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Low temperature hydrothermal pretreatment of algae to reduce nitrogen heteroatoms and generate nutrient recycle streams

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ABSTRACT

A two-stage hydrothermal liquefaction process was used to reduce nitrogen heteroatoms in algae biocrude and generate an aqueous co-product stream for algae cultivation. A low temperature liquefaction pretreatment (PT: 125–225°C, 0.5–30 min) hydrolyzed proteins and partitioned nitrogen to the aqueous phase, which was separated and analyzed for nutrient and toxin concentrations. The retained solids (20%) were subjected to hydrothermal liquefaction (HTL) in the second step at 350°C for 60 min. The low temperature pretreatment was most effective at higher temperatures and holding times (225°C, 15 min), removing 45% of the nitrogen present in the algae solids, while retaining 59% of the solid mass, yet reduced overall bio-oil yields by 35 to 71% relative to single stage HTL. Experiments conducted in two-chamber reactors with increased heating rates, resulted in similar nitrogen removal levels at shorter residence times (250°C, 5 min). When coupled with high-temperature HTL, pretreatment improved the elemental content of the biocrude by increasing the total nitrogen removed and reducing nitrogen heteroatom content by 26–28%, compared to HTL alone. The aqueous co-product stream was analyzed for nutrient availability and indicated potential for recycle and algae cultivation due to its high nitrogen and phosphorus levels, moderate pH, and low inhibitory compound concentrations.

1. Introduction

Algae as a source of liquid fuel production are attractive, since they are renewable, grow rapidly even in adverse conditions, and reduce net greenhouse gas emissions by utilizing CO_2. Although algal biomass production has been commercialized in the past [1], downstream processing and conversion of algal biomass to fuels is still a significant bottleneck in commercialization [2]. Research has focused on the conversion of algal biomass into liquid fuels via drying, followed by catalytic pyrolysis or pyrolysis and catalytic upgrading of the bio-oil, lipid extraction and transesterification, and more recently hydrothermal liquefaction coupled with catalytic hydrotreatment. Liquid fuel produced from algal biomass via transesterification is limited to high lipid algal species and typically requires algae monocultures and biomass drying, which lead to higher production costs. Drying is a highly energy intensive process and negatively affects process economics. Thus, a conversion process that directly converts the wet algae biomass into liquid fuel intermediates is required, which could then be catalytically upgraded to a refinery intermediate. One such conversion technology is hydrothermal liquefaction (HTL), where algae biomass is heated in hot compressed water (~200–370°C, ~4–21 MPa) to generate biocrude, gases, water solubles, and a solid residue [3,4]. The biocrude is subsequently separated and can be potentially upgraded to a product similar to intermediate petroleum refinery streams (e.g., gasoil).

HTL is performed using hot compressed water, since it is a highly reactive medium as it approaches its critical point (374°C, 22 MPa) due to changes in properties such as acidity, solubility, density, dielectric constant and reactivity. HTL acts on lipids, proteins and carbohydrates in algae, transforming them into a bio-oil (biocrude) via three reactions, hydrolysis, depolymerization, and re-polymerization/self-condensation reactions [4]. Protein molecules are hydrolyzed into amino acids followed by deamination (release of NH3) and decarboxylation reactions to form complex hydrocarbons [5]. Biocrude is a dark viscous liquid with an energy value 70–95% of petroleum fuel oil [6]. Thus, HTL converts organic constituents of algae into a liquid biocrude that in theory can be refined to a range of fuels including naptha, diesel, and fuel oil [7,8]. However, due to the presence of proteins in most algae biomass, the biocrude has a large abundance of nitrogen compounds, which pose potential problems in the subsequent upgrading processes, since they can lead to catalyst poisoning and deactivation [9]. The various nitrogen compounds generated in the HTL product biocrude include nitrogen heterocyclics (e.g., pyridine, pyrrole, pyrrolidine, piperdine, and indole) and non-heterocyclics, such as long-chain amines and amides [hexadecanenitrile and hexadecanamides – 6–14].

