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# Recycling nutrients from a sequential hydrothermal liquefaction process for microalgae culture

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ABSTRACT

Nutrient supply and reuse are critical considerations for culturing and processing algae as feedstock for biofuel production. Sequential Hydrothermal Liquefaction (SEQHTL) is used to convert microalgae biomass to biofuel and co-products. Along with biocrude and biochar of the co-products, SEQHTL produces aqueous products with high concentrations of phosphate, organic nitrogen and polysaccharides. In this study, three representative microalgaes, *Chlorella sorokiniana*, *Chlorella vulgaris* and *Galdieria sulphuraria* 5587.1 were evaluated for utilizing the nutrients recovered from the aqueous products of SEQTHL. *C. sorokiniana* and *C. vulgaris* exhibited the ability to hydrolyze polysaccharides, using 77% and 64% of the polysaccharides and removing 94% to 95% of the phosphate, respectively. *G. sulphuraria* on the other hand, could not use the polysaccharides. All three species could completely assimilate ammonia and use 33%–43% of the organic nitrogen. There were no significant differences in terms of lipid contents and composition, *C. sorokiniana* and *C. vulgaris* had higher lipid content (18% of DCW) than what *G. sulphuraria* did (only 10% of DCW). The findings indicate that although being species dependent, it is possible to reuse the nutrients recovered from SEQHTL of algal biomass for algal culture.

## 1. Introduction

Many technologies have been developed for the biorefining algal biomass, such as pyrolysis [1–3] and hydrothermal liquefaction (HTL) [4–6]. These two approaches convert the biomass into bio-oil, gas, and biochar using heat. Pyrolysis is more suitable for dry biomass. In comparison, hydrothermal liquefaction is more suitable for biomass with high water content, since reactions occur in compressed water under subcritical conditions. The most studied process was direct hydrothermal liquefaction in which the biomass feed was heated directly to the target subcritical temperature (DHTL) [7]. During this process though, many high-value products, such as polysaccharides and proteins, can be destroyed and converted to bio-oil and gas through polymerization, depolymerization and condensation reactions at temperature above 300 °C. Non-fuel components such as sterols were mainly converted to complex aromatics that deposited in the biochar fraction. The de-natured high-value products are converted into different chemicals, resulting in unwanted bio-oil qualities with high contents of heteroatom, acidity and high boiling point distribution. This, in turn, increased the complexity and viscosity of the bio-oil [8,9].

As it became clear that high-value co-products in the microalgae biomass has to be fully utilized to improve he economics of algal biofuel, a new concept of hydrothermal liquefaction was developed in 2012 [10–12]. In the modified hydrothermal liquefaction strategy, two-step Sequential Hydrothermal Liquefaction (SEQHTL) was employed to isolate polysaccharides from algae at a low temperature before HTL was used to obtain lipid-rich biomass (Fig. 1) [10–12]. This new technique makes it possible to take the advantages of value-added polysaccharide production that in turn improve the economics of algal bio-oil, and reduced biochar production. Furthermore, fine-tuning process conditions and reaction medium allows SEQHTL to be adaptable to favor extraction of different types of other compounds such as protein in the algal biomass.

Most studies have used about 20% of the feedstock for HTL process, producing large amounts of aqueous co-products with high concentrations of nitrogen and phosphate [7]. However, the aqueous co-products from HTL and SEQHTL are notably different. In SEQHTL, most polysaccharides in the microalgae are removed in the first stage as aqueous

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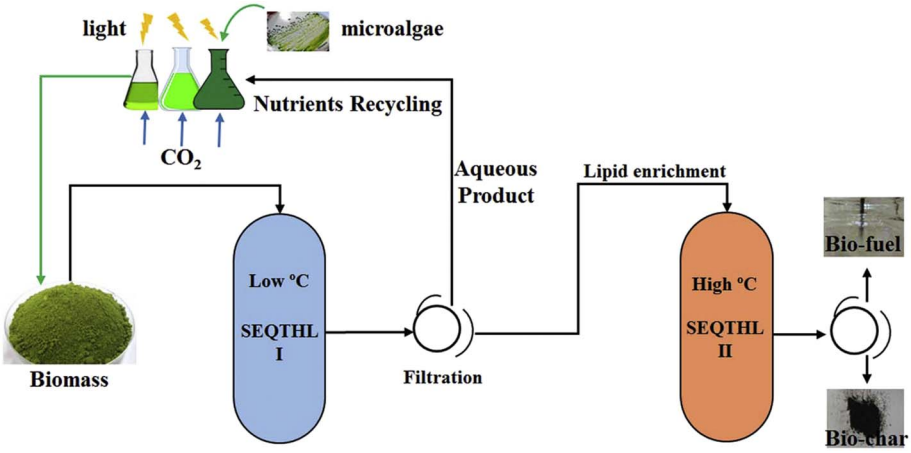


**Fig. 1.** Schematic model of SEQHTL biorefinery for microalgal biofuel and bio-products [10].

co-products, producing 63% less bio-char than HTL [11] and forming relatively low cytotoxic chemical levels [13].

