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Two-stage hydrothermal liquefaction of a high-protein microalga

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ABSTRACT

Hydrothermal liquefaction (HTL) is a promising route for producing renewable fuels and chemicals from algal biomass. However, the protein fraction of the alga gives rise to nitrogen compounds in the oil fraction, which may render the oil unattractive for use in conventional refining processes. We report a two-stage HTL approach with the primary aim of reducing the nitrogen concentration in the bio-crude oil. A mild (<200 °C) pre-treatment step (Stage I) is performed before more severe (250–350 °C) HTL conditions (Stage II) are applied to the microalga *Chlorella* for the production of bio-crude in a batch reactor. The pre-treatment resulted in up to 50 wt.% of the input nitrogen crossing into the Stage I aqueous phase and, following Stage II processing, reductions in the bio-crude nitrogen contents of up to 55%, relative to the direct HTL of untreated *Chlorella* were observed. However, since considerable amounts of the starting material were lost in Stage I, overall lower quantities of bio-crude were isolated after Stage II processing, as compared to single-stage processing. Nitrogen extraction during Stage I is enhanced by the addition of acids (1 N sulphuric or formic acid) but the process remains unselective. Overall, it is concluded that the two-stage approach to reducing the nitrogen content of bio-crudes from a protein-rich alga requires careful evaluation of the trade-off between the desired bio-crude properties and the yield obtained.

1. Introduction

Hydrothermal liquefaction (HTL) has received increasing interest in the past decade as a process for converting biomass to drop-in biofuels and chemicals [1,2]. The HTL of biomass essentially mimics the natural geological processes believed to be responsible for the formation of fossil fuels in a time frame measured in minutes rather than over a geological time span [3].

Reaction temperatures of approximately 250–350 °C, and pressures high enough to maintain the water as a liquid (i.e., 40–250 bar), are generally employed. The biomass feedstock can be processed directly, without an energy-consuming drying step, since water acts both as solvent and catalyst [4]. The HTL of whole biomass yields a range of different products including bio-crude oil, aqueous dissolved chemicals, solid residue, and gas. The product yields and properties vary significantly according to the feedstocks processed as well as the reaction conditions employed.

Extensive research has been conducted processing lignocellulosic biomass under a range of subcritical water conditions [4,5]. More recently, a wide variety of aquatic plants – including micro [6,7] and macro algae [8] – have also been studied. Processing microalgal feedstocks via HTL possesses numerous advantages over other conversion routes, tolerating low cell concentrations (since HTL is a wet processing method), as well as allowing conversion of low-lipid strains, which often have much higher growth rates than those optimised to accumulate high lipid levels [6].

The algal bio-crude produced by HTL has been described as being similar to conventional crude oil, but bio-crude has significantly higher oxygen and nitrogen contents, typically ~10 to 20 wt.% and ~1 to 8 wt.% respectively [8,9], than conventional crude (both elements <1 wt.% [10]). In particular, processing high-protein algae has been found to significantly increase the nitrogen levels of the derived oils. This can impart a number of undesirable properties, including high viscosity and instability towards cross-linking/oligomerisation and can poison catalysts in conventional refining processes [11].

Bio-crude nitrogen derives from the protein present in the algal feedstock. Increasing HTL temperatures enhance the yield of the bio-crude, while also reducing its oxygen content, but these benefits come at the cost of increasing nitrogen content. This has raised the question as to whether it is possible to extract nitrogen-containing components prior to high-temperature HTL (which is carried out typically at temperatures in the range of 300 to 350 °C). In particular, reductions in nitrogen contents of the final bio-crude product are achieved through an

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initial mild HTL (temperatures < 200 °C) as a preliminary treatment, albeit with concomitant reduction in the quantity of the bio-crude generated [12–15]. While these results are promising, the relationships between quality and quantity of the bio-crude produced in such two-stage treatments are poorly understood. Furthermore, previous work was carried out under slow heating conditions and over extended reaction times, conditions likely to be uneconomical in large-scale production plants. Our previous work on continuous [7] and rapid batch heating [8] has shown that HTL of algal biomass can be achieved at significantly shorter reaction times (<10 min) and it is important to understand the trade-offs under these conditions between reducing nitrogen quality through mild hydrothermal pre-treatment and maximising bio-crude yield. Here we note that protein hydrolysis in hot aqueous environments is generally very slow and that the standard laboratory conditions for complete hydrolysis are strongly acidic (typically 6 N HCl at 110 °C for 24 h) — therefore, we have also investigated the use of acidic media for the preliminary hydrothermal treatment, with a view to enhancing the rapid extraction of insoluble peptides prior to HTL for bio-crude production. In this paper we investigate the relationship between the yield and the nitrogen content of bio-crude when a microalgal biomass is subjected to two-stage HTL in which the first stage is at lower temperatures. We also investigate the effect of carrying out the first-stage HTL under acidic conditions, comparing the results from the addition of an inorganic acid (H_2SO_4) with those obtained with the use of formic acid, representing organic acid that could in principle be produced from biomass.

