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Two-step thermal conversion of oleaginous microalgae into renewable hydrocarbons

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HIGHLIGHTS

- Renewable hydrocarbons were produced via hydrolysis–pyrolysis of algal biomass.
- Microalgal fatty acids were completely and selectively extracted from hydrolysates.
- Recovered fatty acids were free of sulfur and low in salts and nitrogen.
- Pyrolysis of microalgal fatty acids yielded deoxygenated renewable hydrocarbons.

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ABSTRACT

The aim of this study was to evaluate the conversion of microalgal biomass to renewable chemicals and fuels through a two-step reaction and separation process. High density *Chlorella protothecoides* culture with 40% lipid accumulation (dwb) was produced in 10 L bioreactors and hydrolyzed in batch stainless steel reactors under subcritical conditions. After hydrolysis, fatty acids free of sulfur and low in nitrogen and salts, were recovered by hexane extraction. The fatty acids were pyrolyzed at 410 °C for 2 h under N_2 yielding *n*-alkanes, α-olefins and internal olefins and low molecular weight fatty acids. This study demonstrated the direct conversion of microalgal biomass into valuable platform chemicals and fuels compatible with the existing industrial hydrocarbon infrastructure.

1. Introduction

Algal biomass can be utilized to produce renewable biofuels either by direct processing or by extracting individual components. The conversion of whole algal biomass into bio-oil is generally carried out in water at temperatures between 200 and 600 °C and at pressures between 5 and 40 MPa (Peterson et al., 2008). The separation of individual components from algae, especially lipids for biofuels, offers a wider range of opportunities for by-products utilization (Chisti, 2007; Rodolfi et al., 2009). However, high infrastructure and operating costs associated with this approach pose significant challenges to the commercial deployment of these technologies.

Typical algal cultures generally are characterized by low cell densities. This requires dewatering, an energy intensive operation which can represent between 30% and 50% of the total microalgal biodiesel cost (Chisti, 2007; Wijffels and Barbosa, 2010; Yeh et al., 2013). As microalgae are generally characterized by small and thick cell wall that require harsh disruption and extraction methods (Halim et al., 2012; Yeh et al., 2013), typical laboratory extraction protocols involve unit operations such as freeze drying, pulverization, cell homogenization, sonication, autoclaving, osmotic shock or microwaving are not good candidates for industrial scale up (Lee et al., 2010).

Renewable fuels produced from thermochemical reactions followed by hydrotreating and hydrocracking have been reported. The typical product distribution from these processes contains high concentrations of nitrogen, sulfur and/or salts. (Ross et al., 2010; Zhou et al., 2010; Anastasakis and Ross, 2011; Jena et al. 2011; Valdez et al., 2011; Vardon et al., 2012; Alba et al., 2012). These elements are undesirable in biofuel and therefore upgrading is required to meet fuel specifications (Duan and Savage, 2011; Alba et al., 2012).

An alternative approach is represented by the possibility of treating algal biomass at temperatures below 300 °C. This approach represents a pre-treatment step known to efficiently disrupt the algal cell wall, eliminating the need to dewatering and separating reaction products while reducing the content of the

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nitrogen and other undesired elements in the bio-oil (Levine et al., 2010; Du et al., 2012). After thermal pretreatment of algal biomass, the aqueous streams separated from the solid pastes, have been successfully used to produce biofuel (Levine et al., 2010; Du et al., 2012). This approach, however, requires further upgrading steps of the bio-oil given the considerable amounts of remaining undesirable elements in the product.

The aim of this work was to address this technological challenge by evaluating the conversion of microalgal biomass to renewable chemicals and fuels utilizing a modified two-step thermal lipid-to-hydrocarbon technology developed in our laboratory (Bressler, 2011). This paper presents an adaptation of this process to high lipid content algal biomass. After hydrolysis, the aqueous stream was separated and the non-aqueous stream was treated with hexane for only recovering the fatty acids which were exclusively used in the pyrolysis reaction.

2. Methods

2.1. Materials

Microalgae *Chlorella protothecoides* (UTEX 256) was directly obtained from a culture collection (University of Texas, Austin, TX). Axenic stocks were stored at room temperature with 25 μmol/m^2 s at 12/12 h light/dark cycles as long storage conditions. All chemicals used for media preparation were purchased from Sigma–Aldrich (St. Louis, MO). Sulfuric acid and hexane (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ) and nitrogen (99.998%) was obtained from Praxair (Missigauga, ON). All chemicals were used as received.

