Review of Recent Reports on Process Technology for Thermochemical

Conversion of Whole Algae to Liquid Fuels

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ABSTRACT

This review considers the recent developments in which there has been a tremendous expansion of the research and development focused on process development for the thermochemical processing of whole algae for the production of fuels. There are several key elements to this expanded interest in thermochemical processing: 1) the processing is applied to whole algae, not just lipid extracts, and as a result higher product yields have been demonstrated; 2) the feedstock composition is not so critical to the process, so that a wider range of algae growth scenarios has been considered; and 3) the envisioned products are actual hydrocarbon fuels, which are infrastructure compatible. Based on these three elements one can envision a more widely expanded utilization with more flexible growth options and more direct market applications of products. Algae can be processed by dry pyrolysis or in water slurry by hydrothermal liquefaction. In either case, the liquid oil product can be hydroprocessed to liquid hydrocarbon fuels. Recovery and recycle of nutrients is possible through treatment of the aqueous byproduct to promote sustainable production of the algae feedstock. In all cases the cost of the algae feedstock is the primary uncertainty in the economic analysis of such processing.

1. INTRODUCTION

In the last five years there has been a tremendous expansion of the research and development focused on the thermochemical processing of whole algae for the production of fuels.ⁱ There are several key elements to this expanded interest in thermochemical processing:

1) Processing is applied to whole algae, not just lipid extracts, and as a result higher product yields have been demonstrated;

2) Feedstock composition is not so critical to the process, so that a wider range of algae growth scenarios have been considered; and

3) Envisioned products are actual hydrocarbon fuels, which are infrastructure compatible.

Based on these three elements one can envision a more widely expanded utilization with more flexible growth options and more direct market applications of products.

This review surveys the most recent developments in whole algae thermochemical conversion to liquid fuels, which address the issues of process development and commercialization. Other recent reviews deal with more fundamental chemical conversion issues and provide a more detailed background.^{ii, iii} This review is more focused on continuous-flow hydrothermal liquefaction (HTL) and process results which show how it can be brought into a commercial application, such as water recycle and biocrude upgrading to hydrocarbon fuels. This review is meant as an update for those already cognizant of the issues of thermochemical processing of biomass and relates to HTL algae processing, specifically.

Thermochemical conversion of algae can be divided into the direct pyrolysis of dry algae and the high-pressure processing of algae in water slurries. In fact, the wet (hydrothermal) processing seems to have the better fit for algae utilization because the algae are grown in very dilute water systems. The partial dewatering of the algae-containing media to the level of 10-20% dry solids, usually accomplished by mechanical means for HTL, is a less energy intensive processing option compared to the thermal

drying to >90% dry solids as is required for pyrolysis. The difference in the required moisture content for the two processing options relates to the value of liquid water in the pressurized system used in HTL, wherein it serves as a heat transfer medium and moderator, while in fast pyrolysis the requirement for boiling off the water in the reactor would result in a large heat sink, which would slow the heating process and interfere with the fast pyrolysis mechanisms. The vast majority of the R&D in thermochemical conversion of algae to fuels is based on hydrothermal processing, and, specifically, HTL to produce a biocrude product. Pyrolysis is relegated to laboratory investigations with little hope for commercial application due to the negative energy balance resulting from the prerequisite feedstock drying.

Thermochemical conversion of algae, in the form of hydrothermal liquefaction, is just moving out of the laboratory to scaled-up pilot plant operations.^{iv} There is no commercial application of algae thermochemical processing to fuels. There are several small algae processing companies basing their business on thermochemical conversion (HTL) of whole algae. For the most part the research and development work is found in universities and national laboratories around the world.

2. THERMOCHEMICAL CONVERSION OF WHOLE ALGAE

2.1. Feedstock Effects

A key element of thermochemical conversion of whole algae is that the thermochemical process is inherently feedstock agnostic. All forms of biopolymers breakdown under thermochemical processing conditions.^v Therefore, whole algae are processed, not just lipid extracts. The chemistry involves a complex of reactions from hydrolysis to dehydration, depolymerization to condensation, and various forms of heteroatom removal to reduce the heteroatom “contaminants” and other trace elements and to concentrate the energy in the algae biopolymers into more hydrocarbon-like structures, which are near fuel quality. A comparative study of algae and other lignocellulosic biomass (pine wood and grape

residue) showed that the biocrude yield was higher from algae.^{vi} As a result, the algae feedstock does not need to be grown under strictly controlled conditions with the intent to maximize the lipid content. In fact, carbohydrate and protein structures, as well as lipids, can be converted directly into fuels by the thermochemical process.^{vii} Of course, lipids produce the highest yield of biocrude, >90 wt%, but proteins and carbohydrates also produce significant yields of biocrude, particularly at higher temperature, 350 °C. Further, it was reported that these two components produced higher biocrude yields when mixed than when tested individually, suggesting interaction which leads to higher biocrude production from whole biomass.^{vii} In addition, the algae species do not need to be carefully controlled, as any algae species, as well as cyanobacteria and other species, can be converted by thermochemical means. The differences in these species appears to have only minimal impact on the on the HTL process with differences in yield indistinguishable from the experimental variation due to the different batch reactor methods used. Mixed algae culture grown in open wastewater treatment systems has shown even better HTL biocrude yield and quality than that from laboratory grown monocultures.^{viii} Other wastewater treatment studies suggest mixed cultures of *Chlorella sp.*, *Scenedesmus obliquus* and cyanobacteria (blue green algae) with several other algal species bio-augmented into the culture including *Chlorella protothecoides*, *Chlorella vulgaris*, *Botryococcus braunii*, *Nannochloropsis oculata*, *Spirulina platensis*, *Scenedesmus dimorphus*, and *Chlamydomonas reinhardtii* produced useful results and provides the basis for the University of Illinois' HTL-based E²-Energy concept.^{ix} The team also reported using mixed-culture algae directly from wastewater as HTL feedstock with reasonable results.^x These advantages for whole algae processing contrast with other thermochemical methods being developed for direct recovery of the lipids as a biodiesel product through transesterification under supercritical conditions^{xi} with and without hydrothermal carbonization pretreatment.^{xii}

HTL of macroalgae has also been studied to a limited extent in the laboratory. University of Leeds performed batch reactor studies with feedstock slurries at 21 wt% dry solids and concluded that

the highest yields of biocrude (10-18 wt%) were derived from *L. saccharina* and *A. esculenta*, while biochar yields were higher (11-19 wt%). By claiming both as energy products, the authors concluded that both species produced energy yields equivalent to anaerobic digestion but greater than fermentation.^{xiii} Higher biocrude yields were reported by the research team at Henan Polytechnic University in China using small batch reactors. They reported up to 32 wt% of solvent-extracted biocrude produced at 370°C when the algae slurry concentration of 23 wt% was used.^{xiv} Other similar limited batch surveys have been reported with both green and brown macroalgae.^{xv} PNNL reported HTL of macroalgae in a continuous-flow reactor. *L. saccharina* was processed over a range of slurry concentrations up to 22 wt% and biocrude yields as high as 28 wt% (58 wt% on carbon basis) were reported, without using a solvent extraction recovery step.^{xvi} Using the PNNL system the biochar yield was reported as part of the mineral precipitate, which was very low (up to 4 wt%). In addition, the PNNL system incorporated catalytic hydrothermal gasification (CHG) for byproduct aqueous processing, wherein another 34% of the carbon of the feed was recovered as a fuel gas product, which had a composition similar to anaerobic digester gas. The PNNL work highlighted a difficulty of macroalgae processing relative to microalgae, i.e., the macroalgae physical structure needs more extensive processing for size reduction to achieve a pumpable slurry for continuous-flow processing. The PNNL study also showed that in HTL, lower concentration feedstocks, at least in the case of macroalgae, result in lower biocrude yields since the water solubility of the biocrude is significant. The effect has also been reported for other feedstocks.^{ii, iv}

