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Microbial utilization of aqueous co-products from hydrothermal liquefaction of microalgae *Nannochloropsis oculata*

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HIGHLIGHTS

- Aqueous co-product from microalgae liquefaction was used for microbial growth.
- Yield, growth rate, and carbon usage data were generated for two model bacteria.
- A microbial side-culture may increase efficiency of a microalgae biofuel operation.

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ABSTRACT

Hydrothermal liquefaction of algae biomass is a promising technology for the production of sustainable biofuels, but the non-oil, aqueous co-product of the process has only been examined to a limited extent. The *aq*ueous phase from liquefaction of the *al*ga *Nannochloropsis oculata* (AqAl) was used to make growth media for model heterotrophic microorganisms *Escherichia coli*, *Pseudomonas putida*, and *Saccharomyces cerevisiae*. Growth rates, yields, and carbon/nitrogen/phosphorus uptake were measured. *E. coli* and *P. putida* could grow using AqAl as the sole C, N, and P source in media containing 10 vol.%–40 vol.% AqAl with the best growth occurring at 20 vol.%. *S. cerevisiae* could grow under these conditions only if the media were supplemented with glucose. The results indicate that in a biorefinery utilizing algae liquefaction, the aqueous co-product may be recycled via microbial cultures with significantly less dilution than previously published methods.

1. Introduction

Hydrothermal liquefaction is a promising technology for producing bio-fuels and substantial research is underway investigating its potential use on several feedstocks, including algae (Greenwell et al., 2009). The process involves heating an aqueous suspension of biomass to 200–350 °C at pressures high enough to keep water in liquid phase. At these temperatures, macromolecules in the biomass break down into smaller molecules that may then repolymerize into a viscous "bio-crude" oil product similar to crude petroleum (Peterson et al., 2008). This type of processing avoids the costly drying steps of other algal biofuel production methods. It also produces bio-crude oil from lipid, protein, and carbohydrate biomass components (Biller and Ross, 2011; Brown et al., 2010). However, previous studies focused primarily on the characteristics and potential uses of the bio-crude oil product of the hydrothermal treatment, while the aqueous co-product remains largely uninvestigated. While not directly upgradable to usable fuels, this aqueous co-product contains up to 45% of the initial carbon of the biomass feedstock and the majority of other components such as nitrogen and phosphorous (Valdez et al., 2012). Disposal of this material as a waste stream would be an energetic and economic burden on a large-scale process from both a nutrient loss and wastewater-processing standpoint. A recent life cycle analysis study concluded, for example, that compared to fuels derived from terrestrial crops, algae fuels may actually cost more energy to produce, a result heavily influenced by fertilizer production energy requirement (Clarens et al., 2009). Efficient utilization of the aqueous co-product is a key factor affecting the overall sustainability of the process since the nutrients contained within it may represent a substantial amount of the energy investment. This

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work examines the composition of this *aq*ueous co-product from hydrothermally treated *al*gae (AqAl) and investigates its utility as a substrate for microbial cell culture.

Recycling AqAl as a media component for algae feedstock cultivation is one potential method for utilizing this material and has been investigated recently. However, algae growth inhibition has been observed at very dilute levels of AqAl concentration (Biller and Ross, 2011; Jena et al., 2011b; Tsukahara et al., 2001). Even if AqAl could be recycled back to an algae growth operation, it would cause accumulation within the system of toxic compounds and substrates not utilizable by the algae. Inserting a microbial growth operation into the process may serve to detoxify the AqAl before it is fed back to the algae. For instance, phenols are known algae growth inhibitors that have been found in AqAl (Biller and Ross, 2011; Jena et al., 2011b), and bacterial cultures have been shown to reduce the concentrations of these compounds in wastewater (Bajaj et al., 2008). Furthermore, microbes grown on AqAl could provide a supplementary source of biomass to the hydrothermal reaction operation, increasing the carbon efficiency of the whole process. It has also been proposed that an anaerobic fermentation step could convert organic carbon in the AqAl to biogas, which could be burned to power the biorefinery facility (Davis et al., 2011).

However, there has been little investigation regarding the quantity, quality, or toxicity of the substrates in AqAl for such an operation. The goal of this study was to investigate the culturability of model microorganisms on media containing AqAl as the sole C, N, and P sources. *Escherichia coli* and *Saccharomyces cerevisiae* were chosen since they have been extensively studied and widely utilized in industrial processes, and each have well-established methods for genetic manipulation and process scale-up. *Pseudomonas putida* has also been studied substantially for its potential in bioremediation and was included in this work due to its robust and versatile metabolism (Nwachukwu, 2001).