2.2. Biomass production

C. protothecoides was selected for this study as model of high density and high lipid content microalgae (Bumbak et al., 2011). *C. protothecoides* was grown in fed-batch regime in a 10 L bioreactor (Biostat B. Sartorius, Germany). Media used for growth was composed of a mineral base (Siegler et al., 2011) with glucose and yeast extract as the main carbon and nitrogen sources, respectively. Lipid accumulation in the microalgae was achieved by maintaining low nitrogen-to-carbon mass ratio in the media (Xiong et al., 2008). The reactor was inoculated at 1.5 OD_{600nm} from a shake flask culture entering the stationary phase. Concentrated solution (400 g/L glucose, 16 g/L yeast extract) was added to the fermentation at irregular intervals to maintain glucose concentration between 10 and 30 g/L to avoid substrate inhibition (Xiong et al., 2008). Aeration rate and stirrer speed were varied between 1–2 vvm and 100–200 rpm respectively, to keep air saturation over 20% (Xiong et al., 2008). When necessary, pure O_2 was mixed with air for the same purpose. During kinetics, glucose concentration was monitored by the GOPOD method (Megazyme, test kit); growth was measured by OD_{600nm} with standard curve for dry biomass, while lipid accumulation was measured by fluorescence with Nile Red (De la Hoz Siegler et al., 2012). At harvesting time, algal biomass was concentrated at 5000g for 10 min in an Avanti, Beckman Coulter centrifuge (Brea, CA) and the slurry stored in a fridge (2 °C) before being used for the hydrolysis experiments. Fermentations were conducted in triplicate to produce enough biomass for the experiments. Algal slurries were pooled and homogenized for use in hydrolysis reactions. This slurry had 27 ± 1% total solids.

2.3. Hydrolysis

Hydrolysis experiments were conducted in two different scale reactors in order to achieve quantities required for either overall product distribution and mass balance determination or quantities for the large scale reaction. In the first case, 15 mL stainless steel batch reactors heated in a Techne model SBS-4 fluidized sand bath with a Techne TC-8D temperature controller (Burlington, NJ) (Maher et al., 2008). Large-scale reactions were conducted in a 2 L batch stainless steel reactor (Parr Series 4530, Parr Instrument Co., Moline, IL). Hydrolysis reactions in both cases were conducted at 280 °C for 1 h with an initial pressure of 500 psi. These conditions were selected based on lipid hydrolysis optimization studies conducted in our laboratory (personal information). The reactors were loaded with 10 g or 600 g algal slurry for the 15 mL and 2 L reactor respectively, purged three times at 500 psi and pressurized to the desired initial pressure with nitrogen. Hydrolysis time was considered when the set temperature was reached and the reaction was stopped by quenching in a water bath for the small reactor and by using an external cooling unit for the 2 L reactor.

2.4. Post-hydrolysis treatment

2.4.1. 2 L reactor

Samples of the gas fraction were collected during the depressurization cycle following reaction completion. Samples of liquid and solid products were collected using plastic containers. A büchner funnel with glass fiber Whatman GF/A filter (Whatman, Maidstone, Kent) was used for initial separation of aqueous fraction and paste. Paste was then successively washed with de-ionized water and hexane and the filtrates were collected in separate containers. Hexane solubles were recovered by evaporating hexane in a rotovapor (Büchi, Flawil, Switzerland). Hexane soluble products were further dried in a convection oven for 2 h at 105 °C to determine their mass gravimetrically. The insoluble paste retained on the filter was dried in a convection oven at 105 °C to a constant weight and this fraction was considered as insoluble solids (Fig. 1).

2.4.2. 15 mL reactor

The mass of gas was determined by measuring the weight of the reactor before and after venting (Maher et al., 2008). The composition of the gas fraction was analyzed on GC–TCD and GC–FID following the method described by Asomaning et al. (2013). The reaction product was collected in pre-weighed 50 mL plastic conical centrifuge tubes (Fisher Scientific, Fairlawn, NJ), frozen at −80 °C and freeze dried for 48 h. The weight of the freeze dried material was used to calculate the mass of the aqueous and soluble products by difference.

2.5. Pyrolysis

Pyrolysis was conducted in 15 mL batch stainless steel microreactors as described by Maher et al. (2008). Briefly, approximately 1 g of hexane soluble lipids was weighed into a clean and dry microreactor which was then closed, checked for leaks, purged with nitrogen, and sealed. The microreactor was heated at 410 °C under constant agitation for 2 h and then immediately quenched in a bucket of water at room temperature. The surface of the microreactor was then cleaned and dried using compressed air to ensure complete removal of sand residues.

2.6. Analysis of products

2.6.1. Proximate

Moisture content was determined gravimetrically by drying in convection oven at 105 °C to a constant weight. Total lipids content was determined gravimetrically following the extraction method described by De la Hoz Siegler et al. (2012). Ash determination was conducted gravimetrically by weighting residues after complete combustion in a muffle furnace at 550 °C. Total nitrogen