2.2. Process Options

As algae are grown in dilute water media, the recovery of the algae for subsequent processing is a critical step. The amount of energy required to concentrate the algae to a form in which it can be effectively processed is a major consideration. The two processing options for thermochemical processing, pyrolysis (temperatures in excess of 500 °C, in the absence of air, for short residence of a

about a second) and hydrothermal (typically 350 °C, 20 MPa pressure for some minutes of residence time), both require removal of the bulk of the water and recovery of algae feedstock at dry solids concentration much above the 0.1 wt%, at which the algae is grown. However, the level of dewatering is an order of magnitude higher for pyrolysis (the feedstock is typically quoted at <10 wt% moisture) and typically requires energy intensive thermal drying of the biomass. Some research has been performed on the use of dried algae in non-aqueous solvents (ethylene glycol and sulfuric acid^{xvii} or ethanol^{xviii}), but the work has not progressed far. On the other hand, the dewatering for hydrothermal processing is more typically accomplished by less energy intensive physical means, as it is meant to concentrate the algae only to a pumpable slurry with typically 15-25 wt% dry solids (75-85 wt% moisture). As a result, little of the process research and development has focused on pyrolysis of algae^{xix} but instead has addressed the use of hydrothermal conditions.^{xx} There have been some more fundamental studies of pyrolysis of algae at very small scale, such as by thermal gravimetric analysis (TGA).^{xxi} Small laboratory reactor studies of slow pyrolysis can also be found.^{xxii, xxiii, xxiv} However, due to the negative energy balance issue for drying the algae prior to pyrolysis, none of this research is moving forward into larger scale or process development. The examples of commercial progress in the thermochemical conversion of whole algae utilize hydrothermal processing.

3. HYDROTHERMAL PROCESSING OF ALGAE

3.1. Process Description Details

Hydrothermal processing is accomplished in a hot, pressurized liquid water environment. Typical operating conditions are about 350 °C and sufficient pressure to maintain the liquid water phase, typically 20 MPa. However, hydrothermal carbonization at lower temperature^{xxv} or hydrothermal gasification at higher temperature or with catalyst^{xxvi} are other options. Gasification is being developed in several labs and will be discussed further below; the path forward for carbonization is unclear and will

not be addressed further here. The slurry of algae, typically at 15-25 wt% dry solids in water is pumped through the hot reaction zone, such that the typical residence time is 10 to 30 min. Pretreatment of the algae by microwave heating prior to HTL has suggested some improvement in biocrude yield and quality, but the effect was found in only one of three species tested.^{xxvii} Following the liquefaction, the biocrude and aqueous phases can be separated. This phase separation is facilitated by removal of any solids phase, whose composition will vary with algae species, reaction conditions and residence time, as demonstrated at Pacific Northwest National Laboratory (PNNL), see Figure 1.^{xxviii} Pressure letdown and heat recovery play into the energy efficiency of the process, and will likely be key elements of design in any commercial process. One method of biocrude recovery often used, particularly with small batch reactor tests, requires the use of chemical treatment and solvents to facilitate the phase separation. These steps are not unlike typical chemistry laboratory methods of organic chemical recovery from water, but are likely to be more expensive to carry out as part of a process.

Figure 1. Process flow diagram for hydrothermal processing of whole algae (from ref xxviii).

Reviews of hydrothermal processing of algae have been published recently by groups active in the research field. The Leeds group considered formation of liquid, gas and solid products^{xxxix} while the Ghent group focused on liquefaction.^{xxx} According to the Leeds group the engineering challenges are straightforward, while the main challenge is the substantial and secure production of the algae feedstock. Relative to HTL, the Ghent group identified research and development issues such as product biocrude recovery and heat-up rate. Their review was performed before there were continuous-flow results in the literature, and they specify that such information is needed to build on all the batch data that has been published. Fortunately, continuous-flow process results are now appearing in the open literature.^{iv}

An interesting recent disclosure suggests that the use of ethanol with the water slurry may facilitate higher biocrude yields.^{xxxi} By using this variant of HTL the authors reported that they were able to increase the biocrude yield from 46 wt% to 57 wt%, while operating at less severe conditions—120 minutes at 280 °C and 10 MPa. However, ethanol recovery was not quantified and the reaction of the solvent into the biocrude cannot be discounted as the explanation of the higher yield. The resulting additional cost for consumed ethanol or process handling losses of solvent could make this option uneconomical.

3.2. Process Parameter Optimization

Essentially all of the batch reactor studies of algae HTL have provided input on the issue of optimum operating conditions. However, all of these studies, which attempt to provide process kinetics based on batch operation using solvent recovery systems for the biocrude, have limited application for continuous-flow processing.

Savage's group has published extensively on this subject using *Nannochloropsis sp.* as the feedstock. In one study^{xxxii} they determined the distribution of organic material as a function of temperature, time and slurry concentration. Approximately 95 wt% of the initial cellular material was

converted to water- and solvent-soluble organic compounds even at the shortest time (10 min) at >300 °C. They found that up to 80% of the energy in the feedstock could be recovered in the biocrude. Up to 80% of the N and P were recovered, but the recovery of the two were maximized at different temperatures. At higher temperature, more of the nitrogen was found in the biocrude, but the nitrogen in the aqueous was more prevalent as ammonia as opposed to organo-nitrogen compounds. Not surprisingly, at higher temperature the P was not found in the aqueous. Its precipitation at hotter hydrothermal conditions has been reported previously. Residual oxygen content decreased as a function of increasing temperature. A subsequent study investigated short residence time with high heat-up rate of the batch reactor. The authors concluded that residence of 1 minute at higher temperature (600 °C and 40 MPa) would also produce a high yield of biocrude equivalent to 5 min or more at 350 °C (20 MPa).^{xxxiii} In a separate study the interplay among the products was modeled over a range of temperature and time. Gas and aqueous products were maximized at longest residence time, with gas formation favored at high temperature and aqueous products favored at lower temperature but essentially plateaued after 30 min. The biocrude production seemed to have a maximum at 400 °C at 5 min residence time, but there was a general plateau from 200 °C to 350 °C with residence times of >30 min at low temperature, but <15 min at the higher temperature range, and the authors concluded that the biocrude yield is highest at temperatures >300 °C and reaction times <20 min.^{xxxiv}

Work at the University of Twente focused on *Desmodesmus sp.* algae in batch reactor tests. Tests from 175 to 450 °C and up to 60 min led the authors to conclude that the maximum biocrude yield of 49 wt% was achieved at 375 °C for 5 min reaction time recovering 75% of the algal calorific value.^{xxxv} Analysis of the cell structures verified that the cell walls remained intact above 225 °C and did not break down until 250 °C. In a companion article the authors presented the analytical data for the biocrude.^{xxxvi} The analyses showed that at temperatures above 300 °C proteins and cellulose break down, giving cyclic dipeptides and amino acids derivatives by pyrolysis-like reactions; carbohydrate derivatives such as

furans; products from the cross reaction of those, such as alkyl-pyrrolidinones, pyrazines, pyrroles and melanoidin-like materials, formed from the reaction of amino acids with sugars; and certain amount of asphaltene-like material.

Optimization in batch reactor tests of HTL with *Phaeodactylum tricornutum* found similar results.^{xxxvii} Specifically, the authors reported a maximum biocrude yield at 350 °C and a reaction time of 15 min, with 83% energy recovery in the biocrude. Higher or lower temperatures resulted in lower energy recovery in the biocrude. Twenty five percent of the nitrogen remained in the biocrude over the range of temperature tested (275-420 °C). Alkali metals remained primarily dissolved in the aqueous phase. Alkaline earths were found exclusively in the solid residue. The solid residue at sub-critical conditions consisted almost exclusively of inorganic material. Whitlockite (calcium phosphate) was identified by powder x-ray diffraction analysis.

