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Valorization of the aqueous phase obtained from hydrothermally treated *Dunaliella salina* remnant biomass

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HIGHLIGHTS

- The valorization potential of *D. salina* remnant by mild HTL was investigated.
- *D. salina* remnant primary consists of carbohydrates (~85 wt%).
- Biomass conversion during HTL mainly occurred into glucose.
- HTL derived glucose was successfully tested as microbial C-source for biotechnology.
- Glucose by-production is beneficial for the process economics of β-carotene production.

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ABSTRACT

Up to 90% of *Dunaliella salina* biomass remains unused after extraction of the main product β-carotene. The potential of mild hydrothermal liquefaction (HTL) to exploit this biomass as a source of valuable by-products was assessed. The results indicate that 80% of the remnant was converted into glucose by mild HTL (100 °C, 0 min). The recovered glucose was successfully used as a carbon source to cultivate biotechnologically relevant microorganisms, namely *Chlorella vulgaris*, *Escherichia coli* and *Saccharomyces cerevisiae*. Furthermore, the analysis of energy demand and operating costs confirms the beneficial effect of mild liquefaction on the overall process economics of algal β-carotene production.

1. Introduction

In recent years, hydrothermal liquefaction (HTL) has become an attractive subject of research, especially in the area of microalgal biofuel generation (Orfield et al., 2014). The approach has the clear advantage to use water as reactant, allowing high moisture contents of the biomass (Yang et al., 2004). In microalgal processes biomass dewatering is one of the most cost-intensive steps in the production. Thus, product extraction technologies which operate efficiently with wet biomass are in great demand. In addition, the bio-oil yield under typical HTL conditions is significantly higher compared to that of conventional extraction because carbohydrates and proteins are partly converted into organic solubles as well (Frank et al., 2013; