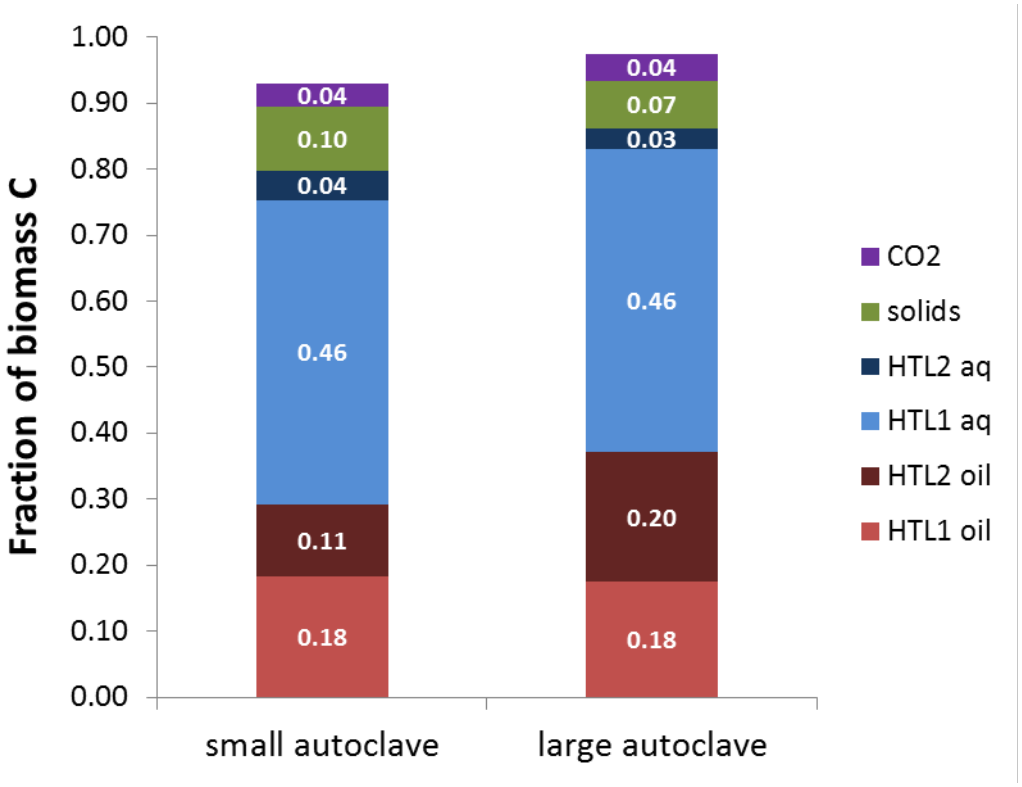

Figure 8. Comparison between fraction of C to several phases obtained in runs in a 0.5-L autoclave and runs with the 1-L autoclave. The 0.5-L autoclave data is the average for runs Sce-300W and Sce-325W. The 1-L autoclave data is the average of 10 runs with temperatures of 300 °C and 325 °C and dwell times of 15 min and 90 min (on average, dwell time did not affect C yield).

1.4 Recycling of nutrients from HTL1 aqueous phase for algae growth

During the HTL process, a significant amount of organic carbon and nitrogen end up in the aqueous phase, resulting in a loss of carbon efficiency[9]. The carbon and nitrogen left in the process water are not suitable to be discharged to the environment without additional treatment. However, they can be recycled and used for algae cultivation[10].

Microalgae have been used as an alternative feedstock for several reasons: first, they don't require arable land; and second, algae have high growth rates per unit area. However, nutrient management of algae is complex. Algae require remarkable quantities of nutrients for growth, including nitrogen, phosphorus, potassium, carbon dioxide, and a wide range of micronutrients. A practical approach is to use process water that contains sufficient/required nutrients for algae cultivation. Since the aqueous phase from HTL contains nitrogen and carbon, it can be fed back to the cultivation system to provide nutrients for microalgae growth. It has been shown that certain strains of algae can grow heterotrophically and prefer an organic carbon substrate over

[9] P. Biller P., Algal Research 1 (2012), 70-76.
[10] Yu G., Energy Environ. Sci. 4 (2011) 4587-4595.

CO_2, while others are mixotrophic and can coincidently drive phototrophy and heterotrophy to use both inorganic (CO_2) and organic carbon substrates [11,12,13].

The initial studies were grouped into three different categories:

- Feasibility of reusing nitrogen from the aqueous phase of HTL1
- Feasibility of growing algae mixotrophically using C from the aqueous phase of HTL1 as a complement C source.
- Feasibility of growing algae heterotrophically using C from the aqueous phase of HTL1 as the single source of C.

1.4.1 Nitrogen recycling study

For the N recycling study, we kept several cultures in parallel, each in a 4-L Erlenmeyer flask. The algae used was *Scenedesmus dimorphous* (UTEX 1237). We bubbled 600 sccm of air with 1 vol% CO_2. The flasks were illuminated with blue fluorescent lamps at an irradiance level of 125 $\mu E \cdot m^{-2} \cdot s^{-1}$, with a photoperiod of 14:10 (light:darkness). These conditions are similar to algae growth studies in the literature. Before starting the actual study, algae was acclimated for 30 days to the growth environment using the full M8 nutrient recipe [14] (Table 4), a nutrient recipe developed to obtain high-density cultures of *C. vulgaris*. The basis for the recipe is to match elemental composition of the medium to that found in biomass. During the acclimation period, we harvested half of the cultures on a weekly basis and added the same amount of culture medium to the flasks. After this period, the full amount of algae cultures was harvested and centrifuged to separate the biomass from the media containing leftover nutrients. Algae were then resuspended in a small amount of water, and were split into several fractions to start new cultures. We started the following cultures in parallel: (A) algae grown using the full M8 recipe; (B) two replicates of algae grown using M8 nutrient levels for all nutrients except nitrate, and using HTL1 water as N source (the amount of HTL1 solution added was enough to obtain the same final N concentration in the culture as in culture A); (C) algae grown using M8 nutrient levels except nitrate, so there is no source of N. We measured the biomass concentration every 3-4 days over a 16-day growth periods.

Table 4. M8 nutrient recipe.

	M8 / $mg \cdot L^{-1}$	M8 / $mol \cdot L^{-1}$
KNO_3	3000	2.97E-02
KH_2PO_4	740	5.44E-03
$NaH_2PO_4.H_2O$	293	1.83E-03
Fe EDTA	10	2.89E-05
$FeSO_4.7H_2O$	130	4.68E-04
$MgSO_4.7H_2O$	400	1.62E-03
$CaCl_2.2H_2O$	13	8.84E-05

[11] Shi X., Biotechnol Prog, 18 (2000) 723–7

[12] Sun N., Process Biochem, 43 (2008),1288–92

[13] Chen F., Process Biochem, 40 (2005) 733–8

[14] R.K. Mandalam and B.O. Palsson, Biotechnology and bioengineering, 59 (1998), 605-611

All the starting cultures had the same dark green appearance that we had observed during the acclimation period. Cultures A and B had the same appearance for the whole 16 days of the experiment. By contrast, the color in culture C (no nitrogen) quickly changed to a light green; a clear difference in color could be appreciated by Day 3. Figure 9 shows a photo of the cultures at the end of the growth cycle, after different amounts had been taken for analysis.

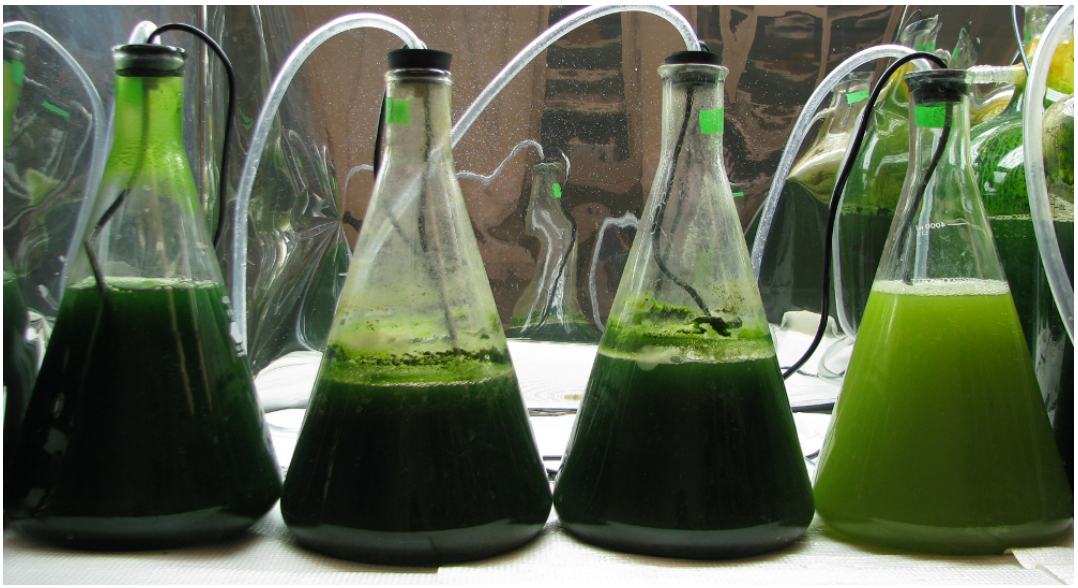

Figure 9. Photobioreactors at the end of the growth cycle to study recycling of N.

Figure 10 shows the evolution of biomass concentration over time. Culture A was grown using the full M8 set of nutrients. The biomass concentration results from this culture (labeled as "N source: NO_3^-") are in agreement with data from measurements performed during the acclimation period. M8 is a recipe rich in nutrients, so the limiting step for algae growth in our laboratory setup was light. It is therefore not surprising that the biomass evolution in Culture A follows a linear trend.

Despite the above-mentioned difference in the color of Culture D, at Day 3 the differences in biomass concentration were small. It is possible that, in Culture D, algae were still able to go through some reproductive cycles using the nitrogen from nitrate anions dissolved in the water trapped in the cake after centrifugation. However, due to the extreme environment, the organisms were stressed. When the nitrogen in the culture was further depleted, algae growth and reproduction ceased due to inability of the algae to synthesize proteins. This hypothesis is in agreement with biomass measurements from Day 6 on (labeled "No N source").

Culture C presented growth rates very similar than that of Culture A. This validates the hypothesis that the nitrogen extracted during the first step of the dual hydrothermal liquefaction treatment is a viable source of nitrogen for algae growth.

At the end of the growth cycle, we separated the algae by centrifugation, washed each cake with DI water, separated it again by centrifugation, and dried each culture separately overnight in a convection oven set at 55 °C. We dried out the centrifugate from the first centrifugation (growth media at the end of the growth cycle) by evaporating the water in a convection oven. We carried out the following analyses:

▪ CHNS elemental analysis of the dry algae and the solid residue from the media. The mass balance of N in the growth media showed that algae consumed 47% of the original N in the aqueous solution. On the other hand, the mass balance in the biomass showed that 35% of the original N in the aqueous solution was incorporated into the algae. The mass balance in the biomass is likely to be more accurate, because part of the N in the final media may be in the form of ammonia or other volatile species that are lost in the evaporation process.

▪ Lipid content of the algae. The results showed that the lipid content did not change during the growth cycle: it was around 22 wt% before and after.

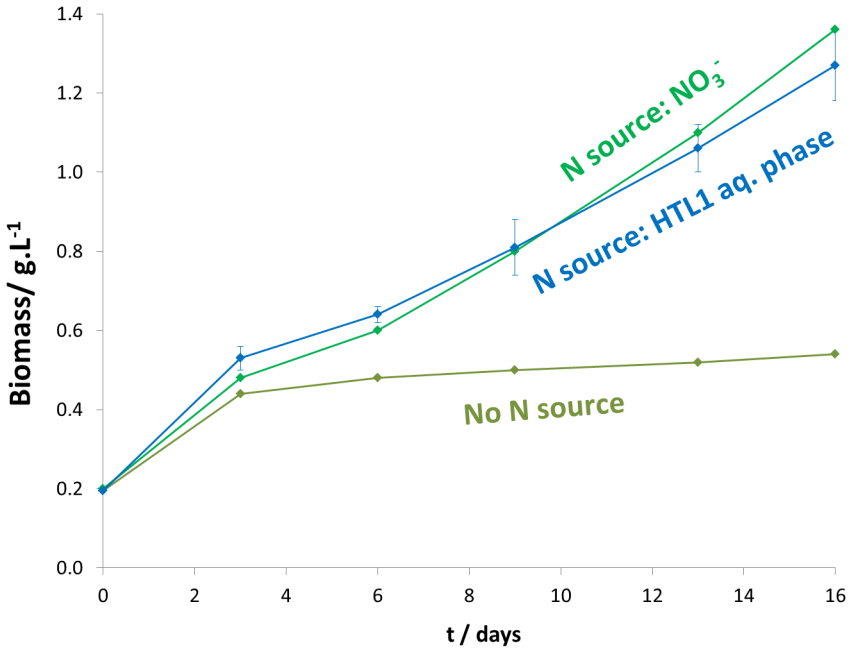

Figure 10. Evolution of biomass concentration over a growth period of 16 days for the study of N recycling from the HTL1 aqueous phase.

1.4.2 Mixotrophic growth of algae using C from the HTL1 aqueous phase

The studies for mixotrophic growth were done in closed photobioreactors without gas bubbling (Figure 11). The algae were kept in suspension by mechanical stirring using a magnetic stir bar. We used these settings to avoid introducing C due to the ~400 ppm of CO_2 in ambient air. We used two sources of C:

▪ Sodium bicarbonate ($NaHCO_3$), to simulate the equilibrium between carbonate, bicarbonate, carbonic acid, and CO_2 that is reached when air with a certain partial pressure of CO_2 is passed through the growth media. We added 1 g/L of $NaHCO_3$, which is sufficient for some algae growth but not to reach the desired biomass concentrations > 1 g/L.

▪ In some of the cultures, we added a small amount of aqueous phase from HTL1. The total dilution factors were 1/35 and 1/70, defined as the ratio between volume of the HTL1 aqueous phase to the total volume of culture. We previously measured the C content in the HTL1 aqueous

phase to be 10 g/L by drying a known volume, weighing the resulting solid residue, and doing CHNS elemental analysis on the residue.

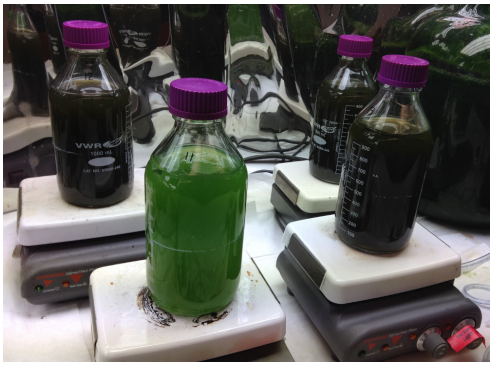

Figure 11. Photobioreactors used in C recycling studies.

The cultures were subject to the same light intensity and light:dark periods described above. Figure 12 shows the evolution of biomass concentration in each of the cultures over a period of 12 days. As expected, the culture with only $NaHCO_3$ growth stopped at about 0.6 g/L, which is the maximum concentration we calculated to be possible with the limited amount of C supplied by adding 1 g/L of $NaHCO_3$. On the other hand, when we added the HTL1 aqueous phase in addition to the 1 g/L of $NaHCO_3$, the final concentrations were higher: 1.0 g/L for a dilution factor of 1/70, and 1.5 g/L for a dilution factor of 1/35. These results showed that algae can be grown mixotrophically, using organic C recycled from HTL1 in addition to the inorganic C supplied in the form of bicarbonate. Assuming the concentration that can be reached from inorganic C is 0.6 g/L, the difference in C for mixotrophic cultures indicates up to 60% of the C in the HTL1 aqueous phase was incorporated in the algae (1/35 dilution case). Note that we stopped the growth cycle after 12 days when the biomass concentration had not leveled, so higher recycling rates are possible (as explained in the next section). For the 1/70 dilution case, the recycled amount was 55% of the C. In conclusion, these results show it is possible to reuse the C present in HTL1 aqueous phase for algae growth.

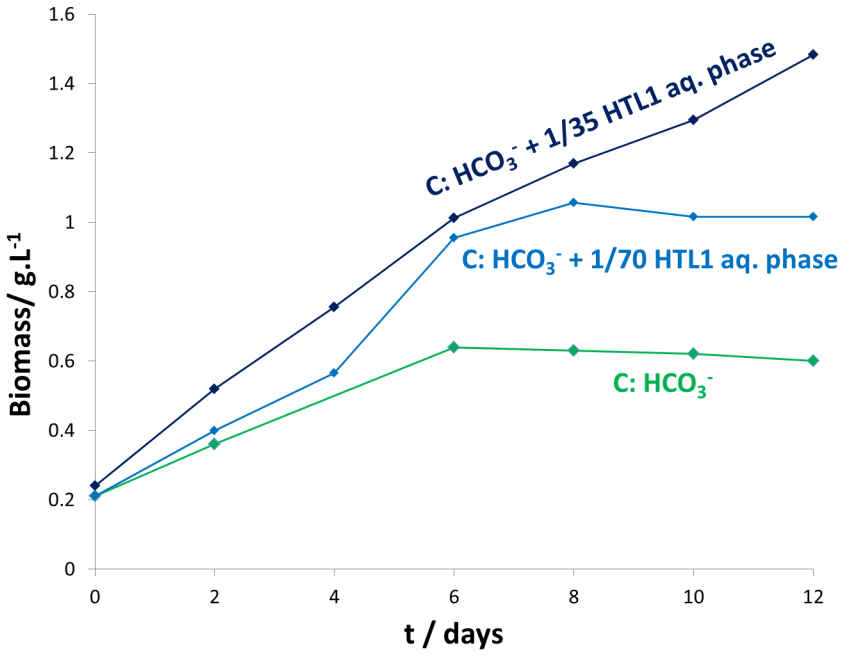

Figure 12. Evolution of biomass concentration over a growth period of 12 days for the study of mixotrophic growth using C from HTL1 aqueous phase.

1.4.3 Heterotrophic growth of algae using C from HTL1 aqueous phase as sole source of C

The studies for heterotrophic growth were done in closed photobioreactors without gas bubbling. The algae were kept in suspension by mechanical stirring using a magnetic stir bar. We used these settings to avoid introducing C due to the ~400 ppm of CO_2 in ambient air. The only source of C came from small additions of aqueous phase from HTL1. The total dilution factors were 1/17, 1/22, 1/33, and 1/66—defined as the ratio between the volume of the HTL1 aqueous phase to the total volume of the culture. We previously measured the C content in HTL1 aqueous phase to be 10 g/L by drying a known volume, weighing the resulting solid residue, and performing CHNS elemental analysis on the residue.

The cultures were subject to the same light intensity and light:dark periods described above. Figure 13 shows the evolution of biomass concentration in each of the cultures over a period of 12 days. The results show a clear relationship between the amount of the HTL1 aqueous phase added and the growth rate and final concentration. Taking into account that no inorganic source of C was supplied to the cultures, these results show that the aqueous phase from HTL1 can be recycled as a C source for heterotrophic growth. In order to quantify the amount of C that went into the algae, we harvested each culture at the end of the growth cycle, separated algae by centrifugation, washed the pellets with DI water, separated the algae from the resulting suspensions by centrifugation, dried the solids in a convection oven, and performed CHNS elemental analysis on the dry algae. The results, summarized in Table 5, show that 65% of the C in the aqueous phase could be reused for algae growth. This is a very encouraging result for implementation of this technology. In addition to optimizing the conditions, we carried out consecutive growth cycles and studied possible inhibitors for growth.