Results in a two-stage HTL process have now been reported wherein the first stage was a lower temperature (200 °C) pass in a semi-continuous reactor system followed by a second pass at 320 °C. A higher yield of biocrude was recovered with a reduced level of nitrogen as more of the nitrogen was released into the aqueous phase byproduct in the first pass.^{xxxviii}

3.3. Catalytic Hydrothermal Liquefaction

Several attempts have been made recently to catalyze the otherwise thermally driven reactions of HTL. The effect of pH on the chemical conversion mechanisms has long been recognized for HTL of biomass.^{xxxix} Ross et al.^{xl} made comparative tests with added base and acids to determine the effect on HTL of *Spirulina*. They determined that the addition of organic acid resulted in higher yield of biocrude with a lower boiling point and improved flow properties, while the addition of base resulted in a more extensive deoxygenation. Subsequent tests by Das et al. suggest that addition of sodium carbonate results in higher yield of biocrude relative to uncatalyzed conditions, while calcium phosphate addition or nickel oxide addition reduced the biocrude yield while increasing the gas yield.^{xli}

The use of heterogeneous catalysts in HTL has also been investigated to a very limited extent by short tests in batch reactor systems. The Leeds group reports that heterogeneous catalysts cause an increase in de-oxygenation, with CoMo and Pt affecting carbohydrate and protein fractions, while Ni deoxygenates lipids and promotes gasification.^{xlii} Savage's group has studied catalytic HTL more extensively^{xliii} and has even devised a special reactor configuration to separate the liquefaction from the catalytic step.^{xliv} In both of these test programs all the catalysts were added to the reactor without any indication of either reduction or sulfiding. There was no analysis performed on the used catalysts. The potential for sulfur poisoning or coke formation on the catalysts was not addressed. The minimal effects reported are probably not important in the long run as catalyst stability in the HTL environment with a sulfur containing feedstock is highly suspect without results showing to the contrary.

3.4. Feedstock testing in HTL

HTL has been used to process a range of microalgae feedstocks in the laboratory. The first report was based on *Botryococcus braunii* by the Yokoyama's group at the National Institute for Resources and Environment in Japan.^{xlv} They followed that work with tests on *Dunaliella tertiolecta*.^{xlvi} Both species were adequately processed to biocrude by HTL at 300-340 °C in batch tests. In the latter case, the authors specifically noted that the biocrude yield was greater than just the lipid content in the feedstock. Zou's group at Tianjin University in China have focused on *Dunaliella tertiolecta* and shown that 37 wt% biocrude yield was possible by processing at 360 °C for 30 min in a batch reactor^{xlvii} while in a second paper^{xlviii} these authors report only a 26 wt% biocrude yield (at 50 min holding time, but only 17 wt% at 30 min) using a slightly different recovery method and with sodium carbonate added as catalyst. Savage's group at the University of Michigan in the U.S. has published extensively of their batch reactor studies with *Nannochloropsis sp.* wherein they concluded that the highest biocrude yield (43 wt%) was achieved after 60 min at 350 °C.^{xlix} Das's group at the University of Georgia also in the U.S. reported similar results (40 wt% biocrude yield after 60 min at 350 °C) with *Spirulina platensis*^{xxxix} while

the group at Paul Scherrer Institute in Switzerland and Aarhus University in Denmark tested *Phaeodactylum tricornutum* also with similar results (39 wt% biocrude yield after 15 min at 350 °C).^{xxxv} A low-lipid algae, *Chlorella pyrenoidosa* was tested at the University of Illinois and found to produce biocrude at only 280 °C with 30 min residence time. The biocrude was reported to spontaneously separate from the aqueous phase, although the batch nature of the test did not allow for clean separation and the biocrude still contained the mineral solids.ⁱ The authors did not report the biocrude yield, but only “refined” oil yield, which is the toluene extract, at 34 wt%. As an alternative to single stage processing, *Chlorella vulgaris* was processed in a 2-stage process, wherein the algae was treated at temperature <200 °C to effectively extract the nitrogen into the aqueous phase, then the remaining algae was treated at higher temperature to form biocrude with a 55% lower nitrogen content. However, the process also resulted in a 20% reduction of yield of biocrude, so that careful consideration of the tradeoffs must be made.^{li} A survey of several species was performed at the University of Ghent in Belgium wherein the authors concluded short reaction times at near critical temperatures converted 50-60 wt% (on a dry, ash-free basis) of the microalgae into biocrude with similar yields and elemental composition of all species tested (*Scenedesmus obliquus* and *almeriensis*, *Phaeodactylum tricornutum*, *Nannochloropsis gaditana*, *Tetraselmis suecica*, *Chlorella vulgaris*, *Porphyridium purpureum*, and *Dunaliella tertiolecta*).^{lii}

In order to better understand the effects of feedstock composition variation, tests have been performed with model compounds and biochemical components. Maillard reactions, condensations of sugars (carbohydrates or carbohydrate fragments) and amino acids (proteins or protein fragments), have been found to occur under the conditions of hydrothermal biomass processing to form oils, including melanoidin-like materials, thus suggesting a method for biocrude production from non-lipid biopolymers.^{liiii} Tester also studied lipid transformation at hydrothermal conditions and found that triglyceride hydrolysis proceeded at sufficient velocity that fatty acid yields could be maximized at short

residence time (<30 min) at up to 350 °C.^{liv} Model development was undertaken at Leeds to develop the relationship between biocrude yield and the biochemical makeup of the algae. Broad agreement was reached between the predictive yields and actual yields for microalgae and showed that the biocrude yield was 5-25 wt% higher than just the lipid content depending on the biochemical makeup.^{lv}

3.5. Biocrude Product Description

A useful biocrude can be produced from HTL, but it is not good fuel in its initial state. The biocrude is oxygenated, acidic, and contains various elements from the original biomass, such as nitrogen and sulfur. HTL biocrude from algae is better than pyrolysis bio-oil, but still needs additional processing before it can be used. The elemental analysis of biocrudes shows a range of composition dependent upon processing severity and feedstock identity. Biller and Ross^{liii} report carbon contents from 68 to 73 wt% for four algae species, while hydrogen varies around 9 wt%. The oxygen content (by difference) ranged from 10 to 19 wt% and the nitrogen from 4 to 7 wt%. These two elements are key to the extent of hydrotreating required to produce useful liquid hydrocarbon fuels. Analysis by gas chromatography/mass spectrometry (GC/MS) indicates that the biocrude contains aromatic hydrocarbons, nitrogen heterocycles and long-chain fatty acids and alcohols. The higher heating values (HHV) are variously reported in the literature in the range from 30 to 38 MJ kg⁻¹.^{xxvii} Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS) has also been applied to the HTL biocrude directly recovered (without solvent extraction) from a continuous-flow reactor system providing data on the heteroatom content of the higher molecular weight material.^{lvi} The biocrudes are about 70 wt% distillable under vacuum and the distillate contained only a minor fraction of the oxygen and essentially none of the trace elements.^{lvii} Further processing of the heavier fraction has yet to be demonstrated.

At this stage of development with the vast majority of information derived from small batch reactors utilizing solvent recovery, an important consideration is the difference between biocrude collected in that manner and that which would be recovered in a continuous-flow unit without solvent

extraction. Savage's group attempted to answer this question and showed, as might be expected, that the biocrude recovered directly by phase separation was higher quality. The solvent extraction recovered more aqueous soluble components, which were higher in heteroatoms and therefore lower in energy content and requiring additional treatment to produce fuel quality material. The yield of biocrude was higher (about 8 wt%) in the work using a solvent extraction method, but the biocrude portion extracted from the aqueous phase had more than twice as much oxygen and nitrogen.^{lviii} The group also compared polar and non-polar extraction solvents and found that the polar solvents (dichloromethane, chloroform) recovered slightly less biocrude, but the recovered product had a higher carbon content and more of the fatty acid product.^{lix} In all cases the more solvent used, the higher the biocrude "yield."