Several approaches to remove nitrogen from feedstock's, such as algae, have been examined. Barreiro et al. [14] examined the effects of enzymatic hydrolysis of proteins on HTL of two algal strains

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(*Nannochloropsis* and *Scenedesmus*). Through cell rupturing, coupled with enzymatic hydrolysis, they were able to extract 44.3% and 62% of the protein from each strain, respectively. This resulted in a net decrease in the nitrogen content of the bio-oil generated from HTL from 5.1% to 4.2% in *Nannochloropsis* at 350°C, with a similar trend observed in Scenedesmus. However, enzymatic hydrolysis removed 54% of the initial solids for *Nannochloropsis* and 81% for *Scenedesmus*, resulting in a significant decrease in the overall biocrude yield. In similar work, a minimum residence time of 2 hours was required with the most favorable results requiring 6 hours making scale-up more difficult to implement [15]. Another technique that has been investigated for reducing nitrogen content is the addition of alkali and organic acids in high temperature HTL [10]. In this study, 1 M alkali or organic acids (Na2CO3, KOH, HCOOH, and CH3COOH) were added to the HTL reaction of *Spirulina* and *Chlorella* microalgal strains. The average nitrogen content in the resulting biocrude was 4.7% for the HTL with alkali and 5.3% for HTL with organic acids; this was coupled with a maximum biocrude yield of 27.3% in *Chlorella* and 20.0% in *Spirulina*. The use of acid catalysts seems to be effective in increasing the quality of the biocrude by removing nitrogen, but the presence of the catalysts may become problematic for downstream processing, requiring additional input energy for separation of the acids before distillation of the biocrude. Hence, there is a need for alternative ways of removing nitrogen without the addition of external reagents such as catalysts, enzymes, or acids.

Levine et al., (2010, 2013) and Lu et al., (2015) used hydrothermal carbonization or HTC (water at 200–325°C; 15–120 min residence times) to pretreat the algae and form hydrolysis solids that could be easily filtered and allowed for separation of lipids from algae without using an external solvent and the need to dry the algae- [16–18]. Moreover, they demonstrated that a nutrient rich aqueous stream could be generated for recycle and algae cultivation. Treatment of the algae under these conditions allowed separation and recovery of the lipids via filtration of the algal solids for subsequent supercritical esterification using ethanol for fatty acid methyl ester generation and recovery of valuable fatty acids. These researchers also observed significant N and P partitioning between the aqueous and solid phase after HTC. These results clearly implied possible benefits for coupling pretreatment (algae hydrolysis at lower temperatures than HTL) with HTL; i.e., easy lipid recovery in the solid phase and partitioning of nitrogen, heteroatoms, and metals/micronutrients to the aqueous phase for reuse and a possible downstream benefit for catalytic upgrading of the HTL algal oil. Development of such a two-stage process would benefit from lower temperatures (≤ 200–225°C) and shorter residence times (≤ 15 min), which would reduce the energy input and required reactor volumes potentially allowing for continuous treatment.

Garcia-Moscoso et al. [19] examined a continuous flash hydrolysis method in a tubular reactor that utilized subcritical water to hydrolyze *Scenedesmus* proteins rapidly. They report that subjecting the algae slurry to subcritical hydrolysis temperatures (305°C) for 10 seconds can extract up to 66% of the nitrogen and percent removal increased from 205 to 305°C. Over this temperature range biomass yield decreased from 52% to 24%. However, heat transfer and flowability become major concerns for the scalability of this process, especially when the solid content is increased and the mechanism of hydrolysis depends on rapid heat transfer.