Table 5. Calculated and measured values for mass balance on heterotrophic growth studies: C input from HTL1 aqueous phase, algae mass variation, C content in algae at end, and percentage of C from HTL1 aqueous phase that was recycled for algae growth.

Dilution factor	C in HTL1 aqueous phase / g	Δm algae / g	wt% C in algae at end	% C recycled
1/66	0.16	0.35	28.6	63
1/33	0.32	0.55	32.7	57
1/22	0.48	0.79	39.5	65
1/17	0.63	0.99	42.0	65

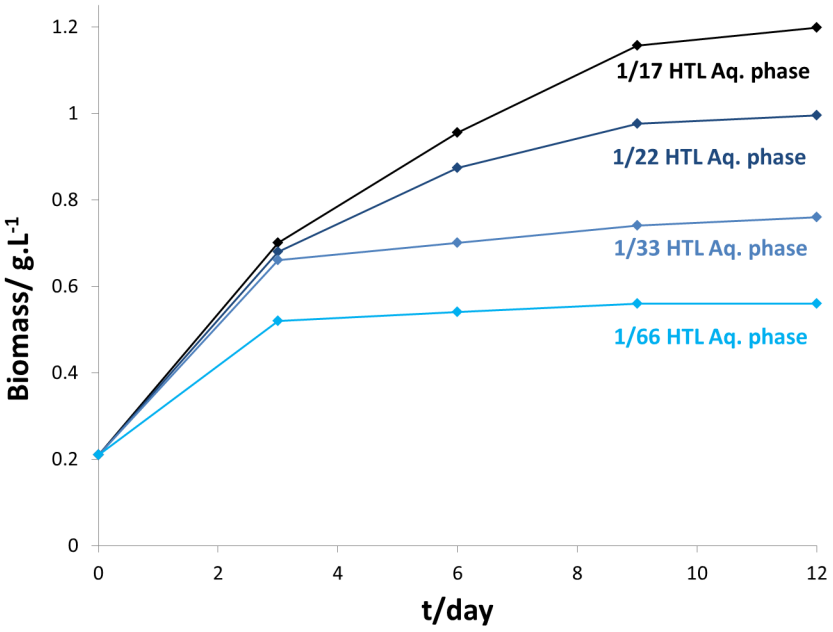

Figure 13. Evolution of biomass concentration over a growth period of 12 days for the study of heterotrophic growth using C from the HTL1 aqueous phase.

The main findings from this study were:

- Nitrogen extracted during the first step of the dual hydrothermal liquefaction treatment is a viable source of nitrogen for algae growth.
- Heterotrophic growth of algae is possible using aqueous phase from HTL1 as the only source of C, with the same growth rates observed when using CO_2 as C source.

Subsequently, we started the study of the long-term effects of recycling HTL1 water for algae growth over a number of cycles, in order to find effects related to accumulation of chemical species in the photobioreactors (PBRs). Several authors described inhibitory effects related to the presence of nickel and organics such as phenol. Scragg[15] showed that some microorganisms are not able to tolerate phenol in the media. Phenol may cause toxicity and stop the growth cycle

[15] A. H. Scragg, Enzyme and Microbial Technology (2006), 39(4), 796-799.

secondary to disruption of the membrane structure through hydrophobic interactions [16,17]. Napan studied the impact of heavy metals from flue gas integration with microalgae production and showed high concentrations of heavy metal could inhibit both growth rate and lipid production [18]. It is possible that Ni leached from autoclave during thermal liquefaction and was present during the process.

We kept six cultures in parallel, each in a 4-L Erlenmeyer flask. Two of the PBRs were used for nutrient recycling study, and the others were used to grow algae given the standard M8 nutrient recipe described in previous reports. These additional PBRs were used to ensure enough supply of algae for the HTL1 process. We used *Scenedesmus dimorphous* (UTEX 1237) that was pre-conditioned for several growth cycles in standard photobioreactors with full nutrient supply. We then centrifuged the algae to separate as much water as possible without damaging the cells. Then, we resuspended the algae in DI water and used it as an inoculant for all the PBRs. Two of the cultures (S1 and S2) were supplied with a modified nutrient recipe consisting of M8 nutrients (except nitrate) and that used HTL1 water as N source (the amount of HTL1 solution added was enough to obtain N concentration levels similar to those obtained using the standard M8 recipe). The rest of the cultures (S3, S4, S5, and S6) were fed with full M8 recipe. The flasks were illuminated with blue fluorescent lamps at an irradiance level of 125 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a photoperiod of 14:10 (light:darkness).We measured the biomass concentration every 3-4 days over a 14/15day growth periods.

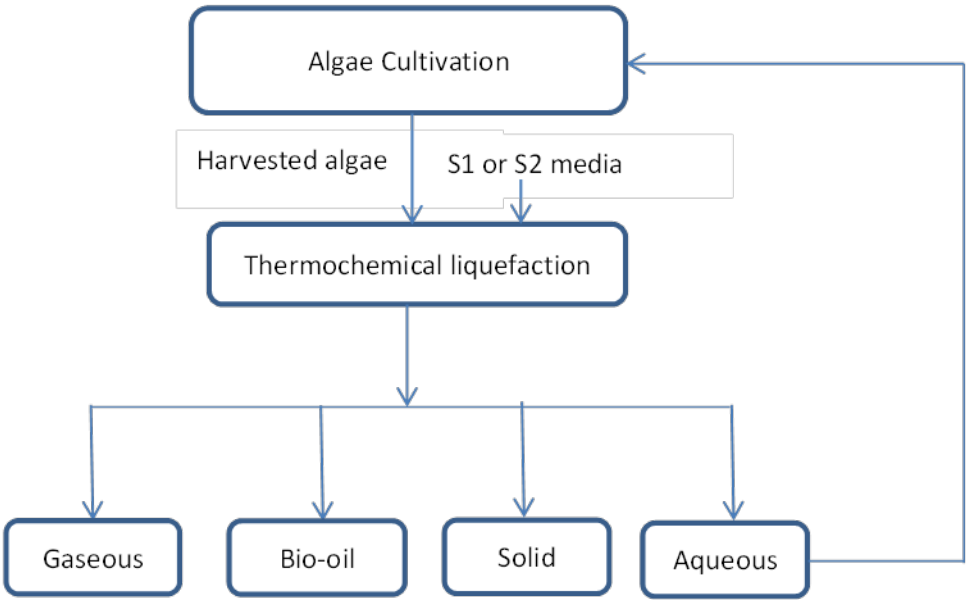

Figure 14. Schematic representation of the work flow.

[16] J. Pittman *et al.*, Bioresource Technology 102 (2011) 17–25
[17] A.H. Scragg, Enzyme and Microbial Technology 39 (2006) 796–799
[18] K. Napan, Algal Research 8 (2015) 83–88

At the end of the growth cycle, 3 L of each culture were harvested and centrifuged in order to separate the algae. We used the algae as a feed for HTL1 in a 0.5-L stainless steel autoclave. In a typical HTL1 run, we used a mixture of DI water and heptane (2:1), and used S1 or S2 media instead of water with the same ratio. The HTL1 products were separated, and the aqueous phase was collected and used for the next growth cycle. The HTL1 water was split into two portions and added to S1 and S2 in small amounts at the beginning of each day for the duration of the growth cycle. The same procedure was performed to harvest the algae and complete the HTL1 runs for the second and third cycles.

Figures 15, 16, and 17 show the evolution of biomass concentration over a growth period of 14/15 days for three consecutive cycles. The growth rates of cultures S1 and S2, which grew using the HTL1 aqueous phase as the C source, were the same as those of the other cultures supplied with CO_2 as the C source. These results show that algae can be grown over multiple cycles using the nutrient recycling scheme that we developed earlier.

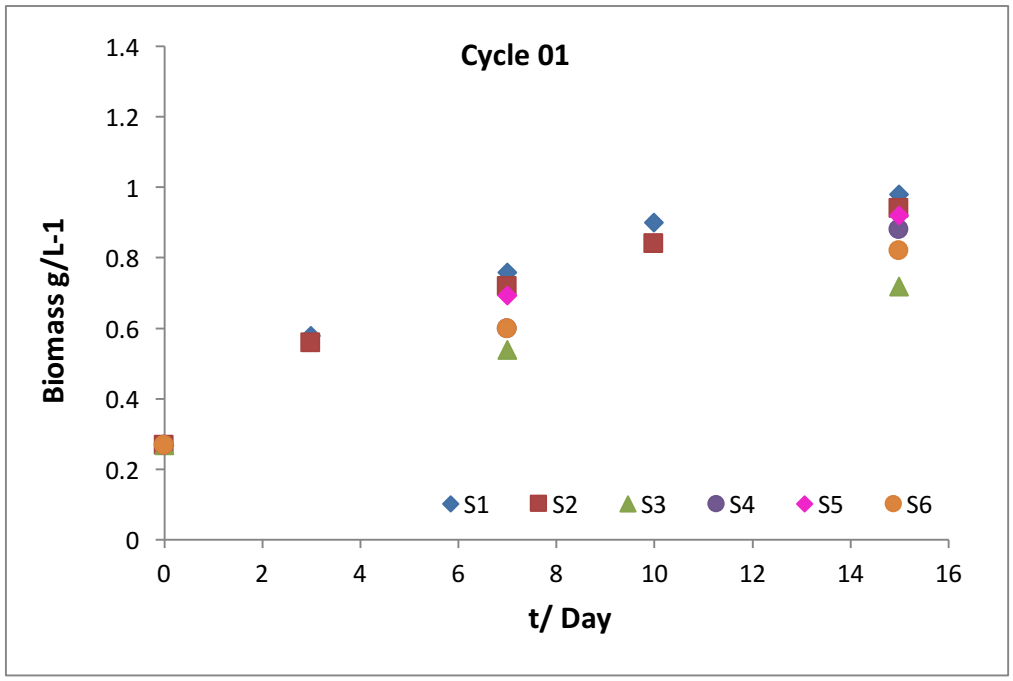

Figure 15. Evolution of biomass concentration over a growth period of 15 days.

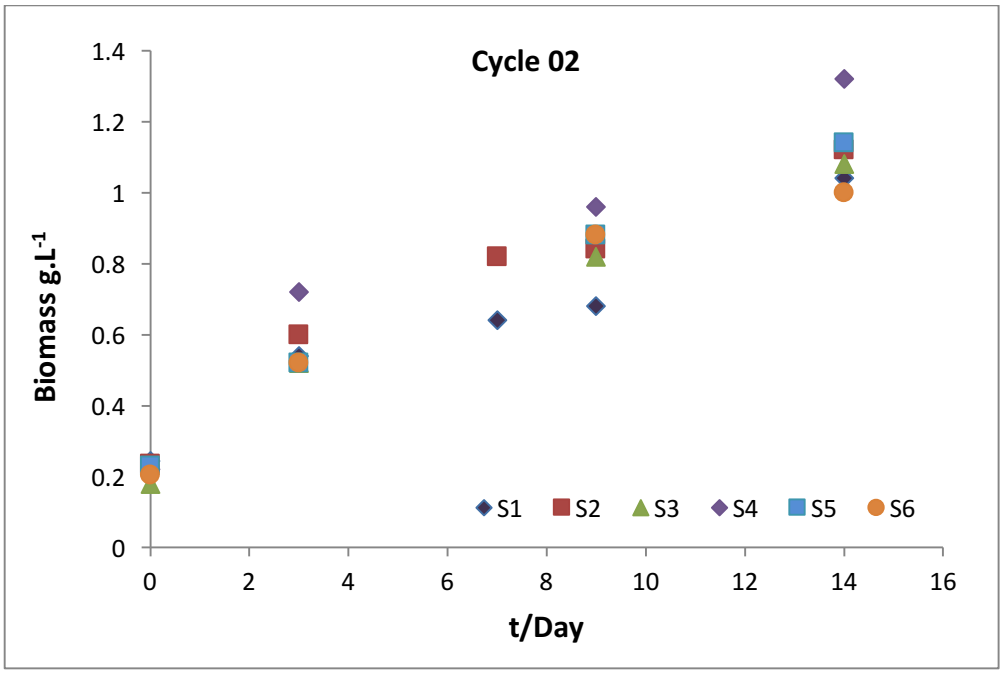

Figure 16. Evolution of biomass concentration over a growth period of 14 days.

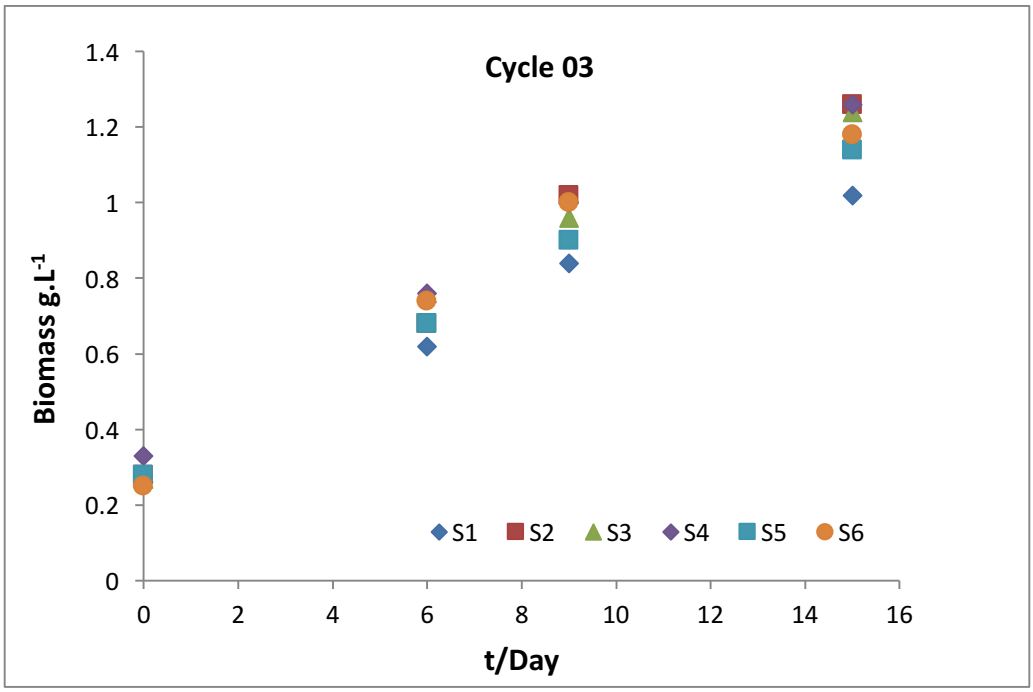

Figure 17. Evolution of biomass concentration over a growth period of 15 days.

We continued the study of the long-term effects of recycling HTL1 aqueous byproducts for algae growth over a number of cycles, focusing on N recycling for this experiment.

For the N recycling study, we kept six cultures in parallel, each in a 4-L Erlenmeyer flask. Two of those (S and S2) were supplied with the HTL1 aqueous byproducts as the N source, and the other four (S4-S6) were grown using the full nutrient recipe (including nitrate as N source). The flasks were illuminated with blue fluorescent lamps at an irradiance level of 125 µE·m-2·s-1, with a photoperiod of 14:10 (light:darkness). At the end of each growth cycle, 75% of each culture was harvested, concentrated to a ~10 wt% sludge by centrifugation, and used as the feedstock for a low-temperature hydrothermal liquefaction step (200 °C, 30 minutes, as described above and in previous reports). The aqueous byproducts of this HTL1 step were supplied to S1 and S2 as the N source in the next cycles.

Figures 18 and 19 show the evolution of biomass concentration over a growth period of 10/13 days for two consecutive cycles. In cycles 1 and 2, cultures S1 and S2 were kept in suspension by bubbling compressed air. During those cycles, the biomass growth rates in S1 and S2 were slightly slower than in the control cultures, but the cultures were nevertheless viable and produced a significant amount of biomass. In the third cycle (Figure 20), there was no air bubbling into S1 and S2, and algae was kept in suspension by stirring. In this cycle, S1 and S2 were not viable. We planned to continue work in this area and determine if the reason was due to the low concentration of organic C to feed the algae, or due to the accumulation of species that inhibit algae growth.

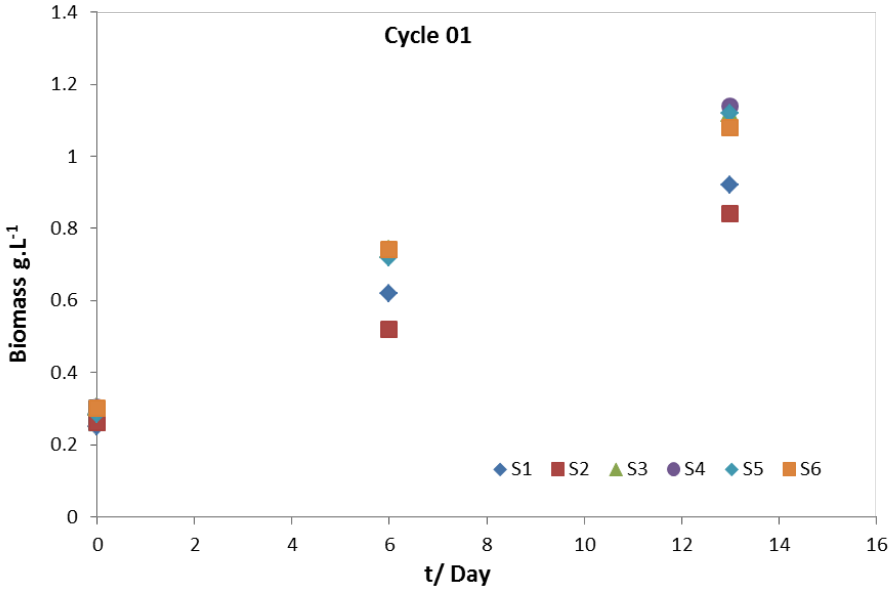

Figure 18. Evolution of biomass concentration over a growth period of 13 days.

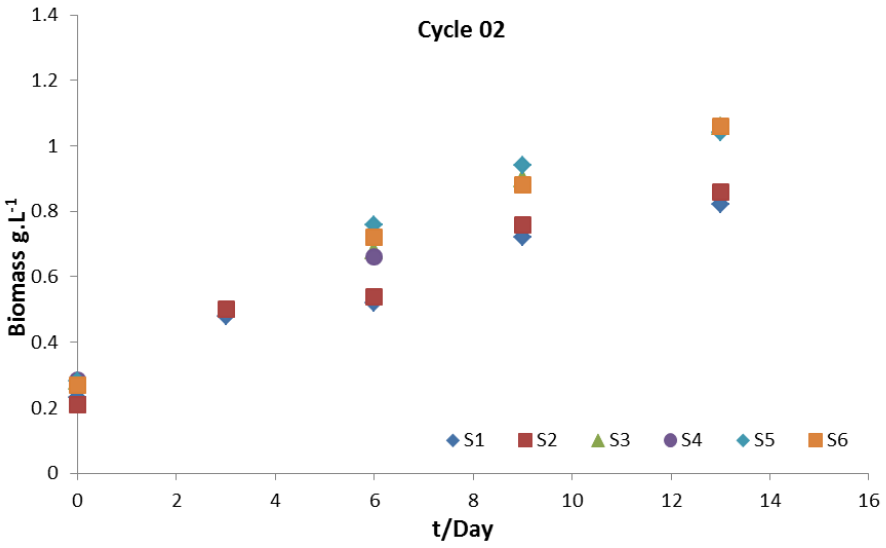

Figure 19. Evolution of biomass concentration over a growth period of 13 days.