3.6. Upgrading of Biocrude to Liquid Fuels

The biocrude derived from HTL of algae may be suitable for use as heavy fuel oil, but significant upgrading is required before it can be used as a transportation fuel. Different methods have been evaluated in the laboratory including catalytic hydrotreatment^{xxvi} and catalytic cracking.^{lx}

Hydrotreating (HT), which is done in a way similar to hydrotreating of fossil crude oil, results in a nearly hydrocarbon product, like a sweet crude oil, when done in a fixed-bed continuous-flow reactor as reported by Elliott et al.^{xxviii} Biocrude hydrotreatment processing was accomplished with a catalyst similar to fossil oil hydrotreating catalysts (cobalt promoted molybdenum sulfide), together with pressure (13 MPa) and temperature (400 °C) similar to other hydrotreaters. Although these results are from relatively short term tests, catalytic hydrotreatment of the biocrude significantly reduced the oxygen to a range of 0.8 to 1.8 wt% in the tests reported. HT resulted in desulfurization and denitrogenation down to nearly immeasurably low levels. Total acid number (TAN) was reduced to below the level of detection due to oxygen removal, but the effect may also result from ammonium (formed by hydrodenitrogenation) neutralization of the remaining acids. The viscosity and density both

correlate with the high hydrogen to carbon atomic ratio 1.85 to 1.98. With such low remaining oxygen content, the solubility of the oil in water was quite low and the carbon content in the aqueous byproduct was very low (0.7-1.4 wt%). The nitrogen content of the aqueous was relatively high, suggesting the presence of a substantial amount of ammonium. The gas products were mostly hydrocarbon, with a tentative identification of ammonia, with little carbon oxides recovered. The result is upgraded oil fairly similar to sweet fossil crude oil. After such upgrading the biocrude could be inserted into a traditional refinery for final conversion to traditional gasoline, jet or diesel fuels. Comparable results have more recently been reported when using a batch reactor system, wherein the authors conclude that the yield of hydrocarbon product by HTL and HT exceeds the amount of lipid originally in the algae (*Chlorella sp.*).^{lxi}

An alternative hydrotreating concept, which has been reported, is based on processing in a supercritical water environment.^{lxii} A range of catalysts have been tested, but only in short time, batch reactor tests with a hydrogen limited environment. Most of the catalysts tested were metals. The level of deoxygenation, denitrogenation, and saturation of hydrocarbons reported was less effective than in the continuous-reactor results.^{xxvi} A key conclusion was that the biocrude was essentially desulfurized by the treatment. A key omission is the analysis of the catalyst following the tests. Sulfur removal by formation of metal sulfides on the catalyst, as would be expected in this type of test, is not a basis for a sustainable process as the sulfiding is a well-known catalyst poisoning process.

A separate report using HZSM-5 (a catalytic cracking catalyst) in the presence of hydrogen but without water was also reported, but again only in a sealed batch reactor.^{lxiii} The authors report that a high yield of aliphatic hydrocarbon product was achieved at 400 °C while a more aromatic product was produced at lower yield at 500 °C. No analysis of the used catalyst was reported, such as to quantify coking, which is a typical result with this type of acidic catalyst.

3.7. Byproduct Water Description and Utilization

In the HTL processing of algae a portion, in the range 25-40 %, of the carbon in the feedstock remains as dissolved organic in the byproduct aqueous. In addition, the aqueous phase contains about 50% of the nitrogen and other soluble minerals and dissolved carbon dioxide. Recycle of this water and potential nutrient stream is another key element in the HTL scheme for algae utilization. Analysis of the dissolved organics has been performed in a few studies, such as FT-ICR MS analysis to evaluate high-molecular weight species and identify nitrogen and oxygen containing elemental compositions^{lvi} and gas chromatography-nitrogen photometric detector (GC-NPD) analysis to more specifically identify nitrogen containing compounds in the aqueous phase.^{lxiv} The toxicity of these compounds was quantified and whole aqueous sample tests verified the potent cell cytotoxic activity with Chinese hamster ovary cell assay with LC₅₀ at 7.5% of aqueous byproduct in DI water. Another study concluded that microbial growth on HTL aqueous phase from *Nannochloropsis oculata* at concentrations up to 10-40% were possible using *Escherichia coli*, *Pseudomonas putida* and *Saccharomyces cerevisiae*, although the *S. cerevisiae* needed glucose supplement.^{lxv} Recycle of the aqueous phase back to the HTL reactor has also been tested and one research team concluded that there was an increase in biocrude yield and that the same level of reaction could be accomplished at lower temperature. Apparently, the soluble organic compounds were further reacted to higher molecular weight compounds, which could then be recovered in the biocrude fraction.^{lxvi}

Other studies have evaluated the recycle of the aqueous byproduct directly to the algae growth pond. An initial study used the aqueous byproduct from *Spirulina* HTL as a growth medium supplement for *Chlorella minutissima*. The supplement contained significant N (ammonium) and P (phosphate), which, at concentrations of 1% or less of the aqueous phase, was utilized at lower levels than when using the control medium alone. At higher concentration (10%) the algae growth was inhibited.^{lxvii} Tests of *Desmodesmus sp.* HTL and regrowth on diluted aqueous phase demonstrated that up to 50% N nutrient replacement can be achieved without reduction in growth. The authors concluded that the lack

of macro-/micro nutrients, other than N or P, such as Mg, in the aqueous byproduct is the main cause of growth reduction rather than toxicity due to insufficient dilution of organic components, such as phenols.^{lxviii} The survey study of *Scenedesmus dimorphus*, *Chlorogloeopsis fritschii*, *Spirulina* and *Chlorella vulgaris* in HTL and algae growth on the aqueous byproduct provides important guidance for future process developers.^{lxix} The aqueous byproduct from the HTL of the several algae species was orders of magnitude higher in nutrients compared to standard growth media. The growth trials showed that heavy dilution of the aqueous phase is necessary to avoid growth inhibition by phenols, fatty acids and nickel. The several algae strains were all able to grow on the aqueous byproduct but required different levels of dilution. Mixotrophic growth was evident such that *Chlorogloeopsis fritschii* (at 400X dilution) and *Chlorella vulgaris* (at 200X dilution) achieved higher biomass yields than in their respective media. A more recent survey of *Scenedesmus almeriensis*, *Nannochloropsis gaditana*, *Phaeodactylum tricornutum* and *Chlorella vulgaris* in HTL and algae growth on the aqueous byproduct provides additional information.^{lxx} These authors report that regrowth is strain specific as *Scenedesmus almeriensis* and *Phaeodactylum tricornutum* were not able to grow satisfactorily. Up to 75% of the nutrients could be replaced by recovered aqueous byproduct for the *Nannochloropsis gaditana* and *Chlorella vulgaris*. A recent study with *Phaeodactylum tricornutum* seems to contradict this result in that adequate growth using recycled HTL aqueous byproduct (25X diluted) was reported. The authors caution that a 4-day adaptation period was required and the addition of subsequent CHG for removal of recalcitrant and potentially toxic organic material may be needed to allow long-term operation.^{lxxi} Another study showed that the aqueous phase from hydrothermal carbonization was also useful for nutrient recycle for algae growth.^{lxxii}

3.7.1. Gasification of Organics

The organic component dissolved in the aqueous byproduct of HTL could be utilized for production of additional fuel by gasification. Clean-up of the aqueous phase by gasification could also

facilitate its reuse for nutrient recycle by removing toxic components. Supercritical water gasification was tested for this advantage but no clear indication of its value was produced, as the aqueous byproduct could be satisfactorily recycled with or without gasification.^{lxxi} CHG at subcritical conditions produces methane and carbon dioxide gases from the dissolved organic residues because of the catalyst.^{xxvi} Thus an important fuel gas byproduct is formed. The product gas can be burned to produce combined heat and power (CHP) for the hydrothermal processing system. The combination of HTL with CHG as a second stage provides a liquid fuel product, while the CHG step cleans up the aqueous byproduct to facilitate its reuse or disposal. In this configuration, the effluent water from HTL is passed to the CHG system for processing of the remaining organics. This remainder contains significant energy, and would also be difficult to dispose of or treat in another way, so CHG presents an ideal second stage. The CHG stage will recover more than 99% of the remaining organic material, leaving only methane gas and clean sterile water as product outputs.