Nutrients in the aqueous products can then be recovered or recycled to increase algae productivity or produce value-added co-products through fermentation or anaerobic digestion [14–22]. In fact, the incorporation of algae processing with nutrient recycling to the culture may increase biomass productivity ten-fold [8]. However, when protein and polysaccharides are otherwise turned into oil directly, the bio-oil from polysaccharides mainly contains cyclic ketones and phenols, as well as proteins composed of pyrazines, pyrroles and amines [9]. Therefore, the properties of the resulting bio-oil are poor, with low energy value.

The overall concept of recycling nutrients, combined with large-scale re-culturing of microalgae, makes it possible for a net harvesting of solar energy and $CO_2$ from the atmosphere to produce algal biofuel. This nutrient recycling strategy also provides a practical solution to the problem associated with potential large fertilizer demands for mass algae culture. However, nutrient recovery from hydrothermal liquefaction of algal biomass is highly dependent on algal species. In this study, we evaluated the potential of using aqueous co-products from SEQHTL to cultivate three representative microalgae, *Chlorella sorokiniana*, *Chlorella vulgaris* and *Galdieria sulphuraria* 5587.1 (GS). Findings of the study may inform the development of sustainable algae culture for biofuel production by recycling essential nutrients and non-fuel carbon from the algal biomass.

## 2. Materials and methods

### 2.1. Materials

Biomass of *C. sorokiniana* 1412.1 was obtained from New Mexico State University and used as the feedstock for the SEQHTL processing. The products of SEQTHL stage 1 were used as the growth medium for three representative species: *C. sorokiniana*, *C. vulgaris* and *G. sulphuraria*.

BG11 was used as the medium for the seeds of *C. sorokiniana* and *C. vulgaris* [23]. A modified Standard Cyanidium medium was used for the seed culture of *G. sulphuraria* [24]. Different dilutions (0 ×, 15 ×, 20 ×, 25 ×, 30 ×) of the SEQHTL aqueous products with 1.5 g $L^{-1}$ $NaNO_3$ were used to evaluate the fit of this fraction. The seed medium for each species was used for the controls.

All analytical grade chemicals were purchased from Sigma Aldrich (USA) unless otherwise noted, including standards of the monosaccharides (rhamnose, arabinose, galactose, glucose, xylose) and palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid. All required reagents for measurements of nitrogen and phosphate content were purchased, along with the procedure manual of the DR/2400 spectrophotometer, from Hach® Company, U.S.A.

### 2.2. Preparation of the aqueous product from SEQHTL

A microalgae and distilled water slurry (100 g for each run) with a ratio of 1:9 was fed to a bomb-type reactor (1 L, Parr4522, Parr Instrument Co.) to prepare the aqueous products according to pre-established conditions [12]. Next, nitrogen bubbling was used to purge the oxygen in the reactor. The reactor temperature was increased to 160 °C at a rate of 7 °C/min, and maintained for 20 min. The reaction pressure was monitored between 110 and 115 psi. Subsequently, the reactor was cooled to room temperature using chilled water. Finally, the aqueous product was prepared by collecting and filtering the aqueous reaction mixture with Whatman quantitative ashless grade-42 filter paper (2.5 μm).

### 2.3. Methods for the cultivation of microalgae

The seed of *C. sorokiniana* and *C. vulgaris* were cultured using the BG11 medium. The seed of *G. sulphuraria* was cultivated using a modified cyanidium medium (MCM) [25] for 72 h. Next, 5% (v/v) cultures with microalgae were transferred to the culture medium and cultivated for 10 days. *C. sorokiniana* and *C. vulgaris* were cultivated under 26 °C. *G. sulphuraria* was cultured on the shaker under 42 °C. All cultures were maintained under a fluorescent artificial light-cycle (12 h: 12 h), bubbled air (0.5% $CO_2$ contained), and in a shaker speed maintained at 150 rpm. To confirm whether the microalgae could utilize the aqueous SEQHTL products, different dilutions (0 ×, 15 ×, 20 ×, 25 ×, 30 ×) of the aqueous product were used as the culture medium for *C. sorokiniana* cultivation.

Next, the composition of the aqueous product samples was analyzed. Every two days, 2.0 mL of sample from each treatment was taken during the culture stage. A portion of the sample (0.5 mL) was used to determine the dry biomass. The remaining 1.5 mL was centrifuged for future analysis. A supernatant was used to measure the polysaccharides, reducing monosaccharide, and total nitrogen ($[NO_3^-]$ and $[NH_4^+]$) content. The precipitation of the cell biomass was stored in a freezer at − 80 °C for future determination of the lipid content. A 15.0 mL sample of the culture medium was used to determine $[PO_4^{3-}]$ at the end of 10 days.

The dry biomass of the microalgae was determined by recovering the wet biomass from the culture medium by centrifugation, then the cells were washed with distilled water and dried at 80 °C overnight until a constant weight was obtained.

All the experiments were carried out with three replicates and were repeated thrice. Data were shown as Means ± Standard Deviation.

### 2.4. Determination of carbohydrate, nitrogen and phosphate concentration

The phenol-sulfuric acid method was used to determine the total