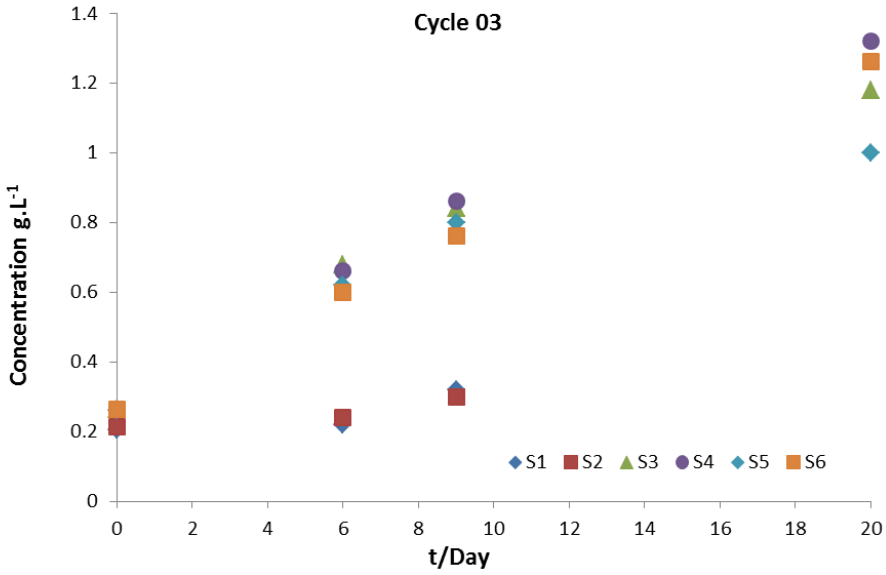

Figure 20. Evolution of biomass concentration over a growth period of 20 days.

We continued the study of long-term effects of recycling HTL1 aqueous byproducts for algae growth over a number of cycles. We simulated a production scheme in which some of the algae cultures are grown using nutrients from external sources, and some of the algae are grown using recycled nutrients. So far, our studies have centered on N and C. The fractions of C and N that end up in the dissolved species in the HTL1 aqueous byproducts are 35-40% and 60-70%, respectively. From those, approximately 2/3 of aqueous C and 1/2 of aqueous N can be recycled as nutrients for algae growth. Thus, we expect that continuous recycling of C will be enough to sustain about 1/4 of the total cultures, and continuous recycling of N could sustain about 1/3 of the cultures.

Our goal was to study whether or not implementation of a nutrient recycling scheme can lead to accumulation of chemical species that inhibit algae growth. This could be either organic molecules or inorganic ions, such as nickel from the vessel and internals in the HTL reactor. For this testing, we operated an algae "mini-farm" with a total capacity of 16 liters. The algae strain was *Scenedesmus Dimorphus*. The flasks were illuminated with blue fluorescent lamps at an irradiance level of 125 μE·m-2·s-1, with a photoperiod of 14:10 (light:darkness). The test was done using the following protocol:

- Cycle 0 was a pre-conditioning cycle in which all 16 liters of culture were grown with full supply of nutrients and bubbling a mixture of air and CO_2.
- Cycles 1, 2, 3, 4, and 5: 12 liters of cultures were grown using full supply of nutrients, and four liters are grown using HTL1 water as source of C and N. These 4 liters are split between three cultures:
    - Culture A (1.5-L Erlenmeyer): this culture was aerated with a mixture of air and CO_2, so only N was recycled.
    - Culture B (1.5-L Erlenmeyer): this culture was aerated with air, but no additional CO_2 was added. In addition to recycling of N, partial C recycling was needed to sustain the average growth rate.
    - Culture C (1-L media bottle, closed and stirred with a magnetic stir bar): in this culture, the only sources of C and N were from the HTL1 water added to the culture.
- Cycles 6, 7, and 8: 12 liters of cultures were grown using full supply of nutrients, and four liters were grown using HTL1 water as source of C and N. These four liters were split between three cultures:
    - Culture A (1.5-L Erlenmeyer): this culture was aerated with a mixture of air and CO_2, so the only N was recycled.
    - Culture B (1.5-L Erlenmeyer): this culture was aerated with air, but no additional CO_2 was added. In addition to recycling of N, partial C recycling was needed to sustain the average growth rate. We had this type of culture for cycle 6 and cycle 7 only.
    - Culture C (1-L media bottle, closed and stirred with a magnetic stir bar): in this culture, the only sources of C and N were from the HTL1 water added to the culture.

At the end of each of these cycles, a large fraction of the culture (>75% overall) was harvested and partially dewatered. The resulting slurry was loaded in the autoclave as a biomass source for the HTL1 step. The aqueous phase was separated and used as source of nutrients for the cultures (50% of it added in the first day, 25% three days later, and 25% three days after that).

Figure 21 depicts the biomass concentration evolution in cycle 5 for cultures grown using HTL1 water as source of nutrients. All cultures grew at a healthy pace, which indicates there was no accumulation of toxic species. The differences in growth rates can be explained by different conditions in each culture, as follows:

- The culture in the bottle (no air or CO_2 added) was inoculated with algae using the "air" culture from cycle 4. The algae were therefore pre-conditioned to grow in an environment containing HTL1 water species, and the fast growth period started soon after inoculation.

- The culture grown with CO_2 addition was inoculated with algae grown without HTL1 water and grew at the expected pace. We did not observe any lag period when HTL1 water was used as sole source of N.
- The culture grown with air but no CO_2 showed a lag period at the beginning, and the growth rate picked up after some days.

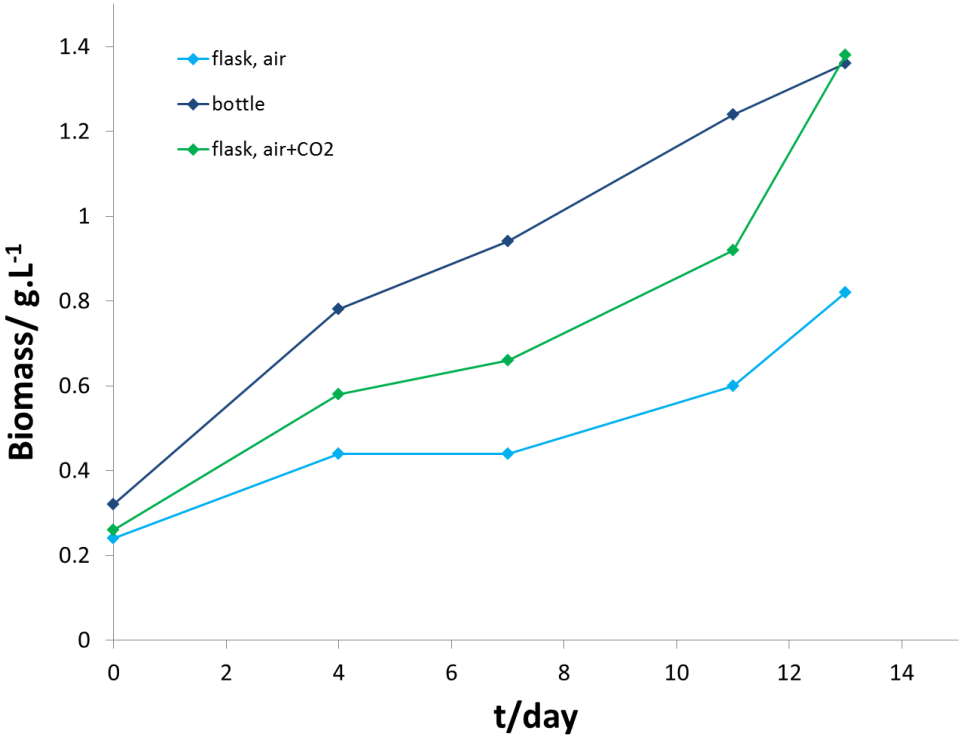

Figure 21. Evolution of biomass concentration for cultures grown using recycled HTL1 water as nutrient source for cycle 5.

Figure 22 shows data similar to that in Figure 21 but for cycle 4. In cycle 4, all cultures were inoculated using algae from the 12 L of cultures with full nutrient supply and no HTL1 water. In this case, a lag phase was observed for the two cultures not supplied with CO_2. It appears there was a period of adaptation in order to switch from CO_2-supported growth to organic C-supported growth.

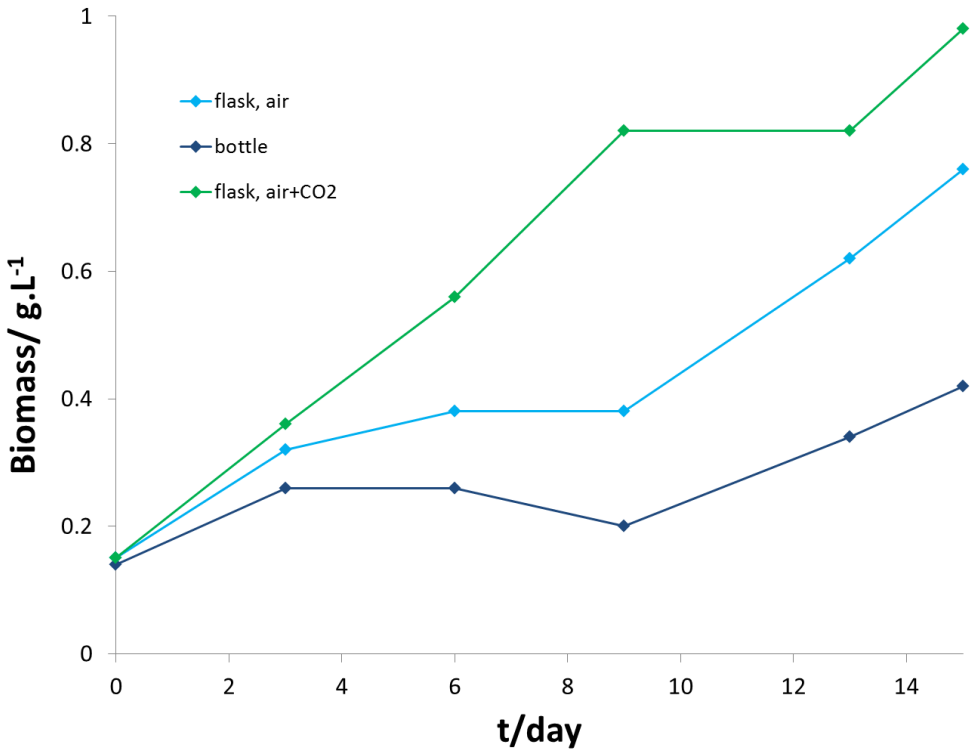

Figure 22. Evolution of biomass concentration for cultures grown using recycled HTL1 water as nutrient source for cycle 4.

Figure 23 depicts the biomass concentration evolution in cycle 8 for cultures grown using HTL1 water as a source of nutrients. All cultures grew at a healthy pace, which indicated there was no accumulation of toxic species. The differences in growth rates can be explained by different conditions in each culture:

- The culture in the bottle (no air or CO_2 added) had been inoculated with algae using the "air" culture from cycle 7. The algae were therefore pre-conditioned to grow in an environment containing HTL1 water species, and therefore the fast-growth period started after 13 days.
- The culture grown with CO_2 addition was inoculated with algae grown without HTL1 water grew at the expected pace. We did not observed any lag period when HTL1 water was used as the sole source of N.

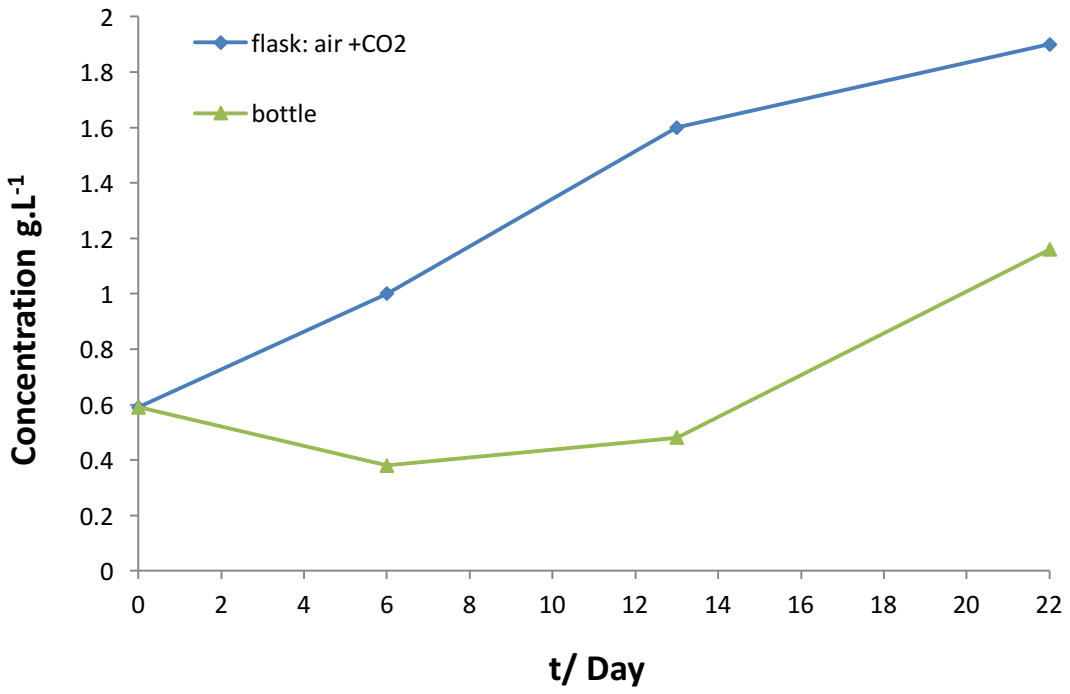

Figure 23. Evolution of biomass concentration for cultures grown using recycled HTL1 water as the nutrient source for cycle 8.

Figures 24 and 25 depict the biomass concentration evolution in cycles 6 and 7 for cultures grown using HTL1 water as the source of nutrients. All cultures grew at a healthy pace, which indicated no accumulation of toxic species. Figure 24 shows similar data as Figure 25. The lag phase was observed for the two cultures that were not supplied with CO_2; this effect was more obvious in cycle 6. It appears that there is a period of adaptation in order to switch from CO_2-supported growth to organic C-supported growth. In cycle 7, as shown in Figure 24, the culture that was supplied by both air and CO_2 had a significant drop in concentration between days 8 and 11; this could be an experimental error during concentration measurement since no color change was observed. The differences in growth rates can be explained by different conditions in each culture:

- The culture in the bottle (no air or CO_2 added) had been inoculated with algae using the "air" culture from cycles 5 and 6 for cultures 6 and 7, respectively. The algae were therefore pre-conditioned to grow in an environment containing HTL1 water species, and therefore the fast-growth period started soon after inoculation.
- The culture grown with CO_2 addition was inoculated with algae grown without HTL1 water and grew at the expected pace. We did not observe any lag period when HTL1 water was used as the sole source of N.
- The culture grown with air but no CO_2 showed a lag period at the beginning, and the growth rate picked up after some days.

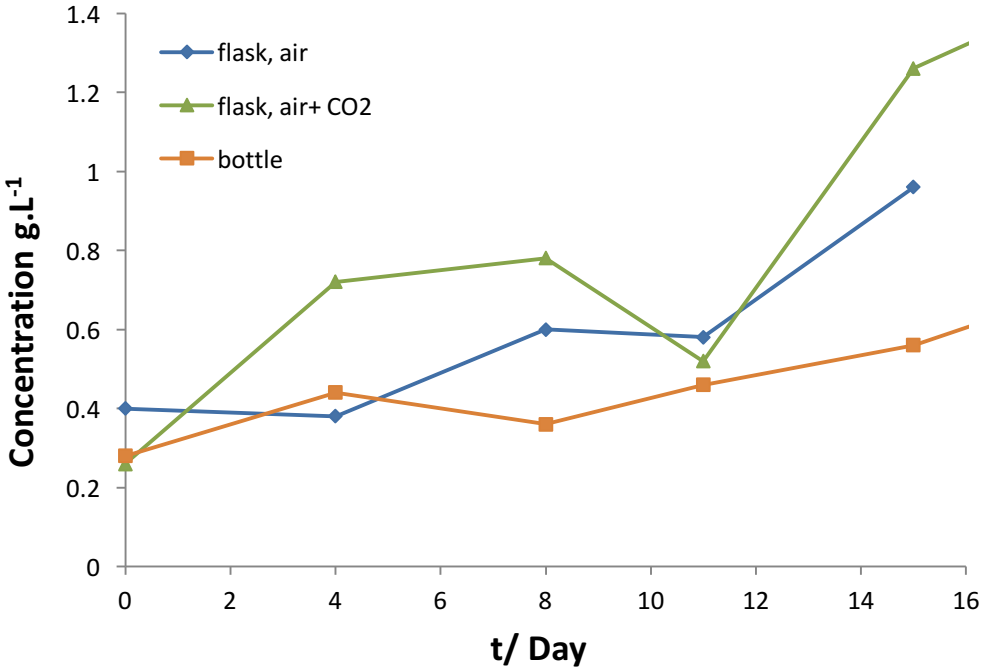

Figure 24. Evolution of biomass concentration for cultures grown using recycled HTL1 water as the nutrient source for cycle 7.

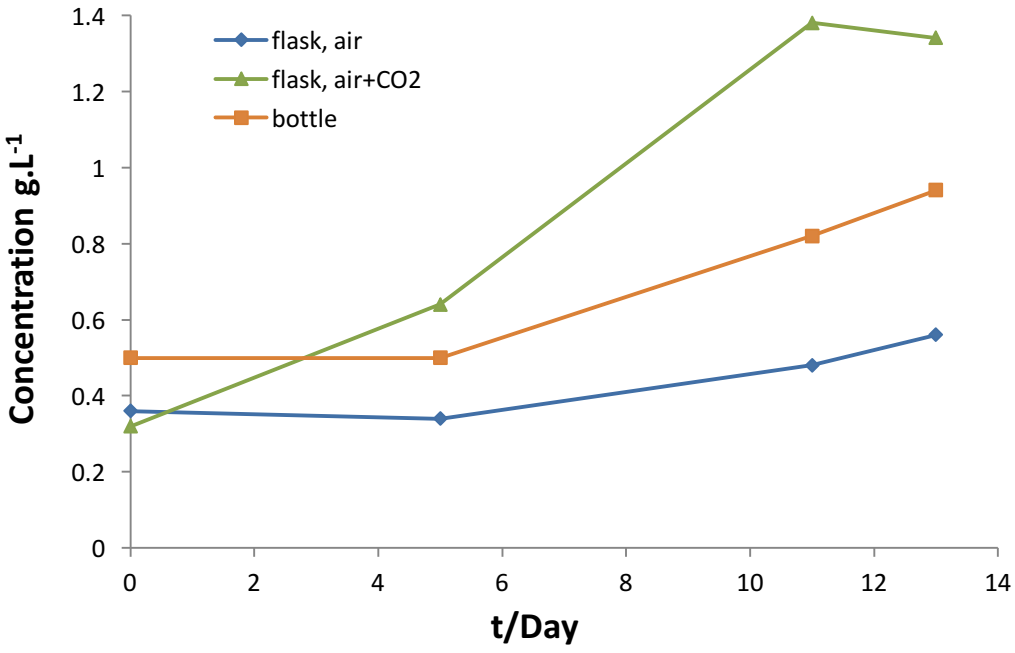

Figure 25. Evolution of biomass concentration for cultures grown using recycled HTL1 water as the nutrient source for cycle 6.

The results showed that algae cultivation using recycled HTL1 water as the source of N and C was feasible. There was no accumulation of toxic species that stopped algae growth. When algae were not supplied with CO_2, there was a lag period for the algae to switch from CO_2-supported growth to organic C-supported growth. In order to maximize plant output, it was necessary to inoculate the cultures with algae already adapted to C-supported growth.

1.5 Model compound study

The process of the liquefaction of the microalgae in subcritical water is complex. Microalgae are broken down into smaller compounds by dehydration, dehydrogenation, deamination, and deoxygenation and further tend re-polymerize, forming oily compounds[19]. Lipids undergo hydrolysis to produce fatty acids, which are further degraded to produce long-carbon-chain hydrocarbons in subcritical water. Proteins, which consist of amino acids bound together through peptide bonds, are hydrolyzed to produce amino acids and some acids. A number of amino acids can be produced during the HTL of the algae. Some of them are asparagine, leucine, cysteine, alanine, valine, etc. These amino acids likely undergo further deamination and deoxygenation. Sato *et al*.[20] studied the HTL of five different amino acids at temperatures between 200-340 °C and a constant pressure of 20 MPa and observed that deamination produces ammonia and organic acids and decarboxylation produces carbonic acid and amines. The decarboxylation and deamination reactions help in removing the oxygen and the nitrogen atoms into the water phase, thereby reducing the nitrogen content in the algae. However, the degraded products of the proteins react with carbohydrates present in the algae through Millard reactions to produce nitrogen-containing cyclic organic compounds such as pyrroles, pyridines, etc.[21] This general reaction network and these complicated reactions provide no clear elucidation regarding which of the constituents are the most likely converted to give the bio-oil yield, how much each of the constituents affects the different phases of the HTL, or what the optimum amount of constituents is to reduce the N content in the bio-oil. Such questions motivated us to study the HTL behavior of the model compounds representing the microalgae. A recent study used model compounds to model the formation of biocrude oil by HTL[22] and developed a model that predicts the biocrude yield. Our study used model compounds to model the formation of biocrude from algae, focusing on the effect of N content of the starting materials on the quality of the oil obtained.

Materials

To study the model compound behavior of the constituents in algae, we purchased the following model compounds: cellulose (acid washed) from Sigma Aldrich, cornstarch (Argo, 100% pure), sunflower seed oil, and dried egg white power were purchased from local grocery stores and distilled water was used. We carried out HTL experiments in a 0.5-L autoclave with constant stirring.

Method

We conducted hydrothermal liquefaction in 16 experiments and varied the compositions of protein, carbohydrate, and lipid (total mass of 40 g) at two different temperature levels (300 °C

[19] X.Z. Yuan *et al*., Energy & Fuels, 23 (2009) 3262–3267.
[20] Sato *et al*., I&EC, 43 (2004) 3217-22.
[21] Toor *et al*., Energy, 36 (2011) 2328-2342.
[22] G. Teri *et al*., Energy & Fuels, 28 (2014) 7501-7509.

and 350 °C) and at two different reaction times (10 min and 60 min) in a 0.5-Lstainless steel autoclave with 210 gm of DI water.

- Total mass of X+Y+Z=40 g. Additional DI water=210 g.
  - X: model of a protein (albumina)
  - Y: model of a carbohydrate (mixture of 50% cellulose and 50% corn starch)
  - Z: model of a lipid (sunflower oil)

Table 6 summarizes the different mixtures. We explored a low range of N content to simulate the feed going to the HTL2 after extraction of N-rich compounds.

Table 6. Model compound mixtures.

Composition	m_x / g	m_y / g	m_z / g
MC01	4	28	8
MC02	8	24	8
MC03	12	20	8
MC04	16	16	8

For reference, a protein content of 10 wt% corresponds to a N content of 1.6 wt%, and 40 wt% of protein corresponds to 6.4 wt%. The typical N content of solids going into HTL2 falls within this range.

Experimental Procedure

Desired composition of model compounds and 210 g of DI water were loaded into a 0.5-L autoclave. The system was purged using pure Ar gas for about 5 min. Once purging was done, the remaining gas was vented out. Then, the system was set on the desired operating conditions (temperature and rpm of the stirrer). Once the target temperature was reached, the reactor was maintained at the target temperature for the desired dwell time. Upon competition of the dwell time, a solenoid valve arrangement made to run cooling water through the reactor was turned on. This cooling water helped lower the reactor temperature. The head-space gas in the reactor vessel was collected through the sample outlet valve. Then, the reactor was opened to the bio-crude product and the water and solvent washes were done both in the reactor vessel and the head to ensure complete removal of solid/oil particulates.

Results

An early qualitative observation in the experiments with high amounts of carbohydrates (MC01) showed that more solids were produced compared to the algae grown in media with less carbohydrates. The amounts of solids produced were seen to decrease with decreasing carbohydrate content. The amount of charred content on the reactor walls followed the same trend.

Figure 26 shows the effect of N concentration in oil as a function of the percentage of protein in the starting mixture.

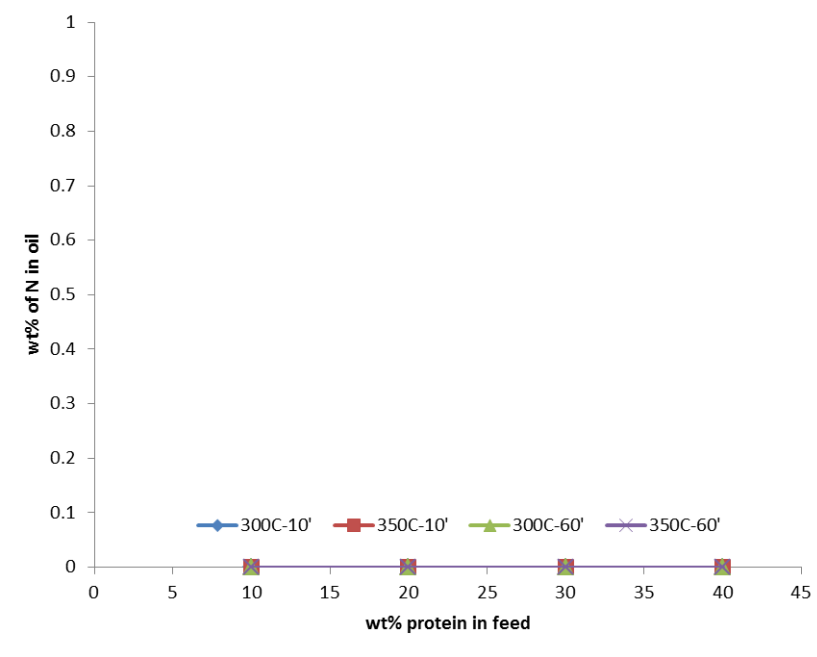

Figure 26. N concentration in oil after HTL treatment of model compound mixtures at two temperatures (300 °C and 350 °C) and two dwell times (15 minutes and 60 minutes).

In addition to the N content in oil, we performed mass balance to determine the distribution of the original biomass into different phases. Figures 27, 28 and 29 show the yields of oil, solids and water-soluble species as a function of model compound composition, temperature and time.

Figure 27 shows that the oil yield increases as the protein content in the model compound mixtures is increased or, in other words, as the amount of carbohydrates in the model compound mixtures decreases. In most cases, increasing the reaction time results in lower oil yields (except at 350 °C and the highest protein content). The formation of solids (Figure 28) follows opposite trends: more solids are obtained at low protein (high carbohydrate) contents in the model compound mixture, and at the same temperature more solids are formed at longer reaction times (especially true for high carbohydrate contents). The reported reactions of carbohydrates in HTL at these temperatures start with hydrolysis of the polymers into oligomers and eventually monomers, followed by recombination of monomers into larger molecules. If these molecules are small in size, they can become part of the bio-oil. However, when given sufficient time to react, they can form larger polymers that form the solid phase. The presence of proteins opens a door to possible reactions between amino acids and sugar monomers (i.e., the Maillard reaction) and we expected that the chemical pathways were more complex than those described above. We did not expect the oils in the model mixture to react with either proteins or sugars.

Figure 29 shows the yield of products in the aqueous phase. The curve for 10-min reaction time (at both temperatures) reflect a different trend than the curve for the 60-min reaction time. The first products have a minimum 20 wt% protein content, and the second products have a maximum of around 30 wt% protein.

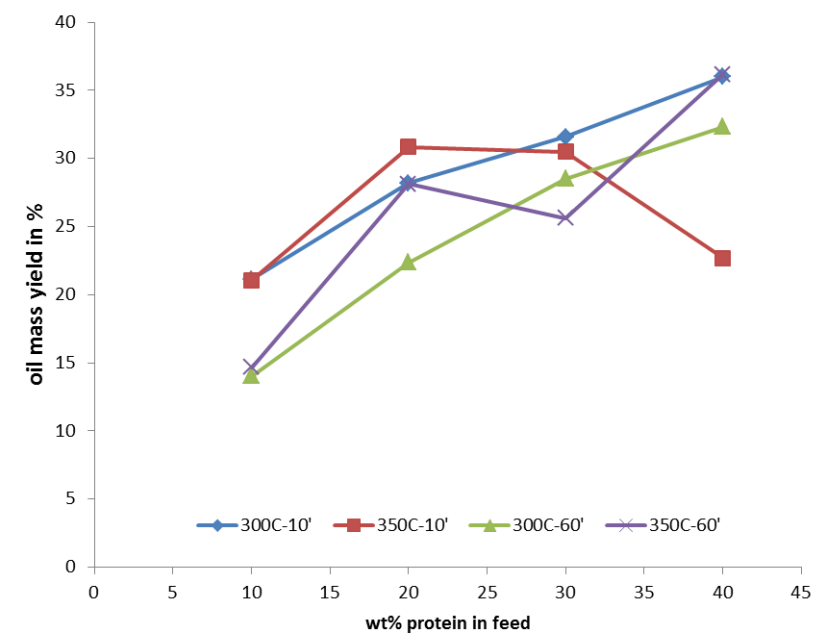

Figure 27. Oil yield after HTL treatment of model compound mixtures at two temperatures (300 °C and 350 °C) and two dwell times (15 and 60 min).

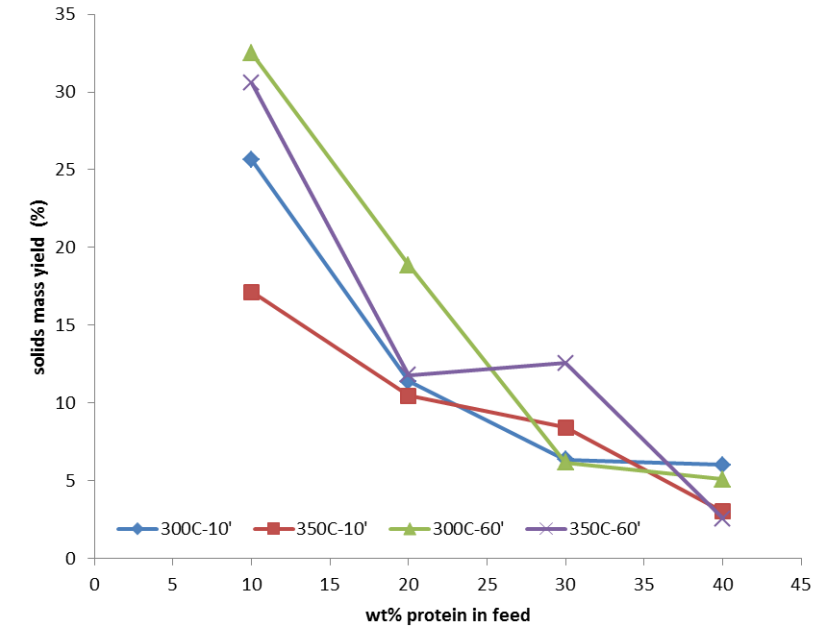

Figure 28. Yields of solids after HTL treatment of model compound mixtures at two temperatures (300 °C and 350 °C) and two dwell times (15 and 60 min).

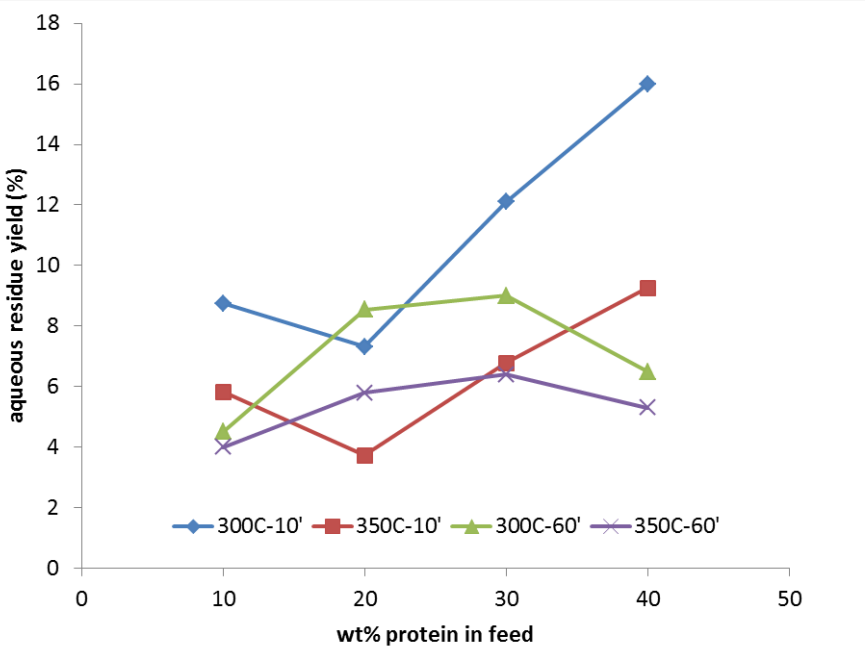

Figure 29. Yields of biomass in the aqueous phase after HTL treatment of model compound mixtures at two temperatures (300 °C and 350 °C) and two dwell times (15 and 60 min).

1.6 Phase separation

One of the tasks in this project that is important for industrial implementation of the technology, is to study separation of phases. In addition to separation of solids, it is important to be able to separate the organic phase (containing bio-oils) from the aqueous phase (containing valuable nutrients for algae growth). We first did a qualitative test to compare the effect of using a different type of solvent in HTL2 and completed two experiments in which the HTL1 step was done using the exact same conditions (composition, time, and temperature). Following this we changed only the type of solvent used: heptane in one experiment, dodecane in the other. After retrieving the products and collecting them in a flask, we observed a significant difference between them (Figure 30). When we used dodecane in HTL2, separation of the products was easy; when we used heptane, we could not distinguish the two phases because an emulsion formed.

Later, we expanded the scope of this study adding a third solvent (decane) and also studied the phase behavior of the emulsion at temperatures between room temperature and 95 °C.

Figure 30. View of products obtained in HTL2 when the solvent in this step was heptane (left), or dodecane (right).

Historically, SRI's previous projects with two-step HTL of algae have resulted in a number of separations issues, particularly after HTL2. A common occurrence was the formation of a "rag layer" between the oil and water phases that was characterized as a complex stable emulsion of oil, water, and solids. After centrifugation, the product was a thick, paste-like cake. This was especially troublesome since this emulsion layer contained a large percentage of the organic species and proved to be difficult to disrupt. The cause of the emulsion was not isolated.

Most current post-HTL separations at the bench scale are accomplished by either gravity separation or solvent extraction. Results have shown that additional oil recovery can be achieved by the addition of salts, suggesting that more efficient separations are possible (Jones *et al*? Pacific Northwest National Laboratory [PNNL-23227]).

A basic separator similar in concept to PNNL's inorganic settler/filtration unit can be built to investigate batch and continuous separation of post-HTL products. This is shown below in Figure 31, which shows a reconfigurable separator that has an artificially high aspect ratio. This allowed us to impose a thermal gradient along the length of the reactor, as well as sample multiple zones, similar to a fractionation column. Early experiments proposed for the reactor included: destabilizing emulsions via temperature, shear reduction, additives, etc.; testing various settling residence times; and various continuous solvent-contacting schemes. One potential trade-off concerned the phase behavior of the HTL products. At high temperatures, oil and water combine to make a single stable phase, which begins partitioning as it cools (Knezevic 2010). The rag layer formation (described above) may be retarded, and temperature may need to be optimized for efficient recovery of the oil phase.

Unresolved issues remain regarding how to efficiently transport hot (350 °C) biphasic flow from the stirred autoclave reactor to the column, how to modify the current setup or how to construct a new setup for continuous high-temperature operation and to monitor the relative levels of the various phases.

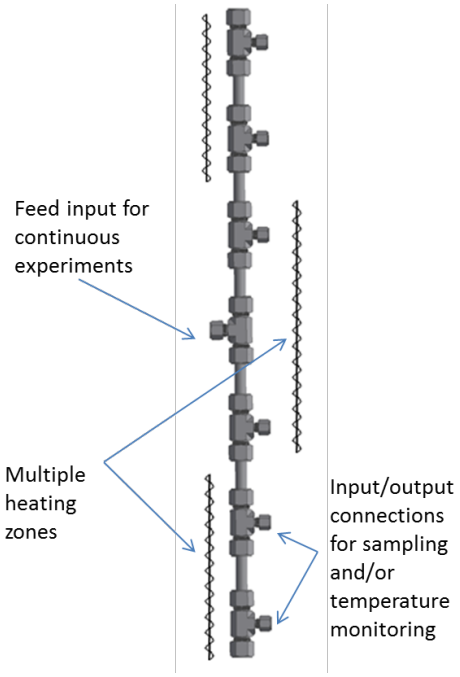

Figure 31. Schematic representation of the separator.

1.7 Parametric study of HTL1

We carried out a parametric study of HTL1, changing reaction temperature, dwell time, and the water:solvent ratio. The objective was to determine the effect of these parameters in the mass distribution between the several phases and its elemental composition.

The experimental design was as follows: the experiment consisted of 20 runs (full factorial design with one factor at five levels and two factors at two levels). Due to the large number of runs, no repetitions were carried out.

The procedure was:

- Load 15 g (on a dry basis) of wet algae into autoclave.
- Add DI water to reach a total amount of water required in that particular run.
- Add heptane to reach a total amount of water required in that particular run.
- Purge with Ar and close valves. Record starting pressure.
- Start stirring at 500 rpm and heat up to the target temperature (once temperature is within 5 °C of target temperature, ramp time is considered finished). During the heat up, enter a set point value higher than target temperature to reduce ramp time.
- Maintain target temperature the amount of time required in that particular run.

- Cool down to room temperature (expedite cooling by removing furnace). Once at room temperature, record end pressure.
- Purge reactor with Ar.
- Retrieve all material from autoclave. If needed, use water to recover material left on walls.
- Separate solids, water phase, and organic phase using standard procedures used.
- Wash solids from with heptane (2 x 25 ml). This heptane can be combined with the water phase previously separated.
- Wash solids from with DI water (2 x 50 ml). This water can be combined with the water phase previously separated.
- Dry aqueous phase, record weight, and do CHNS elemental analysis.
- Dry solids, record weight, and do CHNS elemental analysis.
- Rotovap heptane, record weight of organic phase, and do CHNS elemental analysis.
- If emulsion is formed, dry it and record weight.

The experimental design included three factors:

- Factor 1: temperature. Five levels: 190 °C, 200 °C, 210 °C, 220 °C, and 230 °C.
- Factor 2: dwell time. Two levels: 10 min and 30 min
- Factor 3: solvent (heptane). Two levels: low (42 mL heptane, 168 mL total water) and high (70 mL heptane, 140 mL total water). Note that the volume of water reported here is the total amount of water (water in the algae slurry + water added to the autoclave).

Due to the large number of runs, we did not do repetitions. This allowed us to explore a wider range of conditions at the expense of accuracy in the values obtained. The goal of this experiment was to serve as a general screening. Further experiments in a narrow range of interest would quantify the yields in detail.

The chemical analysis on mass balance between phases and available N/C ratios includes results shown in Figure 32, which depicts the bio-oil C yield in HTL1 alone (fraction of C in the biomass that ends up in bio-oil from the first HTL step). The general trend indicates that the C yield in HTL1 oil increases with temperature: from a yield around 5% at the lowest temperature to around 25% at the highest. The yield increase is probably caused by increased extraction efficiency at high temperature, which may be aided by destruction of structural elements in the cell. As described in a different section of this report, we started some tests to observe effect of operating conditions on algae morphology. At these low temperatures, it is not likely that formation of bio-oil is due to reaction between monomers obtained by hydrolysis of biomolecules. It is expected that the longer reaction time has a positive effect on the bio-oil C yield. The results in Figure 32 show that 10 minutes is too short a reaction time, whereas at 30 minutes the extraction of bio-oil is much higher. We did not observe a yield increase by working with high solvent:water ratios. We planned experiments with reaction times around 30 minutes to assess potential process optimization.

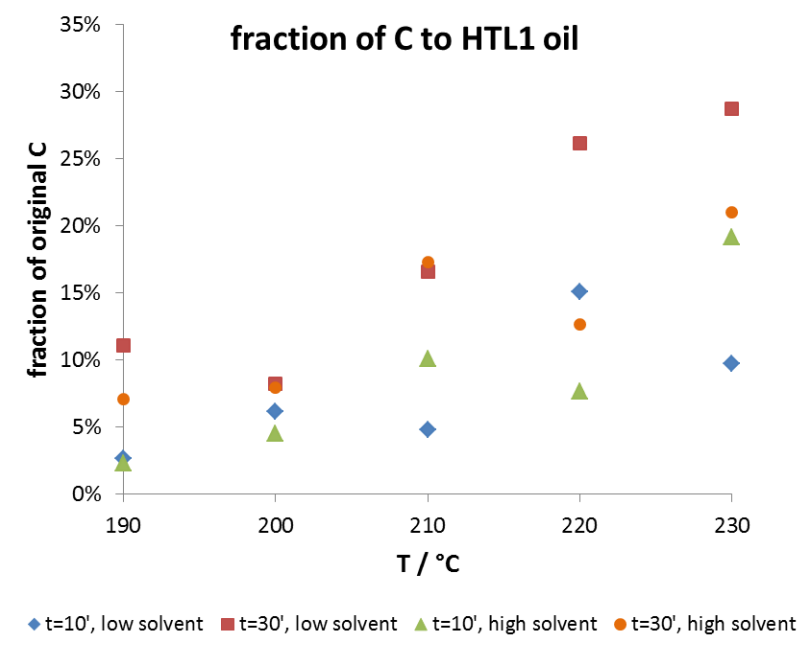

Figure 32. Fractions of algae carbon recovered as HTL1 oil for several temperatures, times, and water:solvent ratios.

Figure 33 depicts the fraction of algae C that remains in the solids after HTL1 and that can be converted to bio-oil in HTL2. The trend in this case is the opposite as the one in Figure 32 because, at higher temperatures, more C is extracted from the solids into the bio-oil. The solids treated at 190 °C contain 50-60% of the original C and, at higher temperatures, this percentage drops to around 35%.

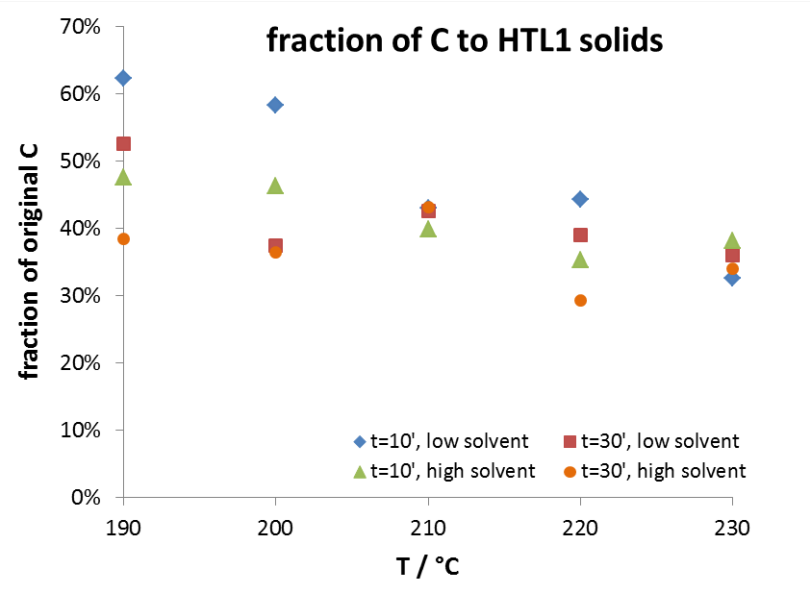

Figure 33. Fractions of algae carbon remaining in HTL1 solids for several temperatures, times, and water:solvent ratios.

Interestingly, the mass extracted into the aqueous by-products remained more or less constant for all temperatures (Figure 34). It therefore seems that by increasing the temperature in HTL1, it is possible to increase the bio-oil yield in this step without increasing the amount of C extracted into the aqueous byproducts. Elemental analysis of these phases is needed to validate this hypothesis.

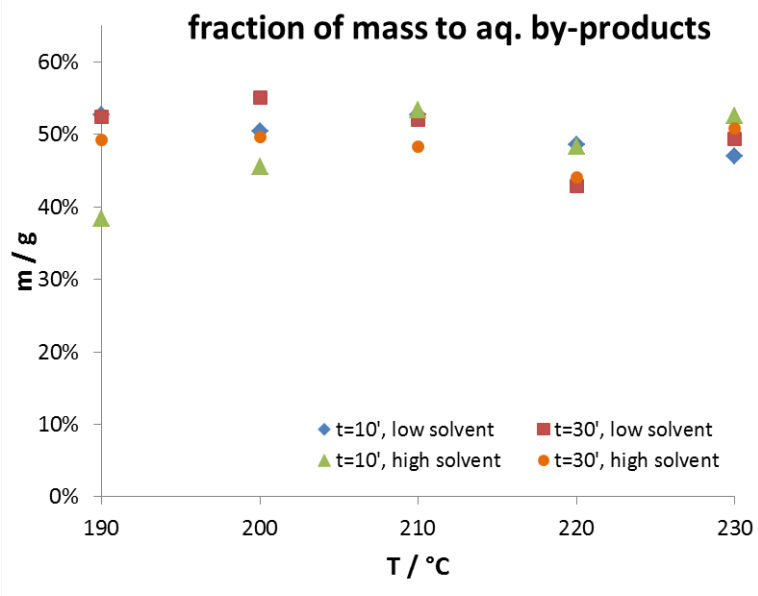

Figure 34. Fractions of biomass found as aqueous byproducts for several temperatures, times, and water:solvent ratios.

An effect of increasing the temperature is the enhancement of decarboxylation reactions that result in the formation of CO_2. Figure 35 shows the fraction of C in the original algae that was estimated to be from CO_2 gas. This calculation is based on the increase in pressure in the autoclave due to the HTL1 process. Pressure was measured before we started heating and again after the system cooled down to room temperature. Although in this set of runs we did not analyze the gas phase, in previous experiments done under similar conditions we found that the gas formed during HTL1 was virtually all CO_2 (CH_4 content was found to be <1 vol%). The results presented in Figure 35 show that by increasing the temperature, the percentage of C transformed into CO_2 in HTL1 increased from 1-2 % to 4-5%.

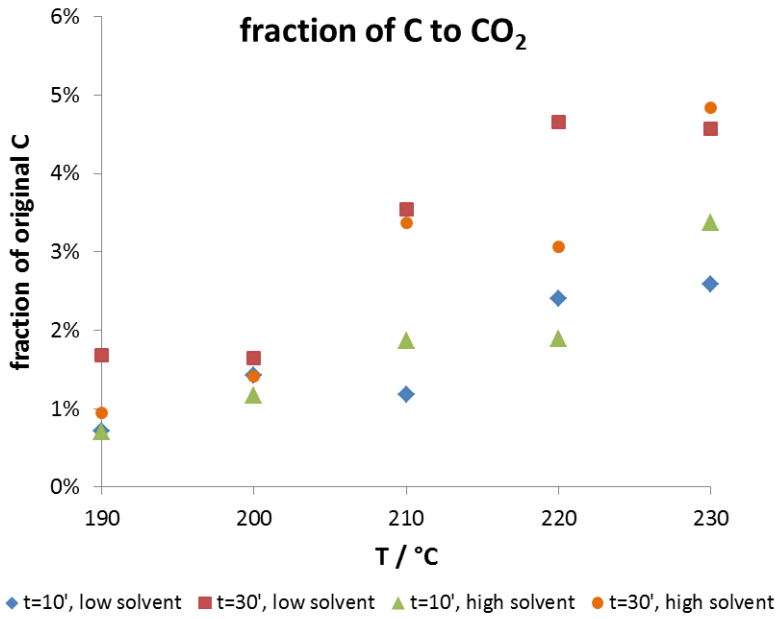

Figure 35. Fractions of algae carbon converted to CO_2 for several temperatures, times, and water:solvent ratios.

In addition to maximizing C yields, an objective of our project was to obtain oils with low N content. Table 7 presents the elemental composition (C, H, N, and S) from the oils extracted in this set of runs. Because bio-oil in HTL1 is obtained at much lower temperatures than conventional HTL processes, the oils from this first step have a low N content, typically well below 1 wt%. Sulfur content is even lower. Most of the oils have C content over 70 wt%, except for those extracted at the lowest temperatures. H content is typically above 11 wt%. From an elemental composition point of view, the quality of these oils meets the targets stated in this project.

The oils obtained in HTL1 are only a fraction of the products in our process. The solids remaining after HTL1 were treated at higher temperature in a second HTL step. The overall mass going into fuel production was, therefore the sum of oil and solids from HTL1. Figure 36 shows the N/C mass ratio of HTL1 oil+solids. At higher temperatures, the N/C ratio in the sum of products decreases from around 0.11 to around 0.08. When converted to atomic ratios, this translates to 1 N atom for 8 of C, and 1 N atom for 11 of C.

Table 7. Elemental analysis results for HTL1 oils produced under different experimental conditions.

run #	temperature	time	solvent	wt% C	wt% H	wt% N	wt% S
CH10-9	190	10	low	65.5	9.7	<0.1	0.1
CH10-2	200	10	low	72.8	10.9	0.1	<0.1
CH10-15	210	10	low	70.2	10.5	0.1	<0.1
CH10-3	220	10	low	74.9	11.2	0.5	<0.1
CH10-17	230	10	low	73.2	10.9	0.5	<0.1
CH10-4	190	30	low	72.6	11.0	<0.1	<0.1
CH10-12	200	30	low	73.5	10.9	0.3	<0.1
CH10-7	210	30	low	74.5	11.2	0.2	<0.1
CH10-19	220	30	low	74.6	11.2	0.7	0.2
CH10-1	230	30	low	76.3	11.1	1.0	0.3
CH10-14	190	10	high	63.9	9.6	0.3	<0.1
CH10-20	200	10	high	69.3	10.3	0.3	<0.1
CH10-18	210	10	high	73.7	11.2	0.4	<0.1
CH10-10	220	10	high	74.2	11.2	<0.1	<0.1
CH10-8	230	10	high	72.6	10.6	0.8	<0.1
CH10-11	190	30	high	74.5	11.3	0.3	<0.1
CH10-13	200	30	high	71.8	11.1	0.3	<0.1
CH10-5	210	30	high	74.8	11.4	<0.1	0.3
CH10-16	220	30	high	74.3	11.2	0.7	<0.1
CH10-6	230	30	high	74.2	11.0	0.9	0.2

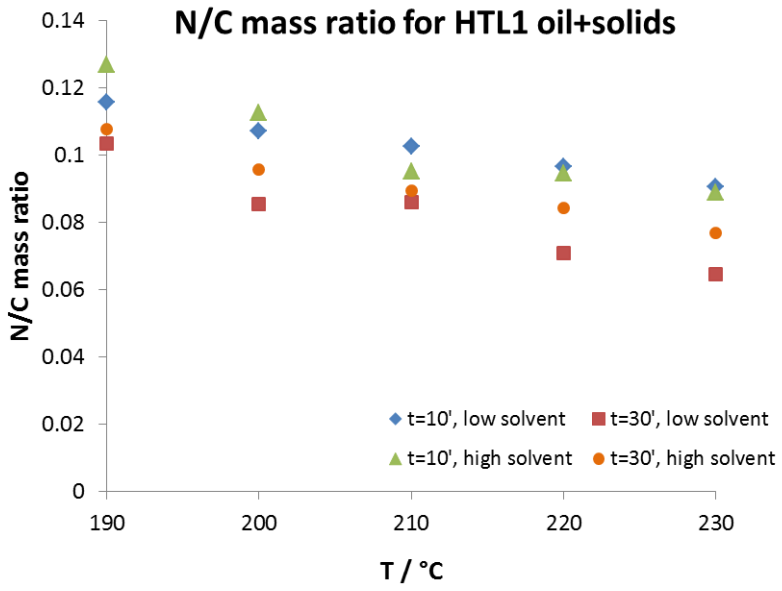

Figure 36. N/C mass ratio for the combined mass of oil and solids in HTL1 (usable phases for production of bio-oil). Results are for several temperatures, times, and water:solvent ratios.

The plan for Q1-2016 included a design of an experiment in which HTL1 was carried out at 220 °C for 30 minutes, and in HTL2 two factors are changed (keeping a reaction time of 15 min):

- Algae type (x3): *Scenedesmus dimorphus* grown in a greenhouse, mix of algae grown in wastewater, and dry *Chlorella*.
- Temperature (x2): 300 °C and 325 °C.

HTL experiments were done in a stainless steel autoclave with an internal volume of 0.5 liter that was equipped with a mechanical stirrer (Parr Instruments Moline, IL). For HTL1 experiments, the algae sources were aqueous slurries of *Scenedesmus dimorphous* obtained by partial dewatering (but not full drying) of algae cultures. This simulates a real biofuel production process and avoids potential disruption of cell walls caused by sample drying. The algae slurries were diluted with DI water and mixed with heptane. Some of the resulting mixture contained 15 g of algae (on a dry basis), 168 ml of water, and 42 ml of heptane (low solvent). Others contained 15 g of algae, 140 ml of water, and 70 ml of heptane (high solvent). By adding a solvent in HTL1, the least polar compounds present in the biomass could be extracted. Heptane was used as a model to simulate recycling of hydrocarbon products as solvents; since heptane has a low boiling point, it allowed easy separation of the biocrude oil by vacuum distillation. The reactor was torqued, purged twice with Ar, and then heated up to the target temperature with a rate of 7 °C/min by means of a resistive furnace; it was maintained at that temperature for a dwell time of either 10 or 30 minutes. At the end of the dwell time, the reactor was cooled down to room temperature (cooling rates for the first 100 °C were higher than 15 °C/min). During the heat up, dwell time, and cooling, the vessel contents were constantly mixed by a programmable mechanical stirrer. The resulting heterogeneous mixture was further processed to collect solid residue, oil, and aqueous byproduct. Solids were separated from the liquid phases by vacuum-assisted filtration, followed by washes with DI water and heptane, and finally dried in a convection oven at 55 °C. The filtrate was transferred to a separation funnel to separate the organic phase from the aqueous phase.

Elemental analysis for nitrogen, carbon, and hydrogen in solid samples was determined using an elemental analyzer (EA, Thermo Scientific Flash 1112) equipped with a thermal conductivity detector. A combustion temperature of 900 °C was achieved in a pre-packed combustion reactor containing chromium oxide and copper (Costech Analytical Technologies, Inc.); a downstream separation column was packed with HayeSep Q polymer with particle sizes in the range 60-80 mesh. Solid samples were ground into a homogeneous fine powder and weighed out into tin capsules (1-3 mg). The samples were run in duplicates. Calibration standards were analyzed daily. In order to determine the N content from non-volatile organic compounds (i.e., proteins and their derivatives) in the aqueous byproducts of HTL1, a known volume of liquid was transferred in a pre-weighed glass dish and the water was evaporated in a convection oven at 55 °C. After recording the residue weight, elemental analysis of the solids was performed using the CHNS analyzer as described above.

At the end of a HTL process there are four phases. First, an organic phase is formed by the bio-oil and, in our case, the solvent used for its extraction. Second, there are solids that are not soluble in organic solvents. At the moderate HTL1 conditions, these solids are algae remains that have not been hydrolyzed or extracted. Cell wall materials are a typical constituent of this phase. Since HTL1 soft solids can be partially converted in HTL2 to bio-oil, they are considered a

valuable product of HTL1. The third phase is formed by the aqueous by-products, which are water-soluble compounds formed mostly by hydrolysis of proteins and carbohydrates. Algae cultivation studies have shown that the C and N in this phase can be recycled for algae growth. Last, there is a gas phase, which is mostly CO_2 when the process is operated at moderate temperatures such as in the HTL1 step. The main goal of low-temperature HTL or HTL1 step is to extract polar nitrogenous compounds into the aqueous phase, so N atoms can be reused for algae growth and don't end up in bio-oil formed at the high-temperature step, or HTL2.

In order to evaluate the effect of temperature, time, and solvent content on the HTL1 process performance, we selected five response parameters: percentage of biomass C that ends up in bio-oil produced in HTL1 (C2O), percentage of biomass C that ends up in solids left after HTL1 (C2S), percentage of biomass C that is extracted in the aqueous by-products (C2W), N/C mass ratio in HTL1 products (NCP) and N/C mass ratio in HTL1 aqueous by-products (NCW). Note that HTL1 products are defined as the sum of bio-oil and solids; bio-oil is ready for hydrotreatment, and solids are treated in the high-temperature HTL2. Table 8 presents the results of an analysis of variance for each of these parameters. The results show that solvent content is the least important of the independent variables explored. On one hand, the p-values for all response parameters are higher than 0.01, which indicates there is less than a 99% confidence interval that the responses between different levels of the factor solvent are significantly different. Especially critical is the high p-value for C2O because extraction of oil is the main function of solvent. One the other hand, the sizes of effects (eta squared) are much lower than those for temperature and time. By contrast, temperature and time have small p-values for all response variables related to bio-oil and solids, and effect sizes that are much higher than those found for solvent. For responses related to the aqueous byproducts, there were no significant differences found for any of the three factors studied in this set of runs. Based on these results, discussion of data from HTL1 parametric study will be limited to the results for the low-solvent case, which is more desirable from a process economics point of view. In future studies, it would be interesting to explore higher algae-to-solvent ratios to determine the minimum amount of solvent required for effective extraction.

Table 8. Results on analysis of variance for five parameters in the HTL1 parametric study: p-values and eta squared (effect size).