CHG can also be used alone to process algal biomass directly to gas products and has been demonstrated in a batch reactor with *Nannochloropsis sp.*^{lxxiii} and in a continuous-flow reactor with *Spirulina*.^{xxiv} By this technology >99% of the COD of the algae slurry can be converted to a fuel gas product. The direct processing of the algae with the preferred metal catalyst is complicated by poisoning of the catalyst by sulfur in the algae. However, use of HTL with mineral separation or appropriate aqueous processing before CHG may allow the complete cycle to be realized.

3.7.2 Recycle of Nutrients

A significant advantage of hydrothermal processing, both HTL and CHG, is that it recovers a high percentage of the nutrients in the original algae (including carbon dioxide), which can be recycled to support new algae growth. Nutrient recycle is achieved in several ways. In the HTL process being developed at PNNL,^{xxvi} one of the process steps is a precipitation/solids separation step, which precipitates minerals, principally phosphate. The phosphorus precipitate is very dense and small in

volume, which can easily be recovered and processed into phosphorus fertilizer. From one third to one half of the nitrogen in the feedstock will appear in the biocrude, but the remainder is present in the effluent water and is recovered later after CHG. Other plant nutrients are also in the effluent water and are recovered later after CHG. Carbon dioxide is also recovered. The gas mixture from the catalytic reactor is a 60/40 mix of methane and carbon dioxide, and the water is saturated with carbon dioxide. Since the water may be returned to the algae growth ponds for the plant nutrients, it will also carry with it the dissolved carbon dioxide. In addition, the carbon dioxide should be able to be separated from the methane in the gas product by conventional means, such as membranes, and returned to the algae growth pond. If the product gas is burned to produce CHP, it can also be scrubbed to recover carbon dioxide. With these steps, nutrient recycle is nearly complete, almost as a closed-loop.

4. STATUS OF COMMERCIAL OPERATIONS

As stated at the beginning of this review, hydrothermal processing of algae to fuels is still primarily a subject of laboratory R&D. The bulk of the research is still performed in batch reactor systems and cannot even be considered actual process development.^{iv}

There are a few examples of the technology coming out of the laboratory into the marketplace.

4.1. Sapphire Energy Inc.

Sapphire^{lxxiv} has been developing their algae liquefaction technology since 2007 and has now moved to a pilot plant scale of operation. Sapphire has three facilities across California and New Mexico. Its headquarters and primary lab are in San Diego, California, there is a Research and Development Facility in Las Cruces, New Mexico. In 2010, the company began construction of the world's first commercial demonstration algae-to-energy farm in Columbus, New Mexico. Construction of Phase 1 (first 100 acres of ponds) was completed on-time and on-budget in 2012 and the company paid back the USDA loan guarantee in 2013. The company has the full technology pathway from algae

growth to harvesting to conversion and fuels marketing. The algae growth system is an open pond design using non-potable water based on non-arable land. With the planned 300 acres of cultivation, the annual product yield is a million gallons of transportation fuels. As part of their patent portfolio (over 300 patents and patent applications) they have a patented process for liquefaction which includes a hydrothermal step with biocrude treatment and recovery including acidification and solvent extraction.^{lxxv} In this manner their process appears as a scaled-up version of the batch laboratory tests but in a continuous-flow arrangement. The biocrude recovery adds considerable complexity to the process. They also have a patent application describing the upgrading of the biocrude product.^{lxxvi} The Sapphire biocrude (“Green Crude Oil”) has been tested in partnership with petroleum refiners, such as Tesoro, in coprocessing with petroleum streams in a range of applications including hydrotreating, catalytic cracking, and delayed coker. As of January 2015 Sapphire announced a shift in market emphasis to nutraceutical products and animal/aquaculture feed.

4.2. Algenol Biotech LLC

Algenol^{lxxvii} uses a proprietary strain of cyanobacteria to produce an ethanol product, which is directly recovered from their photobioreactors. The algae biomass is periodically harvested and processed by hydrothermal liquefaction to produce a biocrude. Algenol has an integrated biorefinery pilot plant in Fort Myers, Florida, with a capacity of 10,000 gal per year of ethanol. After receiving the 2015 Presidential Green Chemistry Award in July, the company underwent a dramatic transition in October when the founding CEO was replaced and a 20% reduction in force of the staff was announced.

In the Algenol process^{lxxiv} algae biomass is dewatered by mechanical means and is fed into the HTL reactor system, which Algenol has developed in collaboration with PNNL. Based on the laboratory work, the HTL biocrude oil is expected to be hydrotreated to produce a liquid hydrocarbon product. The hydrotreated product should contain significantly reduced levels of oxygen, nitrogen and sulfur

compared to the HTL biocrude and can be distilled into diesel, jet fuel and gasoline fractions. Final processing would likely be accomplished at larger scale within a petroleum refinery.

Algenol claims^{lxxiv} its process produces around 8,000 gallons of liquid fuels per year on one wet acre of algal cultivation. The majority of the product is ethanol, but 500 gallons of diesel, 380 gallons of gasoline and 315 gallons of jet fuel result from the HTL process. As a result the technology compares favorably to corn ethanol, which typically yields around 420 gallons per acre per year.

4.3. Genifuel/Reliance

Genifuel Corporation and Reliance Industries, Ltd. were partners with PNNL and others in the National Association for Advanced Biofuels and Bioproducts (NAABB), which coordinated research on the fuels pathway of algae strain development, growth, harvesting, and conversion. Reliance has now contracted with Genifuel to fabricate a 1 ton per day pilot plant for hydrothermal processing of algae biomass to liquid and gaseous fuels. Construction of the pilot plant and start-up has been completed, with delivery to India planned for later in 2015. The hydrothermal processing technology is licensed by Genifuel from PNNL.^{lxxviii} The initial results in the scaled-up system appear to reproduce those found in the laboratory.

4.4. Muradel Pty. Ltd.

Muradel^{lxxix} opened an integrated algae production and hydrothermal liquefaction demonstration plant at Whyalla, Australia, in October 2014 which can produce 30,000 liters per year of biocrude. A planned commercial plant of 1000 hectare would produce 50 million liters of biocrude per year. Muradel uses marine algae grown in seawater on marginal land for their feedstock. They refer to a subcritical water reactor as the means to produce a biocrude and are now testing other biosolids as feedstock, in addition to algae. Earlier they decommissioned a 2-year old algae production pilot plant near Karratha, NWA. The projected cost for biocrude was \$9.90/L using the pilot plant data, but costs are expected at about 1\$/L in a commercial scale plant.

4.5. University of Sydney

A continuous-flow hydrothermal liquefaction pilot plant was designed and built at the University of Sydney in Australia.^{lxxx} Although there is no commercial partner involved in this work, it is a significant element in the process development effort for HTL of algae. The design flow rate of the pilot plant is 15-90 L of algae slurry at 10 wt% dry solids per hour. The process design does not include a biocrude separation technology, but biocrude extraction by dichloromethane is handled batchwise off-line. Processing results for *Chlorella* and *Spirulina* have been published.^{lxxx}

5. TECHNO-ECONOMIC AND LIFE-CYCLE ASSESSMENTS

Although these thermochemical conversion technologies are at an early stage of development, attempts have been made to quantify the economic potential in some cases. Based on the limited data available and the extreme level of interest in algae-based fuels production, a number of studies^{lxxxii} including a technoeconomic assessment (TEA) and several life-cycle analyses (LCA) have been undertaken on the hydrothermal processing option. As stated in the TEA, based on the PNNL efforts, the feedstock cost is significant and the value used is no better than a placeholder, which needs to be better determined for future process assessments. A comparative analysis of the HTL option versus conventional algae oil extraction and biodiesel production based on early literature data suggests that HTL uses biomass more efficiently but has higher emissions and nutrient requirement.^{lxxxii} A later LCA improves on that study by incorporating more information on utilization of the aqueous stream and reports that the HTL algae fuel has considerably lower greenhouse gas emissions than petroleum fuels and even less than corn ethanol. However, the energy return on investment remains lower than for petroleum fuels, but with significant potential for process efficiency improvements^{lxxxiii} based on the study using information from the Sapphire process. A separate LCA confirmed the value of CHG for

improving the GHG emissions as well as the product cost by utilizing the aqueous stream for fuel production and allowing recycle of nutrients.^{lxxxiv} Another LCA suggests that consideration be given to the source of any added carbon dioxide provided to the algae growth environment as well as the perceived difference in effect between ground transport use of the fuel versus atmospheric combustion as jet fuel.^{lxxxv}

CONCLUSIONS

Within the realm of thermochemical conversion technologies used for fuel production from algae, it appears that hydrothermal liquefaction is the leading candidate for near-term commercialization. There are a number of research groups evaluating the technology and several groups are pushing toward commercialization. The differences in the range of species tested and reported in the literature appear to have only minimal impact on the on the HTL process with differences in yield indistinguishable from the experimental variation due to the different batch reactor methods used. Tests in continuous-flow systems should provide more definitive details of product variation with species, but differences are not expected to be great. Clearly the composition of the biomass will impact the composition and yield of the biocrude. The cost of the feedstock is the critical barrier. Utilization of the biocrude product has had only limited study, but the direct hydroprocessing to liquid hydrocarbon fuels appears to be fairly straightforward based on the initial laboratory tests reported. Recycle of the nutrients has been recognized as a key to sustainable operation and hydrothermal processing provides the means to accomplish the recovery of the elements of primary concern, nitrogen and phosphorus. Expanded development and demonstration of the conversion technology is needed in process-representative continuous-flow reactor systems. Scale-up for biocrude production is needed to allow testing of the liquid product at sufficient scale to validate its usefulness.

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- ⁱ Kröger, M.; Müller-Langer, F. Review of possible algal-biofuel production processes. *Biofuels* **2012** 3(3), 333-349.
- ⁱⁱ Guo, Y.; Yeh, T.; Song, W.H.; Xu, D.H.; Wang, S.Z. A review of bio-oil production from hydrothermal liquefaction of algae. *Renewable & Sustainable Energy Reviews* **2015** 48, 776-790.
- ⁱⁱⁱ Chen, Y.; Wu, Y.; Hua, D.; Li, C.; Harold, M.P.; Wang, J.; Yang, M. Thermochemical conversion of low-lipid microalgae for the production of liquid fuels: Challenges and opportunities. *RSC Advances* **2015** 5(24) 18673-18701.
- ^{iv} Elliott, D.C.; Biller, P.; Ross, A.B.; Schmidt, A.J.; Jones, S.B. Hydrothermal liquefaction of biomass: Developments from batch to continuous process. *Bioresource Technology* **2014** 178, 147-156.
- ^v Toor, S.S.; Rosendahl, L.; Rudolf, A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy* **2011** 36, 2328-2342.
- ^{vi} Subagyono, D.J.N.; Marshall, M.; Jackson, W.R.; Chaffee, A.L. Pressurized thermal and hydrothermal decomposition of algae, wood chip residue, and grape marc: A comparative study. *Biomass Bioenergy* **2015** 76, 141-157.
- ^{vii} Teri, G.; Luo, L.; Savage, P.E. Hydrothermal treatment of protein, polysaccharide, and lipids alone and in mixtures. *Energy Fuels*, **2014**, 28, 7501-7509.
- ^{viii} Roberts, G.W.; Fortier, M.-O.,P.; Sturm, B.S.M.; Stagg-Williams, S.M. Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion. *Energy Fuels* **2013** 27, 857-867.
- ^{ix} Zhou, Y.; Schideman, L.; Yu, G.; Zhang, Y. A synergistic combination of algal wastewater treatment and hydrothermal biofuel production maximized by nutrient and carbon recycling. *Energy Environ. Sci.* **2013** 6 3765-3779.
- ^x Chen, W.-T.; Zhang, Y.; Zhang, J.; Yu, G.; Schideman, L.C.; Zhang, P.; Minarick, M. Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. *Bioresource Technol.* **2014** 152, 130-139.
- ^{xi} Patil, P.D.; Gude, V.G.; Mannarswamy, A.; Deng, S.; Cooke, P.; Munson-McGee, S.; Rhodes, I.; Lammers, P.; Nirmalakhandan, N. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresource Technol.* **2011** 102, 118-122.
- ^{xii} Levine, R.B.; Bollas, A.; Savage, P.E. Process improvements for the supercritical in situ transesterification of carbonized algal biomass. *Bioresource Technol.* **2013** 136, 556-564.
- ^{xiii} Anastasakis, K.; Ross, A.B. Hydrothermal liquefaction of four brown macro-algae commonly found on the UK coasts: An energetic analysis of the process and comparison with bio-chemical conversion methods. *Fuel*, **2015**, 139, 546-553.
- ^{xiv} Xu, Y.-P.; Duan, P.-G.; Wang, F. Hydrothermal processing of macroalgae for producing crude bio-oil. *Fuel Proc. Technol.* **2015** 130, 268-274.
- ^{xv} Li, D.; Chen, L.; Xu, D.; Zhang, X.; Ye, N.; Chen, F.; Chen, S. Preparation and characteristics of bio-oil from the marine brown alga *Sargassum patens* C. Agardh. *Bioresource Technol.* **2012**, 104, 737-742; Neveux, N.; Yuen, A.K.L.; Jazrawi, C.; Magnusson, M.; Haynes, B.S.; Masters, A.F.; Montoya, A.; Paul, N.A.; Maschmeyer, T.; de Nys, R. Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae. *Bioresource Technol.* **2014**, 155, 334-341.
- ^{xvi} Elliott, D.C.; Hart, T.R.; Neuenschwander, G.G.; Rotness, L.J.; Roesijadi, G.; Zacher, A.H.; Magnuson, J.K. Hydrothermal processing of macroalgal feedstocks in continuous-flow reactors. *ACS Sustain. Chem. Eng.* **2013** 2(2), 207-215.