2. Methods

The study began with the hydrothermal reaction of algae biomass and separation of the aqueous phase (AqAl) from the solid and oil products. This AqAl was used to formulate cell culture media where it was the only C, N, and P source. These media were used to aerobically culture bacteria *E. coli* and *P. putida* in 50 mL tubes. After incubation, cells were separated from the supernatant and concentrations of C, N, and P compounds in spent media were compared to those of the initial, fresh media. Cultures were also grown in 96-well microplates in order to discern growth kinetics (through periodic measurement of optical density). This experimental setup is summarized in Fig. 1. The growth of the yeast *S. cerevisiae* on AqAl was investigated as well, though not as thoroughly due to poor initial performance. Specific procedures for each step are as follows.

2.1. Hydrothermal liquefaction

Nannochloropsis oculata (a strain often used for hydrothermal liquefaction studies due to high bio-crude oil yields and commercial availability) algae slurry was purchased from Reed Mariculture Inc. as the source of biomass. The material was ∼35 wt.% solids (the remainder as water), and composed of 59 wt.% proteins, 14 wt.% lipids, and 20 wt.% carbohydrates on a dry basis, as reported by the supplier. Deionized water was added to the slurry to adjust it to 20 wt.% solids content before reaction. For each reaction, 150 mL of 20 wt.% slurry were loaded into a Parr 4570 Pressure Reactor with a calculated total volume of 283 mL. A LC Miller PR-15AB induction coil heater was used to raise the temperature to 350 °C and then hold it for a period of one hour. The average heat-up time for each reaction was 8.7 min and the temperature was maintained within 2 °C of the target. The reactor was agitated by an impeller at 800 rpm and reached a stable pressure of 100 bar during the reaction. After one-hour of reaction time, the reactor was cooled to room temperature and the gas product was vented. The contents of the reactor (consisting of solid, aqueous, and oil products) were mixed with 100 mL of dichloromethane (Optima grade, >95% purity) and transferred to a glass separatory funnel. The dichloromethane-soluble product fraction was defined as the "biocrude oil" product and the remaining water-soluble phase as the aqueous product (AqAl). This is a common practice among researchers investigating hydrothermal liquefaction of algae (Biller and Ross, 2011; Jena et al., 2011b). After one hour of equilibration time, the AqAl was decanted from the oil phase. The AqAl was further purified by vacuum filtration through a Corning 0.2 μm cellulose acetate filtration apparatus in order to remove residual oil and solids. This final product was considered "raw AqAl". This entire reaction and separation process was repeated three times in order to generate enough AqAl for bacterial growth experimentation and the resulting batches were combined. Hydrothermal liquefaction reactions to generate AqAl for yeast growth tests were done on a separate date, and varied slightly from the above method in that they used a 15 wt.% algae slurry. Previous work has indicated that distribution of products for hydrothermal

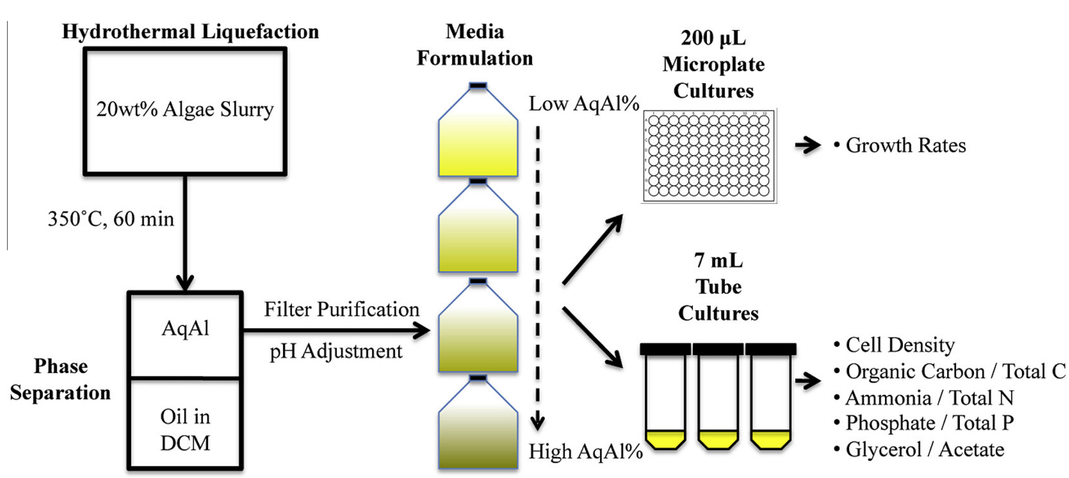

Fig. 1. Experimental flowchart for bacterial growth studies illustrating material generation steps and the data acquired from each culture study.