Chakraborty et al. [19] proposed a sequential hydrothermal extraction that utilizes both low-temperature (160°C) and high-temperature (300°C) hydrothermal liquefaction on *Chlorella sorokiniana* microalgae to both reduce nitrogen present in the generated bio-oil and to extract value-added polysaccharides. Through their two-stage batch process, they reduced the nitrogen present in their bio-oil from 1.14% using direct HTL to 0.78% using sequential HTL. Their algae feedstock was grown heterotrophically in fermentors, which yielded a low nitrogen feedstock (2.91%, initially). The addition of the low-temperature HTL decreased the bio-oil yield from 27.8% in direct HTL to 23.4%. Miao et al. [21] tested a similar sequential HTL of yeast (180°C followed by 220°C) and reduced the nitrogen in bio-oil from 1.13% using direct HTL to 0.51% using sequential HTL; their yeast feedstock contained a nitrogen content of 2.65%, which is significantly lower than many algal feedstocks due to the high protein content normally found in algae. This two-stage process had very little effect on the biocrude yield, which was ~58.3% for both direct and sequential HTL. In both Chakraborty et al. [20] and Miao et al. [21], the feedstocks studied were grown under ideal conditions. Realistically, large-scale algae cultivation must be performed under economically feasible conditions, balancing optimization of feedstock characteristics with economics and energy inputs.

Converting algae to fuel intermediates via HTL is appealing because the feedstock is renewable and energetically favorable since it utilizes a wet feedstock. Lack of a suitable method to reduce nitrogen in HTL bio-oil or biocrude can limit the widespread implementation of this technology. Although techniques involving enzymatic, acid or alkaline hydrolysis are effective to a certain extent in treating algae biomass prior to HTL, they potentially require costly downstream separations. Utilization of low-temperature pretreatment has been suggested, but limited studies on its effect on mass-produced algae have been performed. In principle, utilization of such a pretreatment could hydrolyze proteins without significantly hydrolyzing lipids, causing the degradation products to become water soluble and removing nitrogen from the solid phase [16,17,22,23]. The solids retained from the hydrothermal pretreatment could then be utilized as a low-nitrogen feedstock for high-temperature hydrothermal liquefaction to produce biocrude with a favorable elemental composition and the aqueous co-product phase from the pretreatment can be evaluated for characteristics that would allow nutrient recycle for algae cultivation [24–27]. Very recently, Jazrawi et al., [28] published evidence that a two stage HTL (<200°C, 250–350°C) process reduced the nitrogen content in biocrude by 55% relative to HTL alone for low lipid/high protein pond cultivated *Chlorella vulgaris*. It is also desirable to know the effect of this pretreatment stage on the bio-oil heteroatom composition, micronutrient, and inhibitor levels ultimately produced via HTL, since evidence suggests algae bio-oil is only economically feasible if the aqueous co-product generated from HTL is recycled for nutrient supplementation for algae cultivation, which is not presently very well-known [29].

This work expands on the results of Jazrawi et al. [28] and evaluates the use of a low-temperature hydrothermal pretreatment (PT) step over a wider temperature and residence time range as a nitrogen reduction method for three different algae feedstock's. Additionally, the pretreatment effect on protein, micronutrient, and inhibitor levels in the aqueous phase and heteroatom levels in the biocrude is presented.

2. Experimental

2.1. Materials

Freeze-dried *Spirulina platensis* was obtained from Earthrise Nutritionals LLC (Calipatria, CA), and *Nannochloropsis* sp. was obtained from Reed Mariculture ("Nanno 3600″, strain CCMP525). A consortium of three algal strains (UGA Consortium henceforth), namely *Chlorella sorokiniana*, *Chlorella minutissima*, and *Scenedesmus bijuga*, were grown and harvested for use in this study as well. Please note, that it was our original intent to grow three algae strains in the raceway. Subsequent analysis of the final harvested product indicated that only two strains predominanted - *Chlorella sorokiniana* and *Scenedesmus bijuga*.

2.2. Algae growth

A monoculture of the constituent strains were first grown in 20 L carboys under controlled conditions in a growth chamber at 25 ± 1°C for 12 h with alternating light–dark cycles; the light intensity was 100 μmoles m^{-2} s^{-1} with continuous air bubbling. The final consortium was prepared by mixing equal proportions (*v*/*v*) of each individual strain