- ^{xvii} Zou, S.; Wu, Y.; Yang, M.; Li, C.; Tong, J. Thermochemical catalytic liquefaction of the marine microalgae *Dunaliella tertiolecta* and characterization of bio-oils. *Energy Fuels* **2009** *23*, 3753-3758.
- ^{xviii} Huang, H.; Yuan, X.; Zeng, G.; Wang, J.; Li, H.; Zhou, C.; Pei, X.; You, Q.; Chen, L. Thermochemical liquefaction characteristics of microalgae in sub- and supercritical ethanol. *Fuel Proc. Technol.* **2011** *92*, 147-153.
- ^{xix} Hognon, C.; Delrue, F.; Texier, J.; Grateau, M.; Thiery, S.; Miller, H.; Roubaud, A. Comparison of pyrolysis and hydrothermal liquefaction of *Chlamydomonas reinhardtii*. Growth studies on the recovered hydrothermal aqueous phase. *Biomass Bioenergy* **2015** *73*, 23-31.
- ^{xx} Jena, U.; Das, K.C. Comparative evaluation of thermochemical liquefaction and pyrolysis for bio-oil production from microalgae. *Energy Fuels* **2011**, *25*, 5472-5482.
- ^{xxi} Chow, M.C.; Jackson, W.R.; Chafee, A.L.; Marshall, M. Thermal treatment of algae for production of biofuel. *Energy Fuels* **2013**, *27*, 1926-1950.
- ^{xxii} Campanella A.; Muncrief, R.; Harold, M.P.; Griffith, D.C.; Whitton, N.M.; Weber, R.S. Thermolysis of microalgae and duckweed in a CO₂-swept fixed-bed reactor: Bio-oil yield and compositional effects. *Bioresource Technol.* **2012** *109*, 154-162.
- ^{xxiii} Aysu, T. Catalytic pyrolysis of *Eremurus spectabilis* for bio-oil production in a fixed-bed reactor: Effects of pyrolysis parameters on product yields and character. *Fuel Proc. Technol.* **2015** *129*, 24-38.
- ^{xxiv} Zeng, Y.; Tang, J.; Lian, S.; Tong, D.; Hu, C. Study on the conversion of cyanobacteria of Taihu Lake water blooms to biofuels. *Biomass Bioenergy* **2015** *73*, 95-101.
- ^{xxv} Heilmann, S.M.; Davis, H.T.; Jader, L.R.; Lefebvre, P.A.; Sadowsky, M.J.; Schendel, F.J.; von Keitz, M.G.; Valentas, K.J. Hydrothermal carbonization of microalgae. *Biomass Bioenergy* **2010** *34*, 875-882.
- ^{xxvi} Elliott, D.C.; Hart, T.R.; Neuenschwander, G.G. Chemical Processing in High-Pressure Aqueous Environments. 9. Process Development for Catalytic Gasification of Algae Feedstocks. *Ind. & Eng. Chem. Res.* **2012** *51*, 10768-10777.
- ^{xxvii} Biller, P.; Friedman, C.; Ross, A.B. Hydrothermal microwave processing of microalgae as a pretreatment and extraction technique for bio-fuels and bio-products. *Bioresource Technol.* **2013** *136*, 188-195.
- ^{xxviii} Elliott, D.C.; Hart, T.R.; Schmidt, A.J.; Neuenschwander, G.G.; Rotness, L.J.; Olarte, M.V.; Zacher, A.H.; Albrecht, K.O.; Hallen, R.T.; Holladay, J.E. Process development of hydrothermal liquefaction of algae feedstocks in continuous-flow reactor. *Algal Research*. **2013** *2*(4), 445-454
- ^{xxix} Biller, P.; Ross, A.B. Hydrothermal processing of algal biomass for the production of biofuels and chemicals. *Biofuels*, **2014**, *3*(5)603-623.
- ^{xxx} Barreiro, D.L.; Prins, W.; Ronasse, F.; Brilman, W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects.. *Biomass Bioenergy* **2013**, *53*, 113-127.
- ^{xxxi} Zhang, J.; Zhang, Y. Hydrothermal liquefaction of microalgae in an ethanol-water co-solvent to produce biocrude oil. *Energy Fuels* **2014** *28*, 5178-5183.
- ^{xxxii} Valdez, P.J.; Nelson, M.C.; Wang, H.Y.; Lin, X.N.; Savage, P.E. Hydrothermal liquefaction of *Nannochloropsis* sp.: Systematic study of process variables and analysis of the product fractions. *Biomass Bioenergy* **2012** *46* 317-331.
- ^{xxxiii} Faeth, J.L.; Valdez, P.J.; Savage, P.E. Fast hydrothermal liquefaction of *Nannochloropsis* sp. to produce biocrude. *Energy Fuels* **2013** *27*, 1391-1398.
- ^{xxxiv} Valdez, P.J.; Savage, P.E. A reaction network for the hydrothermal liquefaction of *Nannochloropsis* sp. *Algal Research* **2013** *2*, 416-425.
- ^{xxxv} Torri, C.; Alba, L.G.; Samorì, C.; Fabbri, D.; Brilman, D.W.F. Hydrothermal treatment (HTT) of microalgae: Detailed molecular characterization of HTT oil in view of HTT mechanism elucidation. *Energy Fuels* **2012** *26*, 658-671.
- ^{xxxvi} Alba, L.G.; Torri, C.; Samorì, C.; van der Spek, J.; Fabbri, D.; Kersten, S.R.A.; Brilman, D.W.F. Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept. *Energy Fuels* **2012** *26*, 642-657.
- ^{xxxvii} Christensen, P.S.; Peng, G.; Vogel, F.; Iversen, B.B. Hydrothermal liquefaction of the microalgae *Phaeodactylum tricornutum*: Impact of reaction conditions on product and elemental distribution. *Energy Fuels* **2014** *28*, 5792-5803.

- ^{xxxviii} Prapaiwatcharapan, K.; Sunphorka, S.; Kuchonthara, P.; Kangvansaichol, K.; Hinchiranan, N. Single- and two-step hydrothermal liquefaction of microalgae in a semi-continuous reactor: Effect of the operating parameters. *Bioresource Technol.* **2015** *191*, 426-432.
- ^{xxxix} Elliott, D.C. Hydrothermal processing. In: *Thermochemical Processing of Biomass: Conversion into Fuels, Chemicals and Power*, Robert C. Brown, ed., **2011**, 200-231, John Wiley & Sons, Ltd., Chichester, UK.
- ^{xl} Ross, A.B.; Biller, P.; Kubacki, M.L.; Li, H.; Lea-Langton, A.; Jones, J.M. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel* **2010** *89*, 2234-2243.
- ^{xli} Jena, U.; Das, K.C.; Kastner, J.R. Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*. *Bioresource Technol.* **2011** *102*, 6221-6229.
- ^{xlii} Biller, P.; Riley, R.; Ross, A.B. Catalytic hydrothermal processing of microalgae: Decomposition and upgrading of lipids. *Bioresource Technol.* **2011** *102*, 4841-4848.
- ^{xliii} Duan, P.; Savage, P.E. Hydrothermal liquefaction of a microalga with heterogeneous catalysts. *Ind. Eng. Chem. Res.* **2011** *50*, 52-61.
- ^{xliv} Yang, L.; Li, Y.; Savage, P.E. Catalytic hydrothermal liquefaction of a microalga in a two-chamber reactor. *Ind. Eng. Chem. Res.* **2014** *53*, 11939-11944.
- ^{xlvi} Dote, Y.; Sawayama, S.; Inoue, S.; Minowa, T.; Yokoyama, S.-Y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel* **1994** *73*(12) 1855-1857.
- ^{xlii} Minowa, T.; Yokoyama, S.-Y.; Kishimoto, M.; Okakura, T. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. *Fuel* **1995**, *74*(12) 1735-1738.
- ^{xlvii} Zou, S.; Wu, Y.; Yang, M.; Li, C.; Tong, J. Bio-oil production from sub- and supercritical water liquefaction of microalgae *Dunaliella tertiolecta* and related properties. *Energy Environ Sci* **2010** *3*, 1073-1078.
- ^{xlviii} Zou, S.; Wu, Y.; Yang, M.; Imdad, K.; Li, C.; Tong, J. Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta*. *Energy* **2010** *35*, 5406-5411.
- ^{xlix} Brown, T.M.; Duan, P.; Savage, P.E. Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. *Energy Fuels* **2010** *24*, 3639-3646.
- ⁱ Yu, G.; Zhang, Y.; Schideman, L.; Funk, T.L.; Wang, Z. Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. *Trans. ASABE* **2011** *54*(1) 239-246.
- ⁱⁱ Jazrawi, C.; Biller, P.; He, Y.; Montoya, A.; Ross, A.B. Maschmeyer, T.; Haynes, B.S. Two-stage hydrothermal liquefaction of a high-protein microalga. *Algal Research* **2015** *8*, 15-22
- ⁱⁱⁱ Barreiro, D.L.; Zamalloa, C.; Boon, N.; Vyverman, W.; Ronsse, F.; Brilman, W.; Prins, W. Influence of strain-specific parameters on hydrothermal liquefaction of microalgae. *Bioresource Technol.* **2013** *146*, 463-471.
- ⁱⁱⁱⁱ Peterson, A.A.; Lachance, R.P. Tester, J.W. Kinetic evidence of the Maillard reaction in hydrothermal biomass processing: Glucose—glycine interactions in high-temperature, high-pressure water. *Ind. Eng. Chem. Res.* **2010** *49*, 2107-2117.
- ^{liv} Johnson, M.C.; Tester, J.W. Lipid transformation in hydrothermal processing of whole algal cells. *Ind. Eng. Chem. Res.* **2013** *52*, 10988-10995.
- ^{lv} Biller, P.; Ross, A.B. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technol.* **2011** *102*, 215-225.
- ^{lvi} Sudasinghe, N.; Dungan, B.; Lammers, P.; Albrecht, K.; Elliott, D.; Hallen, R.; Schaub, T. High resolution FT-ICR mass spectral analysis of bio-oil and residual water soluble organics produced by hydrothermal liquefaction of the marine microalga *Nannochloropsis salina*. *Fuel* **2014** *119*, 47-56.
- ^{lvii} Eboibi, B.E.-O.; Lewis, D.M.; Ashman, P.J.; Chinnasamy, S. Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation. *Bioresource Technol.* **2014** *174*, 212-221.
- ^{lviii} Xu, D.; Savage, P.E. Characterization of biocrudes recovered with and without solvent after hydrothermal liquefaction of algae. *Algal Research* **2014** *6*, 1-7.
- ^{lix} Valdez, P.J.; Dickinson, J.G.; Savage, P.E. Characterization of product fractions from hydrothermal liquefaction of *Nannochloropsis* sp. and the influence of solvents. *Energy Fuels* **2011** *25*, 3235-3243.
- ^{lx} Li, Z.; Savage, P.E. Feedstocks for fuels and chemicals from algae: Treatment of crude bio-oil over HZSM-5. *Algal Research* **2013** *2*, 154-163.