	Temperature		Time		Solvent	
	Pr(>F)	^{2}	Pr(>F)	^{2}	Pr(>F)	^{2}
% of C to oil (C2O)	2.3E-06	5.6E-01	7.9E-05	2.8E-01	4.3E-01	5.7E-03
% of C to solids (C2S)	4.8E-11	6.7E-01	1.2E-08	2.7E-01	3.8E-02	9.2E-03
% of C to aqueous (C2W)	9.3E-01	5.1E-04	7.4E-01	7.1E-03	3.9E-01	4.9E-02
N/C in products (NCP)	4.0E-08	5.8E-01	1.4E-06	3.1E-01	2.9E-02	2.7E-02
N/C in aqueous (NCW)	2.1E-01	1.1E-01	4.0E-01	4.5E-02	4.6E-01	3.5E-02

Figure 37 shows the distribution of biomass C amongst the four phases after HTL1 treatment of algae slurries at different times and temperatures. For most experiments, the amount of C accounted for in the different phases fell between 80% and 90%. The remaining of the original C is likely lost during handling of the products (i.e., on walls of autoclave, or as volatile organics in the aqueous phase). The results in Figure 38 show a clear tendency toward increasing oil extraction at longer dwell times and high temperatures. Although increasing oil extraction at this stage is desirable, there are two factors to take into account. First, longer dwell times will result in higher capital costs due to larger size equipment needed for the same throughput. Second, as temperature increases, larger percentages of N are found in the recovered oils. Table 9 presents the elemental composition for oils obtained in HTL1 under different conditions. While oils obtained at the low end of the explored temperature range have N contents below or close to the detection limit of our instrument (0.1 wt% N), oils extracted at 200-230 °C have N contents in the range 0.5-1.0 wt%. The results clearly indicate that higher temperature and longer dwell times result in higher N content in oil. The mechanism for incorporation of N into algae-derived bio-oils is reported to start with the Maillard reaction between amino acids and monosaccharides produced by hydrolysis of proteins and sugars[23]. In the 190-230 °C temperature range, the rate of all processes increases with temperature. Table 9 also shows that, in general, bio-oils extracted in HTL1 have high C and H contents (>70 wt% and >10 wt%) and low sulfur contents.

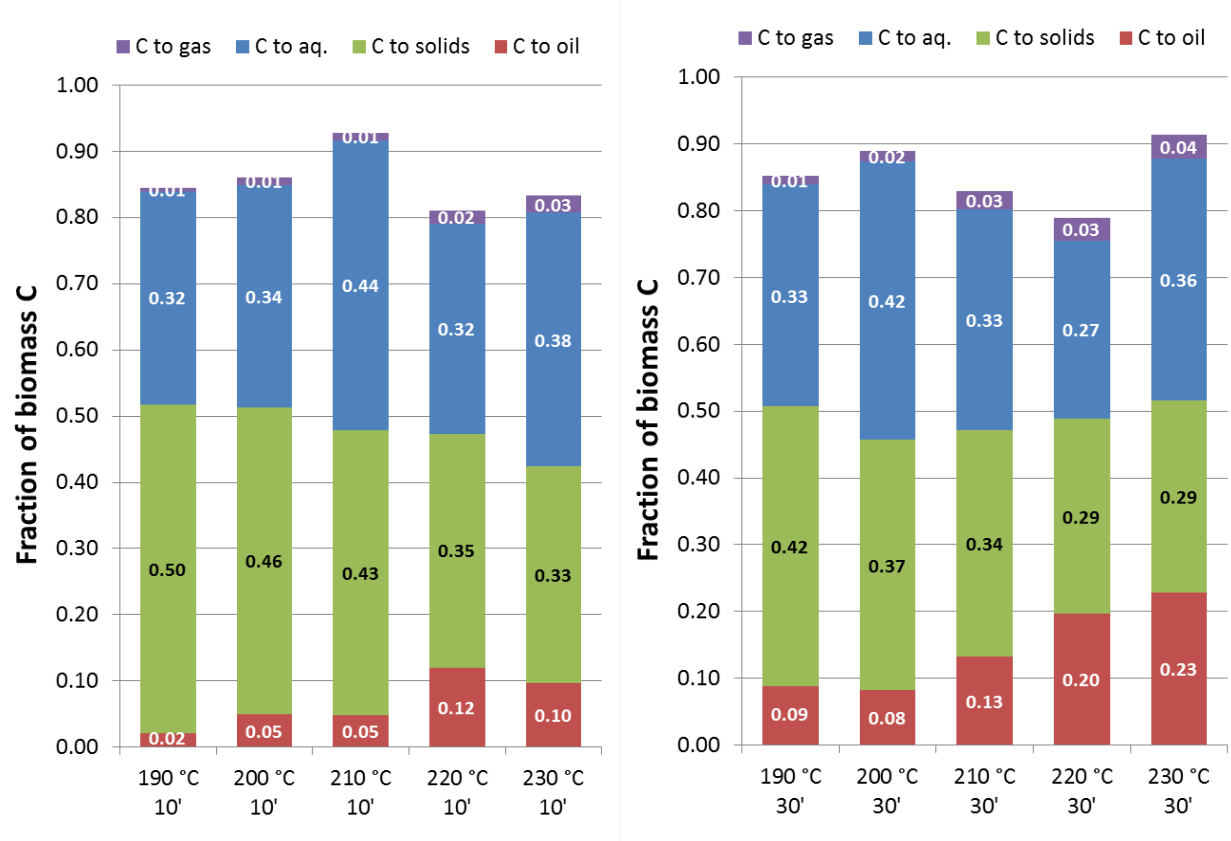

Figure 37. C distributions between phases in HTL1.

[23] T. Saqib *et al.*, Energy 36 (2011) 2328-2342

Table 9. Elemental analysis results for HTL1 oils produced under different experimental conditions

T / °C	t / min	solvent	wt% C	wt% H	wt% N	wt% S
190	10	low	65.5	9.7	<0.1	0.1
200	10	low	72.8	10.9	0.1	<0.1
210	10	low	70.2	10.5	0.1	<0.1
220	10	low	74.9	11.2	0.5	<0.1
230	10	low	73.2	10.9	0.5	<0.1
190	30	low	72.6	11.0	<0.1	<0.1
200	30	low	73.5	10.9	0.3	<0.1
210	30	low	74.5	11.2	0.2	<0.1
220	30	low	74.6	11.2	0.7	0.2
230	30	low	76.3	11.1	1.0	0.3

Figure 37 also shows the fraction of C remaining in the soft solids that are transferred to the high-temperature step (HTL2). In this case, the trend is opposite to that observed for the fraction of C going into oil. As time and temperature increase, the amount of C that remains in the solids decreases. This is to be expected based on the C-to-oil results because, as more C is extracted in the bio-oil, less remains in the solids. Interestingly, the sum of C going into oil and C remaining in the solids remained more or less constant, in the range 45-52% (except for a slightly lower value at 230 °C and 10 minutes). The fraction of C extracted in the aqueous phase did not show a particular correlation with reaction time or temperature. This suggests that the chemical processes responsible for transferring C into these phases have fast kinetics even in the lowest temperature explored. As explained above, the aqueous byproducts from HTL1 are a valuable stream that can be used as a source of N and C for algae growth. Figure 37 also shows that a small fraction of biomass C is converted to CO_2 due to decarboxylation of fatty acids. There is an increase in CO_2 formation at longer times and higher temperatures. This is of limited importance because it represents only a small fraction of the biomass C, and decarboxylation is bound to happen anyway during the high-temperature HTL2. In summary, if the goal of process optimization is to maximize C in the products (oil plus solids), the data summarized in Figure 37 does not show a clear benefit toward increasing dwell time or temperature. On the other hand, if extraction of oil at this step is desired, higher temperatures and longer dwell times should be used, taking into account the associated increase in N content in the oils.

After HTL1, the biomass is mainly distributed between products (bio-oil and soft solids) and soluble species in the aqueous by-products. As stated in the introduction, the main objective of the two-step HTL process is to obtain bio-oils with low N content. Ideally, the HTL1 step should extract as much of the N into the aqueous byproducts while limiting the amount of C co-extracted in this phase. Figure 38 compares N/C mass ratios of the biomass in each of these two groups. The N/C mass ratio in the biomass used in this study is also depicted. It is clear that the HTL1 step is effective in extracting N preferentially in the aqueous phase, producing products

with lower N/C ratios than the original biomass. Moreover, the data in Figure 38 shows that, in the studied range of temperatures, increasing the temperature results in products that have progressively lower N content. It is worth noting that a typical N/C mass ratio in proteins is around 0.31, which is close to the values found for the aqueous phase residues after HTL1 extraction at temperatures above 210 °C. Thus, further optimization of this step is unlikely to yield significantly improved results.

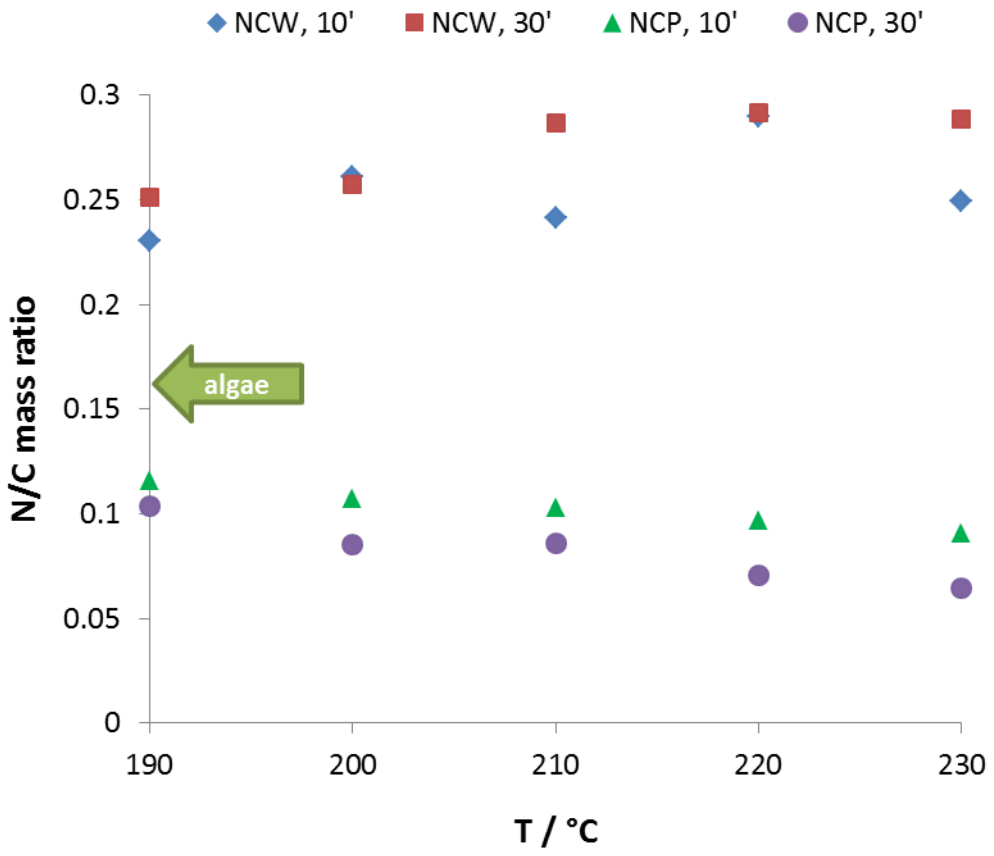

Figure 38. N/C mass ratios in HTL1 products (NCP) and N/C mass ratio in HTL1 aqueous byproducts (NCW) at 2:1 water:solvent ratio and two different reaction times: 10 and 30 min.

1.8 Effect of HTL1 conditions on algae morphology

The low-temperature step (HTL1) of SRI's two-step HTL process serves multiple purposes. It extracts the lipid content of the algae to protect it from the high-temperature step (HTL2), it allows for separation of water-soluble nitrogenous compounds, and it begins the production of some of the useful HTL products. For the algae entering HTL1, no additional processing is done beyond concentrating the incoming material to a slurry with approximately 10 wt% solids. Purely solvent-based Soxhlet extraction techniques have relatively low oil yields, especially when compared to mechanical and supercritical CO_2 techniques[24]. In HTL1, we hypothesize that the algae cells are undergoing thermal lysis at these conditions, which has been reported in the

[24] P. Mercer *et. al*., European Journal of Lipid Science and Technology, 113 (2011) 539-547.

literature to have excellent disruption characteristics as compared to mechanical, microwave, and laser treatment[25].

To date, few studies have been performed to examine morphological changes at the cellular level of algae after exposure to hydrothermal liquefaction conditions[26] [27]. Having physical evidence of the disruption or decomposition will aid the optimization of HTL1. Also, our current autoclave-type setup for doing batch experiments—while allowing for study of a wide range of pressures, temperatures, mixing, and composition characteristics—does not allow for rapid heat up. The relatively slow heating rate prevents us from accurately assessing the effect of temperature at short timescales. For transitioning our process to continuous operation for more commercially relevant scales, the required residence time is a key factor in managing capital costs of reactors. Therefore, the study was aimed at informing both our understanding of the underlying physical phenomena in HTL1 and the optimization of reaction methodology for current and future operations. In this study, temperature, reaction time, and solvent loadings were examined.

Experimental

The apparatus used for these experiments consisted of a Techne SBS-4 fluidized sandbath (50-600°C) and several 15-mL tubing reactors (Swagelok ½" OD 316SS tubing, 0.049" wall) with provision for remote handling of up to six reactors simultaneously. The stainless steel tubing reactors are purged with argon prior to each run and checked for leak tightness by adding positive pressure of inert gas and immersion in soap solution. A calibration reactor was also fabricated with a high-temperature thermocouple (ceramic connector, type K) and pressure transducer (0-5000 psig) to allow for accurate assessment of the temperature and pressure profiles during a run. After the heating step of each reaction was completed, the reactors were quenched in a water-ice bath until the internal temperature of each reactor was below the ambient temperature. After collecting the products in a vial, they were then stored in a refrigerator at 40°F. No attempts to recover additional material from the tubing walls with solvent were made.

This first series of runs was designed to approximate reaction conditions similar to previous studies while also examining the effect of faster heating rates not currently possible with the current autoclave equipment. However, due to the small working volume of each tubing reactor, only a small amount of algal material could be reacted in each tube. Previous attempts at combining multiple tubes into a single batch for workup resulted in poor reproducibility due to the eventual failure of the pressure seals on the tubing compression fittings and the difficulties of extracting all the material due to the high surface-to-reactant ratio. In this experiment, tubing reactors were used a minimum of times and data points were discarded if the reactor showed signs of leakage during the run. Another important point is that these reactors were not agitated in the same manner as the stirred autoclaves, so it was important to establish a baseline compared to the autoclave experiments.

The initial reaction conditions were based on a parallel study done in the 0.5-L, CH10 (190-230°C, 10-30 min, 1:2 - 1:5 solvent:water loading). In this case, a larger initial bracket was chosen for temperature since we could more rapidly iterate on these experiments. The parameters

[25] J. R. McMillan *et. al.,* Applied Energy, 103 (2013) 128-134.
[26] C. Jazrawi *et. al.*, Algal Research, 8 (2015) 15-22.
[27] L. Garcia Alba *et. al.,* Chemical Engineering Journal, 228 (2013) 214-223.

for this first study were: 150-250°C. 5-25 min. 1:2-1:5 solvent:water loading. The solvent was heptane.

Results

To prepare for analysis via scanning electron microscopy (SEM), a small amount of the wet solids from each sample needed to be dried and fixated on SEM mounts. A standard method for preparing delicate samples is critical point drying, CPD, (Botes 2002). However, in the absence of that particular apparatus on site, we investigated alternative processes that could be performed with available materials and equipment. To start, we used oven drying and lyophilization (freeze drying) on small aliquots of the solid slurry.

Oven drying is known to produce undesirable results, particularly with the starting algae material. The high heat and large surface tension effects as the water in the slurry evaporates can cause major disruption of the cell walls. Although the heat effects can be mitigated by using vacuum ovens and lower temperatures, the surface tension effects remain, which is why CPD is typically used. In CPD, the water is replaced by supercritical CO_2 and then forced to undergo a vapor-phase transition. Since the surface tension of a supercritical fluid is zero, when the CO_2 turns into a vapor and ultimately leaves the cell, the morphology of the cell is essentially undisturbed. In lieu of purchasing a critical point dryer, we decided to investigate lyophilization and chemical methods, which utilize similar physical phenomena to more gently dry substances for SEM.

First, a sample of the fresh starting algae slurry was vacuum-oven dried at 60°C for 24 hours. A small amount of this material was then mounted on carbon tape and gold-sputtered. A similar mounting procedure was used for all samples after drying. Micrographs of this material are shown below in Figure 39.

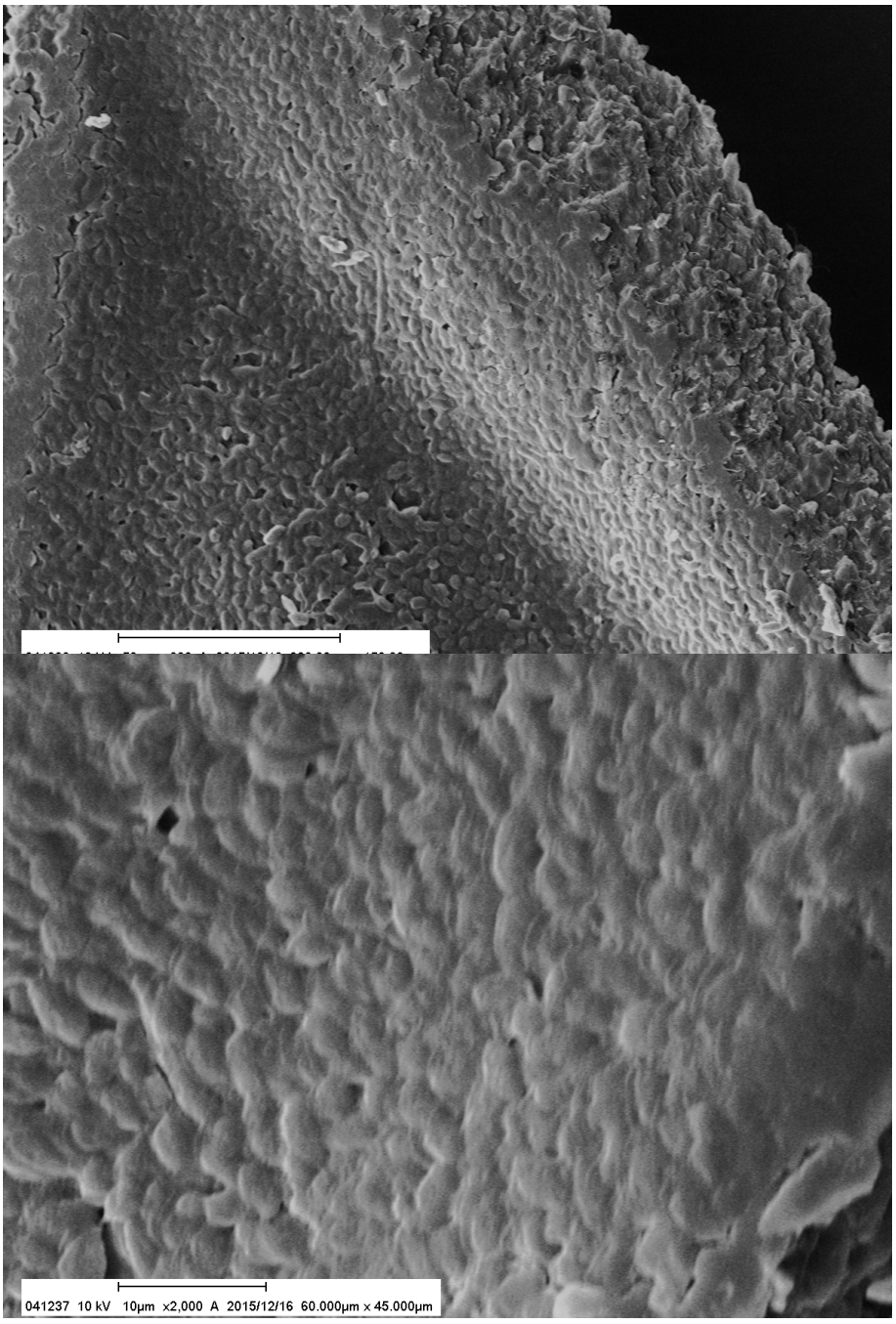

Figure 39. SEM micrographs of oven-dried starting material. Top: 600x magnification, bottom: 2000x magnification.