2. Materials and methods

2.1. Algal biomass

The microalga investigated (*Chlorella vulgaris*, purchased from Synergy Natural Limited) was grown in freshwater ponds before spray drying and packaging in powder form. The biochemical composition (lipids, protein, carbohydrates) was provided by the supplier. Moisture content was determined by weight loss from a ~3 g sample upon heating in an oven at 105 °C for 2 h while the ash content was determined from the residual mass obtained after heating ~3 g of dried sample in a muffle furnace for 3 h at 550 °C. The elemental analysis (C, H, N and S, with O determined by difference) was obtained using a commercial analyser (Flash EA 1112, CE Instruments, UK). The proximate and elemental analyses are reported in Table 1 as the average of duplicate assays, the maximum deviation between runs being ±0.3%. These analyses are typical of a low-lipid, high-protein microalga.

Table 1
Analysis of *Chlorella* feedstock.

Analyses	*Chlorella*
Proximate (wt.% as received)	
Ash	6.0
Moisture	5.2
Elemental (wt.% daf)[a]	
C	53.5
H	7.4
N	11.0
S	0.5
O[b]	27.6
Biochemical (wt.% as received)	
Carbohydrates	25
Protein	60
Lipids	4

daf = dry ash free.
[a] Average of duplicates; variation < 0.3%.
[b] By difference.

2.2. Reactor setup

Reactions were carried out in a small-scale (20 mL) rapid-heating/cooling batch reactor system described previously [8]. Briefly, the reaction volume is formed by a 120 mm length of ¾″ outer diameter stainless steel 316 tube (wall thickness 1.65 mm), closed at one end and connected to gas supplies via a length of ¼″ tubing. Swagelok fittings are used throughout, enabling rapid dismantling and reassembly of the system. The reactor is loaded with biomass/water (see below) and purged with nitrogen before being pressurised, also with nitrogen, to ~90 bar. It is then immersed in a pre-heated fluidised sand bath (Techne SBL-2D) to raise the temperature to the desired experimental set-point. The temperature in the reactor is measured through a centrally located K-type thermocouple — typically, the internal temperature approached within <10 °C of the set-point within 1–2 min. The reaction times reported include these heat-up periods. Transport of water vapour out of the reactor volume is inhibited by restricting the opening in the connecting tube to an annular opening of 0.165 mm over a 50 mm length of the ¼″ connecting tubing. At the completion of the reaction period the reactor volume, still attached to the connecting tubing, is plunged into an ice bath, causing the temperature to decrease to <20 °C within 30 s.

2.3. Processing methodology and analytical techniques

Fig. 1 describes the two-stage experimental protocol and nomenclature used in the following descriptions. In all cases, the feed to the first stage is a suspension of nominally 20 wt.% dry *Chlorella*, prepared by mixing ca. 2.5 g of dry biomass with 10 mL of aqueous solvent. The feed to the second stage is then made up as a 10% aqueous suspension of the dry solids produced in the first stage. Bio-crude is produced only in the second stage; the first stage did not produce any identifiable oil phase (<1 wt.% yield, as determined by dichloromethane (DCM) extraction, see below). Gas production in Stage I is also expected to be negligible [16].

First stage treatments were performed using three different solvents (distilled water, 5 wt.% sulphuric acid, and 5 wt.% formic acid — the acids are each approximately 1 N) at temperatures in the range of 100–200 °C and at residence times of 5 and 15 min. The product mixture was subsequently collected and centrifuged to separate the Stage I Solid and Aqueous Phase.

The Stage I Solids fraction was dried, weighed and analysed for its elemental composition and ash content. Solid yields were then calculated based on the recovered weight and are reported as a fraction of the dry feedstock. The experiments were conducted in duplicate, for which the maximum absolute error for the solid yield was <2%; only average values are reported.

The Stage I Aqueous Phase was analysed for total organic and inorganic carbon (TOC and TIC, IL550 TOC, Hach-Lange, Germany). The analyses were carried out in duplicate, for which the maximum relative deviation was <2%, and we report the average result. Total nitrogen (TN) was determined by colourimetry (test cuvettes LCK338, Hach-Lange, Germany).

The treated *Chlorella* solids obtained from Stage I were dried, mixed with distilled water, and returned to the reactor for the second stage of the process. All the runs in Stage II were processed with a 10 wt.% feed concentration (1.0 g dry solids with 9 mL distilled water). Reactions were carried out over a range of temperatures (250–350 °C) with a constant reaction time (10 min) and the product yields and quality were determined.

The Stage II HTL oil–water–solid product mixture was decanted and the reactor washed using DCM and distilled water (30 mL each in 15 mL aliquots). The resulting mixture was separated in a separating funnel followed by filtration and DCM solvent evaporation to afford a bio-crude oil, a solid residue and an aqueous phase — as described