- ^{lxi} Biller, P.; Sharma, B.K.; Kunwar, B.; Ross, A.B. Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel* **2015** *159*, 197-205.
- ^{lxii} Bai, X.; Duan, P.; Xu, Y.; Zhang, A.; Savage, P.E. Hydrothermal catalytic processing of pretreated algal oil: A catalyst screening study. *Fuel* **2014** *120*, 141-149.
- ^{lxiii} Li, Z.; Savage, P.E. Feedstocks for fuels and chemicals from algae: Treatment of crude bio-oil over HZSM-5. *Algal Research* **2013** *2*, 154-163.
- ^{lxiv} Pham, M.; Schideman, L.; Scott, J.; Rajagopalan, N.; Plewa, M.J. Chemical and biological characterization of wastewater generated from hydrothermal liquefaction of *Spirulina*. *Environ. Sci. Technol.* **2013** *47*, 2131-2138.
- ^{lxv} Nelson, M.; Zhu, L.; Thiel, A.; Wu, Y.; Guan, M.; Minty, J.; Wang, H.Y.; Lin, X.N. Microbial utilization of aqueous co-products from hydrothermal liquefaction of microalgae *Nannochloropsis oculata*. *Bioresource Technol.* **2013** *136*, 522-528.
- ^{lxvi} Ramos-Tercero, E.A.; Bertuccio, A.; Brilman, D.W.F. Process water recycle in hydrothermal liquefaction of microalgae to enhance bio-oil yield. *Energy Fuels* **2015** *29*, 2422-2430.
- ^{lxvii} Jena, U.; Vaidyanathan, N.; Chinnasamy, S.; Das, K.C. Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. *Bioresource Technol.* **2011** *102*, 3380-3387.
- ^{lxviii} Alba, L.G.; Torri, C.; Fabbri, D.; Kersten, S.R.A.; Brilman, D.W.F. Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae. *Chem. Eng. Jour.* **2013** *228*, 214-223.
- ^{lxix} Biller, P.; Ross, A.B.; Skill, S.C.; Lea-Langton, A.; Balasundaram, B.; Hall, C.; Riley, R.; Llewellyn, C.A. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. *Algal Research* **2012** *1*, 70-76.
- ^{lxx} Barreiro, D.L.; Bauer, M.; Hornung, U.; Posten, C.; Kruse, A.; Prins, W. Cultivation of microalgae with recovered nutrients after hydrothermal liquefaction. *Algal Research* **2015** *9*, 99-106.
- ^{lxxi} Bagnoud-Velásquez, M.; Schmid-Staiger, U.; Peng, G.; Vogel, F.; Ludwig, C. First developments towards closing the nutrient cycle in a biofuel production process. *Algal Research* **2015** *8*, 76-82.
- ^{lxxii} Du, Z.; Hu, B.; Shi, A.; Ma, X.; Cheng, Y.; Chen, P.; Liu, Y.; Lin, X.; Ruan, R. Cultivation of a microalga *Chlorella vulgaris* using recycled aqueous phase nutrients from hydrothermal carbonization process. *Bioresource Technol.* **2012** *126*, 354-357.
- ^{lxxiii} Guan, Q.; Wei, C.; Savage, P.E. Hydrothermal gasification of *Nannochloropsis sp.* with Ru/C. *Energy Fuels* **2012** *26*, 4575-4582.
- ^{lxxiv} www.sapphireenergy.com
- ^{lxxv} Cranford, R.J.; Aravanis, A.M.; Roussis, S.G. Process for the Recovery of Oleaginous Compounds from Biomass. U.S. patent No. 8,192,628 B2, June 5, 2012.
- ^{lxxvi} Goodall, B.L.; Aravanis, A.M.; Behnke, G.A.; Cranford, R.J.; Sajkowski, D.J. Methods of Preparing Oil Compositions for Fuel Refining. U.S. patent Application 2012/0116138 A1, May 10, 2012.
- ^{lxxvii} www.algenol.com
- ^{lxxviii} Oyler, J. Hydrothermal Processing of Algae: Fuels and Recycled Plant Nutrients. ABO Algae Biomass Summit 2012 <http://www.genifuel.com/text/20120927%20Genifuel-ABO%202012%20Hydrothermal%20Processing.pdf>
- ^{lxxix} www.muradel.com
- ^{lxxx} Jazrawi, C.; Biller, P.; Ross, A.B.; Montoya, A.; Maschmeyer, T.; Haynes, B.S. Pilot plant testing of continuous hydrothermal liquefaction of microalgae. *Algal Research* **2013** *2*, 268-277.
- ^{lxxxi} Jones, S.B.; Zhu, Y.; Snowden-Swan, L.J.; Anderson, D.B.; Hallen, R.T.; Schmidt, A.J.; Albrecht, K.A.; Elliott, D.C. Whole Algae Hydrothermal Liquefaction: 2014 State of the Technology. PNNL-23867, Pacific Northwest National Laboratory, Richland, Washington, USA. June 2014.
- ^{lxxxii} Frank, E.D.; Elgowainy, A.; Han, J.; Wang, Z. Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. *Int. J. Life Cycle Assess.* **2013** *18*(1) 137-158.
- ^{lxxxiii} Liu, X.; Saydah, B.; Eranki, P.; Colosi, L.M.; Mitchell, B.G.; Rhodes, J.; Clarens, A.F. Pilot-scale data provides enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. *Bioresource Technol.* **2013** *148*, 163-171.

^{lxxxiv} Orfield, N.D.; Fang, A.J.; Valdez, P.J.; Nelson, M.C.; Savage, P.E.; Lin, X.N.; Keoleian, G.A. Life cycle design of an algal biorefinery featuring hydrothermal liquefaction: Effect of reaction conditions and an alternative pathway including microbial regrowth. *ACS Sustain. Chem. Eng.* **2014** *2*, 867-874.

^{lxxxv} Connelly, E.B.; Colosi, L.M.; Clarens, A.F.; Lambert, J.H. Life cycle assessment of biofuels from algal hydrothermal liquefaction: The upstream and downstream factors affecting regulatory compliance. *Energy Fuels* **2015** *29*, 1653-1661.