The oven-dried material formed large clumps with highly distorted material. It was very difficult to differentiate between individual cells at high magnification. Although it was possible the solids heated in excess of the oven drying temperature would not be further disrupted, the surface tension issues and resulting clumping precluded the use of any oven drying for SEM samples.

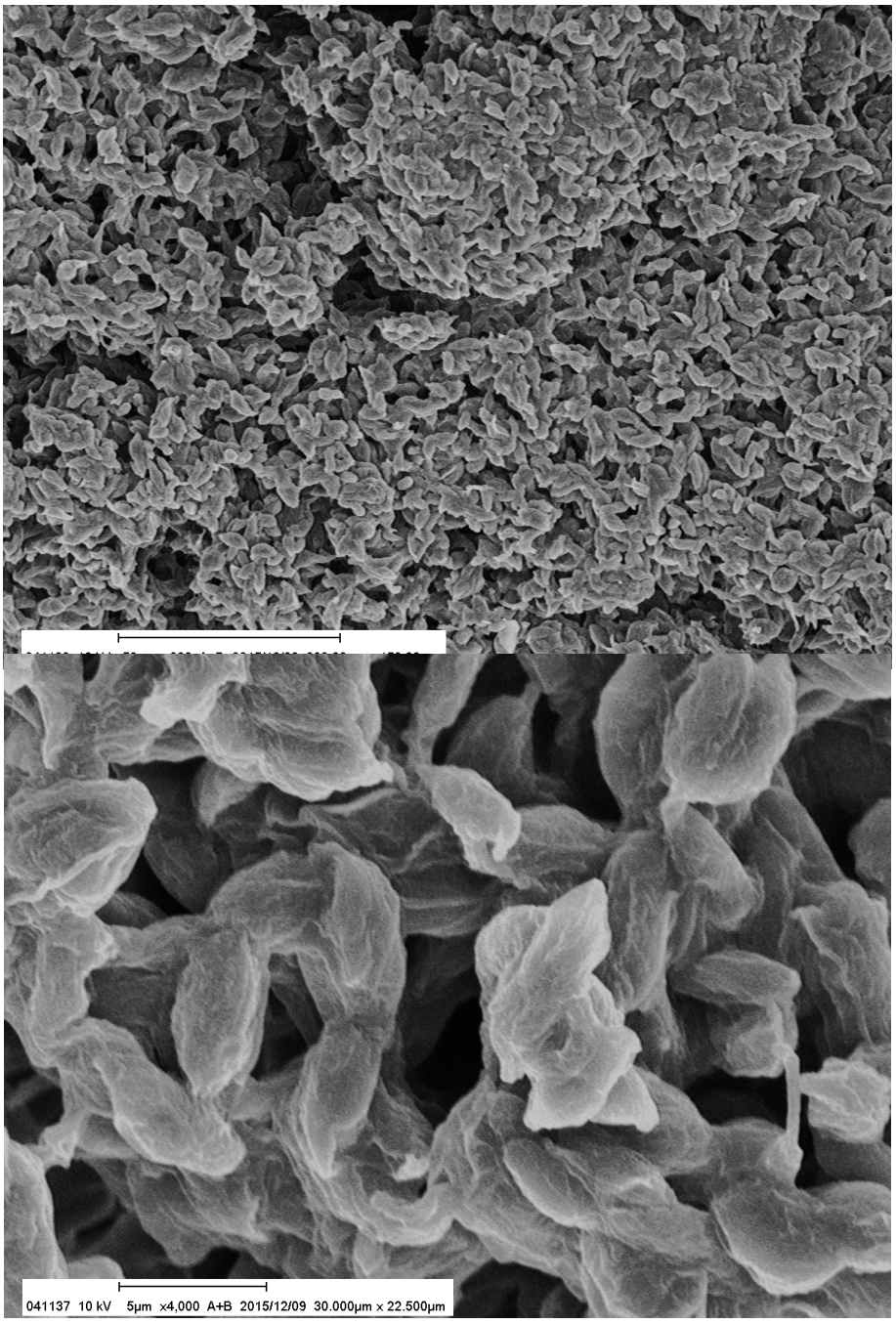

Figure 40. SEM micrographs of lyophilized starting material. Top: 600x magnification, bottom: 4000x magnification.

In comparison, the lyophilized material shown in Figure 40 showed much more distinct cell structure. The overall shape is consistent with optical micrographs we took of live specimens, as shown in Figure 41. However, there does seem to be some distortion of the specimens as well as some "melding" of cells that were side-by-side. The clumping and mild distortion were unresolved issues.

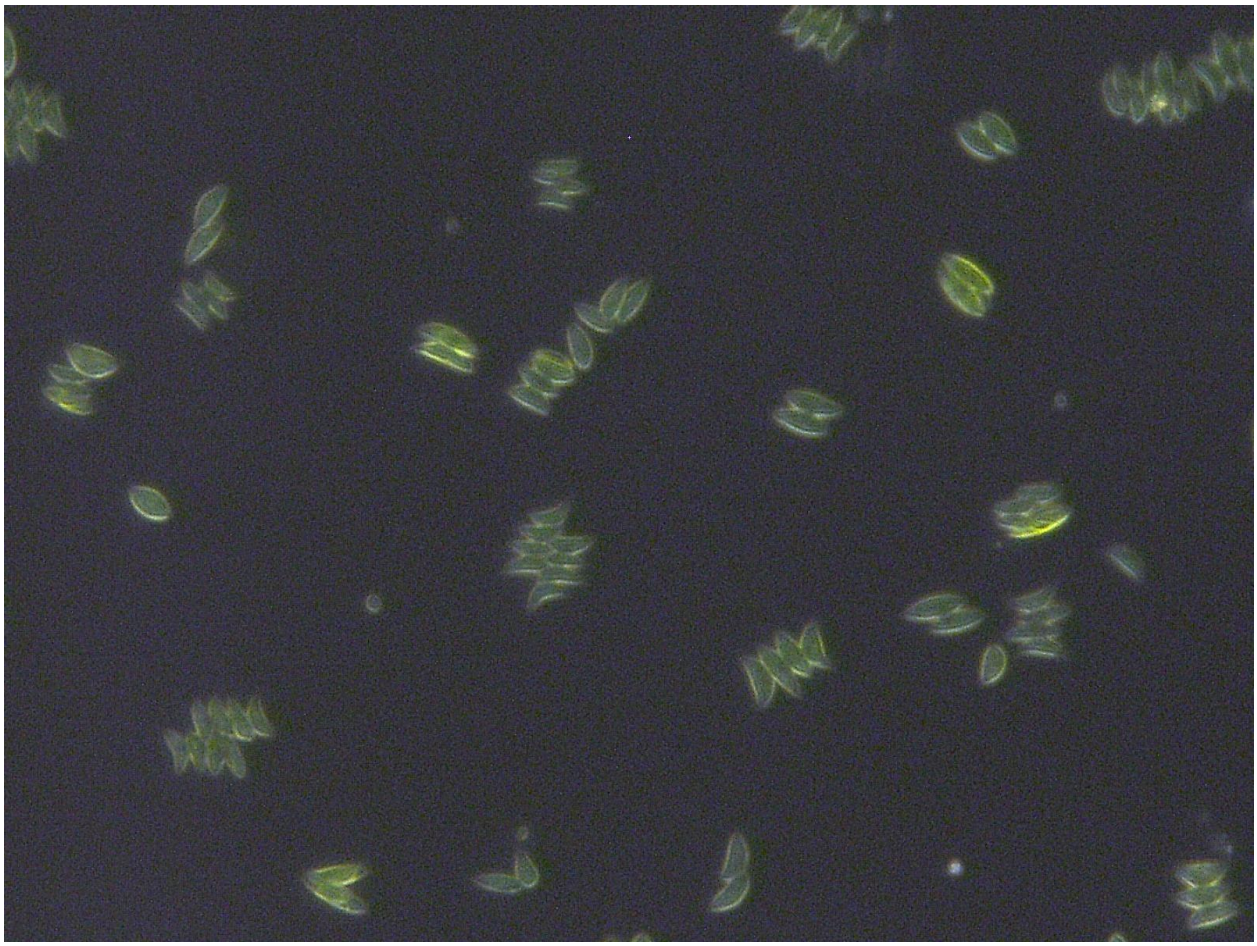

Figure 41. Optical micrographs of a live sample of *Scendesmus dimorphus* at 1000x magnification.

For further fixation, we investigated various chemical methods (Jung 2010) more suitable for our samples. In the meantime, we also prepared additional lyophilized samples for SEM of the HTL1 samples for qualitative analysis. In an attempt to counteract the clumping issue, we tried lyophilizing a dilute suspension of the sample onto a glass slide to encourage more even dispersion of the cell clumps (Figures 42 and 43).

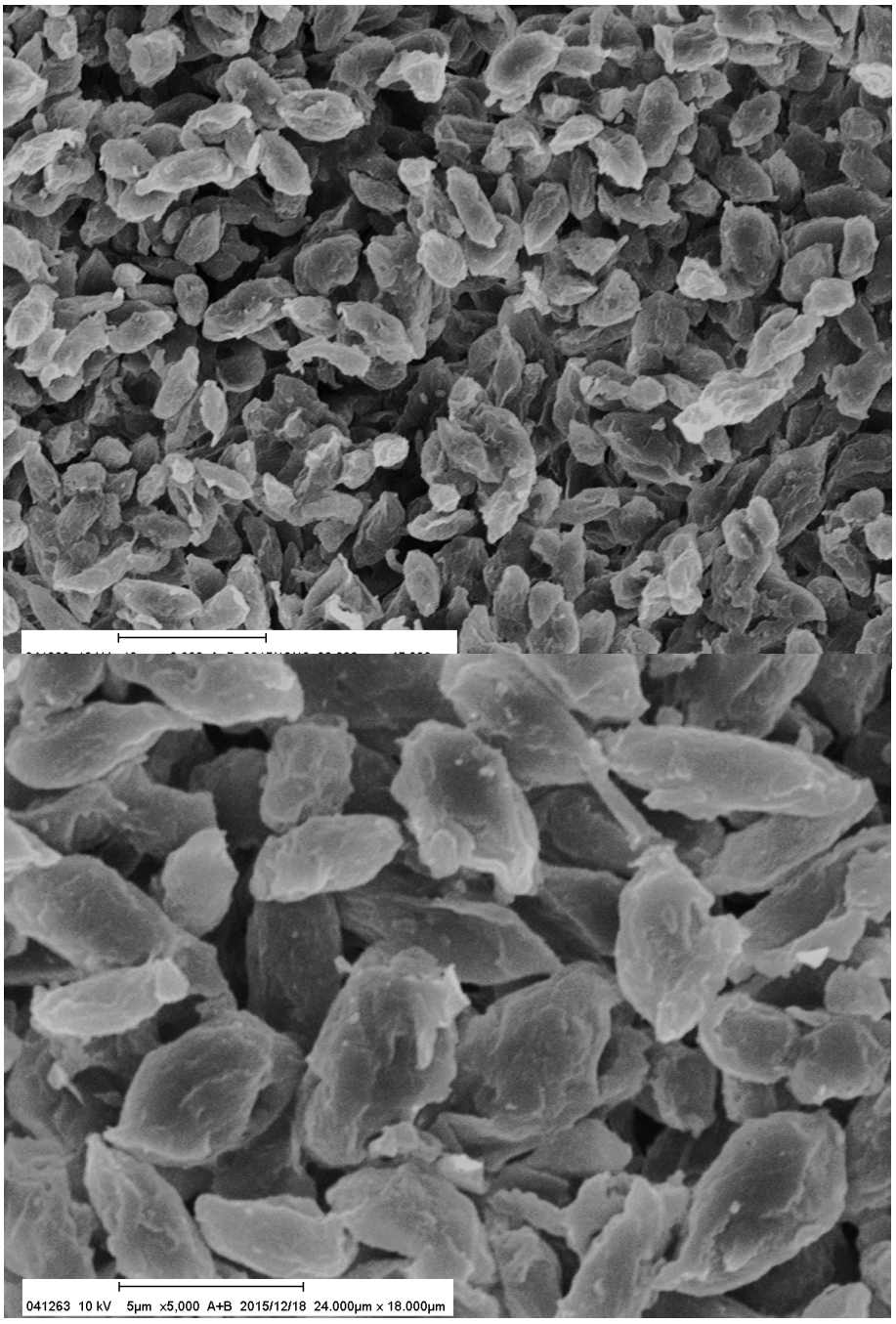

Figure 42. SEM micrographs of lyophilized HTL1 sample (150°C, 5 min, no solvent). Top: 2000x magnification, bottom: 5000x magnification.

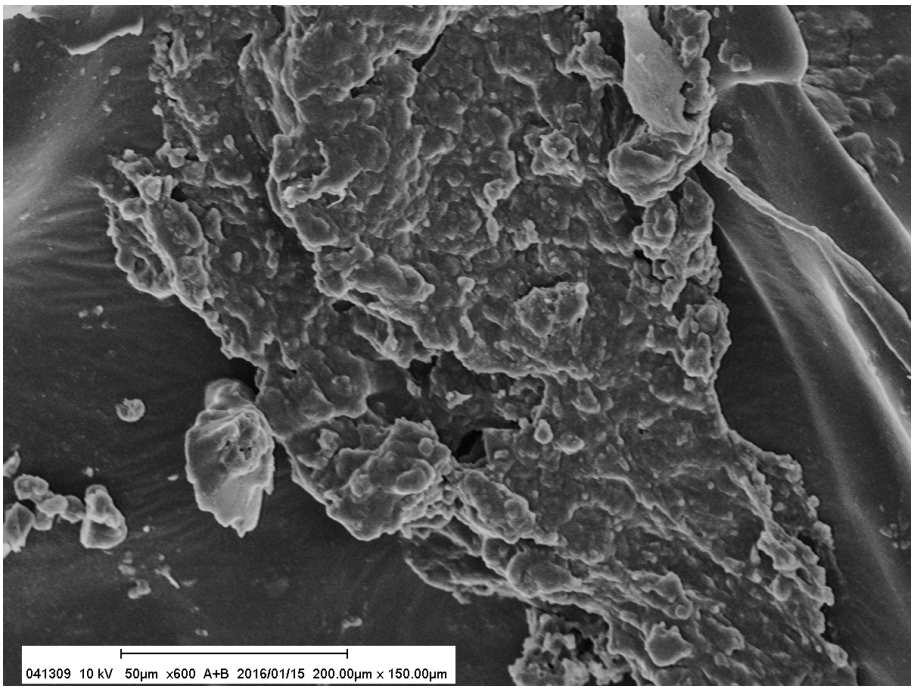

Figure 43. SEM micrographs of lyophilized HTL1 material (250°C, 15 min, no solvent), 600x magnification.

In the 150°C/5-min experiment, we can see that the algal cells seem to have largely the same appearance as the starting material; however, in the 250°C/15-min experiment the cells began to completely disintegrate into a non-discrete mass.

1.9 Initial hydroprocessing of HTL1 biocrude oil

The purpose of this study was to provide an initial assessment of the processability of a biocrude oil produced by SRI International's HTL1 low-temperature reactor. This included processing a sample of the HTL1 biocrude under nominal conditions over a commercial hydrotreating catalyst and observing the conversion of the feed to products so that we could begin defining the appropriate parameter space for this feed material.

For this initial study, SRI provided UDRI with approximately 39.6 g of an HTL1 product. This was diluted with 354.5 g of n-heptane, giving a total volume of approximately 500 mL. The catalyst chosen for this initial study was a sulfide NiMo/alumina catalyst from Albemarle (KF-860). This catalyst was chosen based on previous experience with similar catalysts for heteroatom removal from heavy hydrocarbon liquids. The reactor used for this study was a down-flow trickle-bed reactor similar to the unit shown in Figures 44 and 45.

A general schematic of the reactor system used for this program is shown in Figure 44, and a photograph is shown in Figure 45. Note that the reactor may be fitted with different inlet gases and product tanks depending on the specific configuration system and includes separate gas and liquid feeds with heated transfer lines, a heated catalyst reactor, heated product collection tanks, and a gaseous vent that can be monitored with various online and offline analyzers.

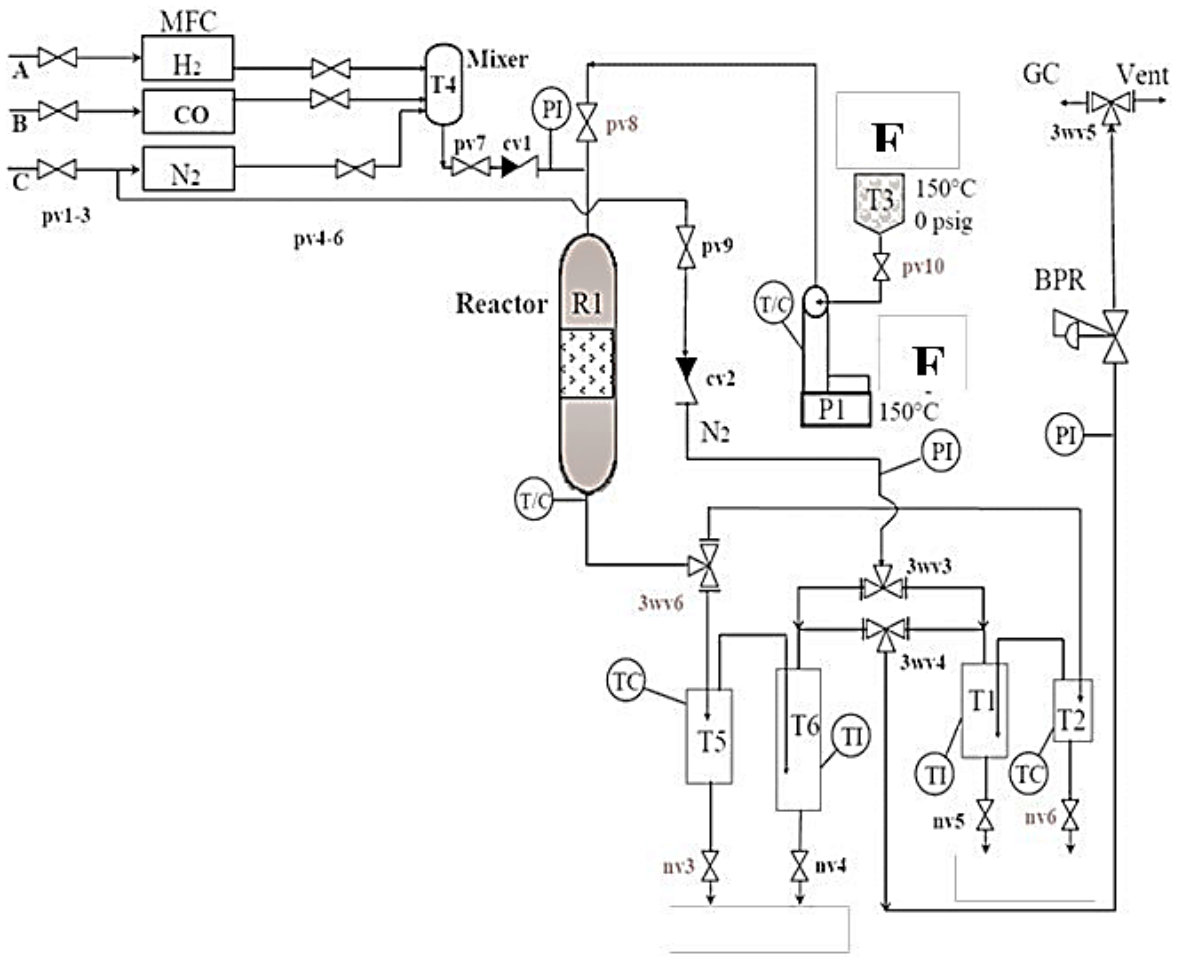

Figure 44. General schematic of the trickle-bed catalyst reactor.

The reactor used in this study was a 1/4" diameter by 24.5" long 316 stainless steel tube vertically oriented inside a three-zone furnace (Applied Testing Systems, Inc.). The temperature of the 6" central heating zone was controlled independently through thermocouples that are spot welded to the exterior of the reactor tube at the center of each heated zone. The length of the tube within the furnace was wrapped with aluminum foil to assist in evenly distributing the heating loads. Reactor pressure was controlled by a back- pressure regulator downstream of the product receiver. Gases were supplied from regulated cylinders that were also used to pressurize the system. Gas flow rates were precisely controlled with 5850i Brooks mass flow controllers. Liquid feeds could be heated to avoid crystallization in a 1-L feed tank and charged into a heated ISCO-500D syringe pump. The liquid and gas feeds combined at the top of the fixed-bed column and mixed while flowing through approximately 6" of 54-mesh silicon carbide before contacting the catalyst-containing portion of the bed. For the 1/4" diameter reactor tube, catalysts could be used as the crushed and sieved extrudates or, more commonly, the crushed extrudates diluted with silicon carbide. (Diluting the catalyst provides superior temperature control in the presence of strongly exo- or endothermic reactions and reduces channeling through the bed.) For our study, the catalyst was crushed, sized, and diluted 1:1 with silicon carbide. The lower section of

the reactor was similar to the inlet section with a volume of 54 mesh silicon carbide resting on a plug of quartz wool on a 20-µm sintered stainless steel filter at the exit of the reactor tube.

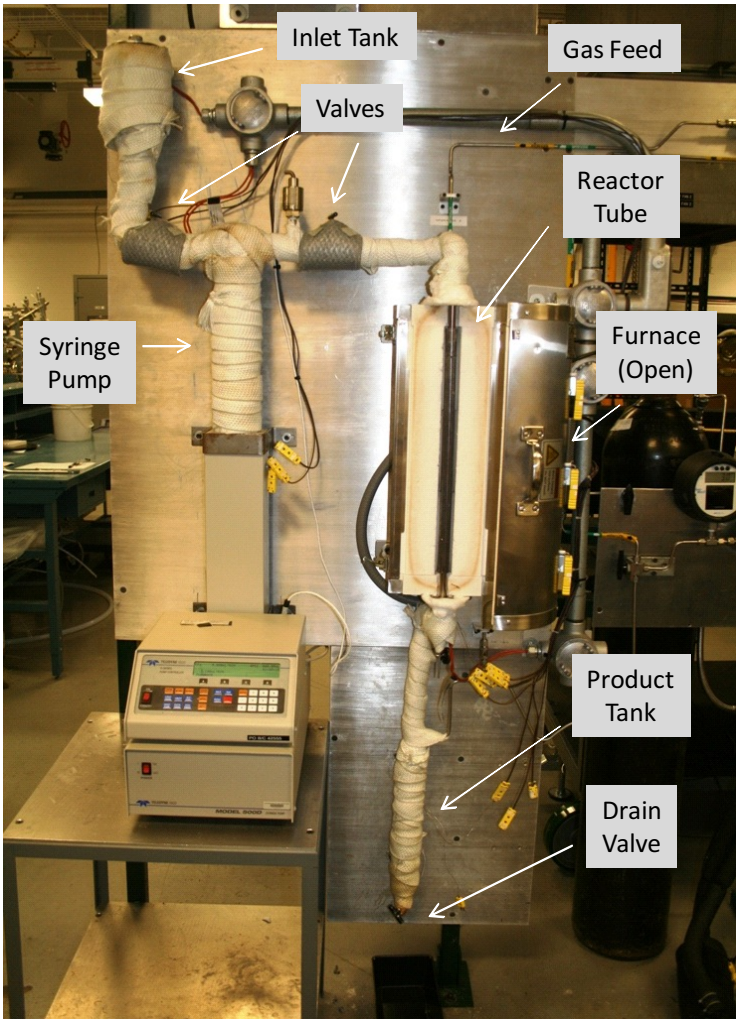

Figure 45. Photograph of an example reactor configured for heteroatom removal.

For this study, the KF-860 NiMo catalyst was sulfided after the reactor was prepared and loaded with catalyst and silicon carbide. Briefly, the reactor was mounted in a horizontal furnace, supplied with a flowing mixture of hydrogen and hydrogen sulfide at atmospheric pressure, and heated gradually over 2 hours to 350 °C, held for 2 hours, cooled to room temperature, and then capped for transfer to the reactor furnace.

For the initial test with the HTL1 biocrude, the reactor was heated to 350 °C and supplied with hydrogen at 900 psi at a targeted 71.2 mL/min. (Molar ratio of H2:feed = 20:1, assuming feed density is 0.9 g/mL and feed MW = 226 g/mol.) The HTL1 biocrude was fed at a rate of 0.04 mL/min, giving a liquid hourly space velocity (LHSV) of 2/hr. The initial product from the reactor appeared clean and clear. The HTL1 biocrude feed rate was then changed to 0.02 mL/min, giving an LHSV of 1/hr. The initial product from these conditions appeared yellow and clear. However, after approximately 48 hours on-stream, an uncontrolled pressure rise was

noted in the feed pump, indicating a blockage in the feed line. Heating the feed line to 90 °C had no effect, so the run was stopped and the reactor removed for inspection. The feed tube to the reactor was found to be plugged with a dark, solid mass starting at the point where the feed entered the first heated zone of the reactor furnace. The entrance to the catalyst bed was found to be filled with a similar dark mass. This was thought to be the product of a polymerization reaction of the polymer feed as it was heated from ambient to 350 °C at the entrance to the reactor. Since it was clear that the HTL1 biocrude was not being quantitatively transported through the reactor, it was not likely that the collected product reflected the true conversion of the feed: thus, it was considered invalid for the purposes of evaluation and analysis.

TGA and differential scanning calorimetry (DSC) analyses of the HTL1 biocrude and the material collected from the inlet zone of the reactor showed a small exotherm (possibly indicating the onset of polymerization) starting at approximately 300 °C. Based on this observation, a second attempt to process the HTL1 biocrude was made using a reactor temperature of 280 °C at 900 psi with an LHSV of 1/hr. Under these conditions, the reactor pressure remained stable and the initial was clear and lightly colored as shown in Figure 46. However, after ~27 hours of operation, the product turned quite dark, suggesting a sharp decline in catalyst activity. To determine whether the catalyst activity could be recovered by increasing the temperature, the reactor was operated for 24 hours at 300 °C. This gave only a slight visual improvement in the appearance of the product. The run was ended, and the reactor was dismantled to inspect the feed inlet line and the entrance to the reactor. Both were found to be clean and clear and showed no signs of the blockage observed at 350 °C.

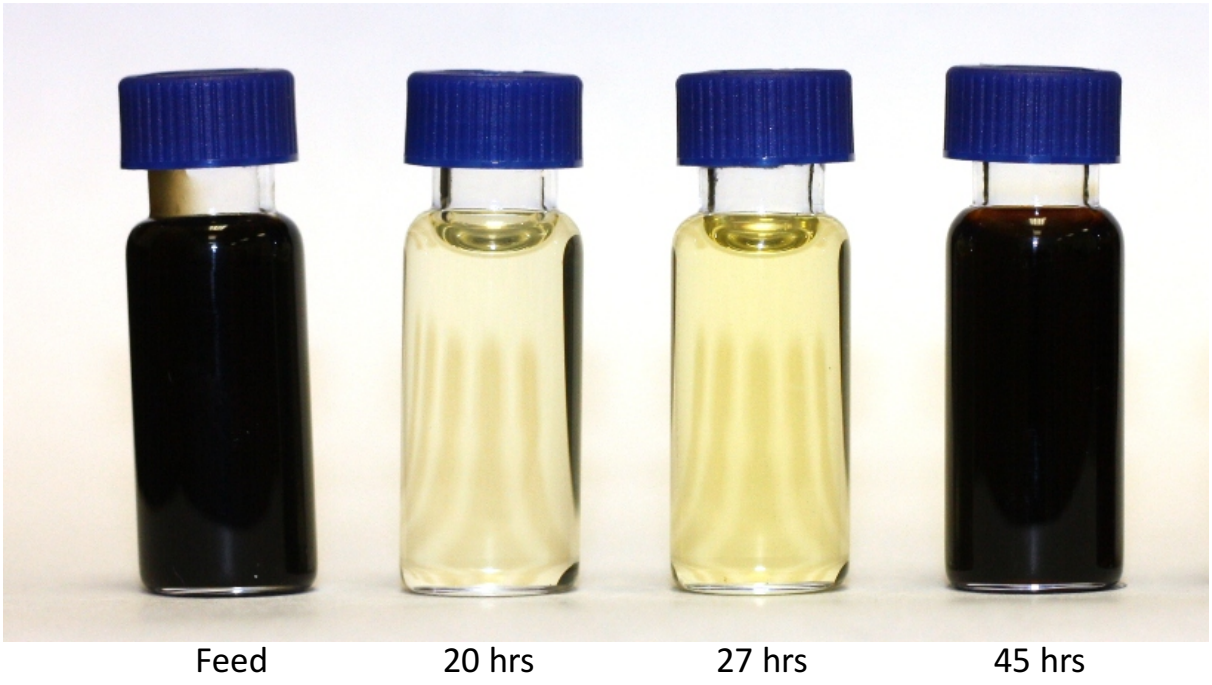

Figure 46. Sample of the HTL1 biocrude feed and the hydrotreating reactor product along with the reactor temperature and time on-stream at 280 °C with 900 psi hydrogen and an LHSV of 1/hr.

Figure 47 summarizes the GC-MS analysis of the HTL1 biocrude feed and products with the sulfided KF-860 NiMo at 280°C and 900 psi hydrogen and 1/hr LHSV. This illustrates that the initial catalyst activity is quite high giving a product that is predominantly within the carbon range of jet turbine fuel (C8-C18) and consisting of linear alkanes that are suitable as a jet fuel blending stock. However, as the catalyst activity declines a reduction in the overall yield of the normal alkanes is noted along with an increase in the production of alkenes and high molecular weight alkanes. There may also be components present in the later product that are too heavy for analysis by GC-MS.

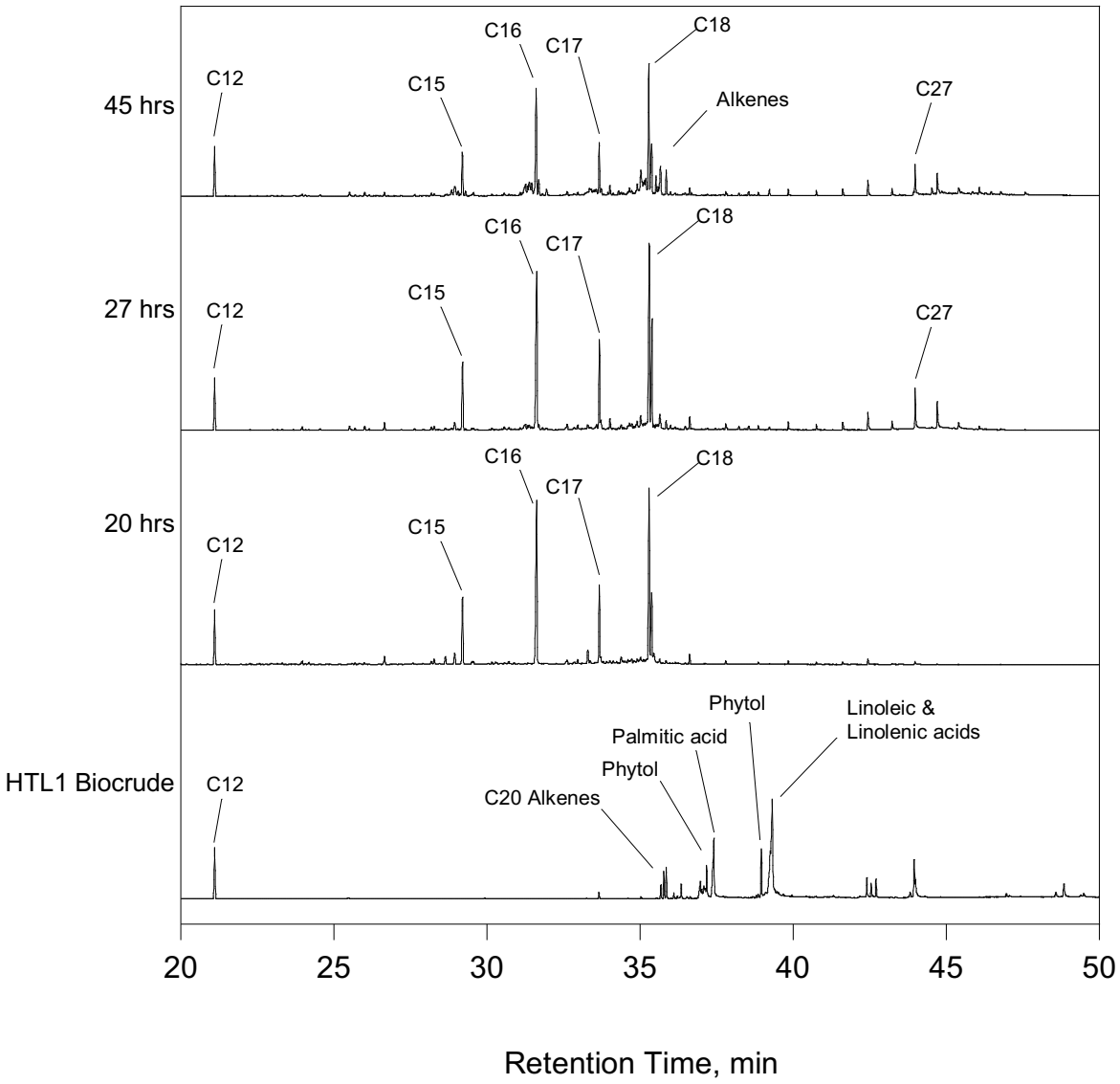

Figure 47. Summary of the GC-MS analysis of the HTL1 biocrude feed and products with the sulfided KF-860 NiMo at 280 °C and 900 psi hydrogen and 1/hr LHSV.

The results show that a sulfided NiMo catalyst operating under modest conditions of temperature, pressure, and space velocity can convert the HTL1 biocrude into products suitable for use as a blending stock with conventional jet turbine fuel. However, the catalyst lifetime was quite short, and the mechanism of catalyst deactivation will need to be understood and mitigated. The HTL1 biocrude may also be susceptible to polymerization at temperatures above 300 °C. This should be further explored to better define the limits of the parameter space for processing the HTL1 biocrude.

1.10 Manuscript on nutrient recycling

Although a manuscript was submitted to Energy and Fuels (a draft was presented in Appendix A of Q3 2015 quarterly report), the reviewers required significantly more laboratory work before acceptance. Because this project was prematurely closed out, there were no resources to perform the requested studies. A shorter communication may be considered in the future.

2. Technical milestones and deliverables

Milestone M4.1: "Collaboration plan finished (including NDAs and CRADAs)"

Milestone 1.1.1 "Analytical chemistry protocols are finalized". A report with the analytical protocols was submitted in the Q3 2015 quarterly report.

We developed a technical collaboration plan based on the activities that involved more than one of the entities in the project:

- Algae production: SRI and AS coordinated this task (points of contact were: Jordi Perez and Peter Valdez). We had expected at AS's site in Alabama would be used on site for continuous experiments and that it would be shipped to SRI for HTL tests in autoclaves.
- Oil upgrading: SRI, UDRI, and AS coordinated shipments of biocrude oils obtained in different reactors under different conditions (points of contact were: Jordi Perez, Steven Zabarnick, and Peter Valdez). We established the minimum amount for meaningful tests was 100 mL of sample, with 20 wt% of oil content in a hydrocarbon solvent.
- Continuous reactor operation (lab and pilot): SRI and AS coordinated to schedule work using the AS facilities in Alabama (points of contact were: Jordi Perez and Peter Valdez).
- Life cycle assessment (LCA) and greenhouse gas (GHG) studies: SRI, Los Alamos National Laboratory (LANL), and AS coordinated the exchange of information required for the studies at LANL (points of contact were: Jin-Ping Lim, William Kukic, and Peter Valdez).

We established a schedule for periodical (most of the times monthly) conference calls with all team members.

On May 26, 2015, we finalized the subcontract with UDRI.

As of project close out, we did not have a signed contract with AS.

The summary of project milestones is provided in Table 10.

Table 10. Project Milestones and Deliverables

Milestone Number* (Go/No-Go Decision Point Number)	Milestone Description (Go/No-Go Decision Criteria)	Milestone Verification Process (What, How, Who, Where)	Anticipated Quarter (Quarters from Start of the Project)	Percentage complete
M 4.1	Collaboration plan finished (including NDAs and CRADAs)	Report sent to DOE	1	75%
M 1.1.1	Analytical chemistry protocols finalized	Report sent to DOE	2	100%
M 1.4.1	Batch dual-HTL process demonstrated with C efficiency >65% and N content in oil <1.3 wt%.	Report sent to DOE	3	65%
G 2.3.1	One successful HTL run in the lab-scale continuous reactor with C efficiency >65% and N content in oil <1.3 wt%.	Report sent to DOE	4	0%
M 5.1.1	Batch dual-HTL process demonstrated with C efficiency >75%, N content in oil <1 wt%, H content in oil > 9 wt%, and O content in oil <15wt%.	Report sent to DOE	6	0%
M 6.1.1	One successful HTL run in the lab-scale continuous reactor with C efficiency >75%, N content in oil <1 wt%, H content in oil > 9 wt%, and O content in oil <15wt%.	Report sent to DOE	7	0%
M 7.1.1	Recommendations for implementation and scale-up completed	Report sent to DOE	8	0%
M 8.1.1	Final report submitted	Report sent to DOE	8	100%

SECTION II - PROJECT OUTPUT

We presented some of the results at the Bioenergy 2015 conference on June 23rd and 24th in two formats:

- A poster entitled "Multi-step hydrothermal liquefaction (HTL) of algae with nutrient recycling" by Nasim Ehterami, Tripura Mulukutla, Jin-Ping Lim, Fran Tanzella, Jordi Perez was presented, The poster can be downloaded at: http://www.sri.com/sites/default/files/publications/chase_bioenergy_2015_poster_v05.pdf
- A presentation entitled "Algae-to-fuel: integrating thermochemical conversion, nutrient recycling and wastewater" by Jordi Perez is as described on SRI's website: http://www.sri.com/work/projects/hydrothermal-liquefaction-pathways-low-nitrogen-biocrude-wet-algae

Invention disclosure titled "Use of Biosolids for Dewatering Microalgae Cultures" was filed as SRI #P7302.

An informal collaboration with PNNL was established. The main points of contact were Karl Albrecht and Daniel Howe. We sent samples from our aqueous phases (HTL1 and HTL2 for the work so that they could characterize aqueous phases from several HTL processes).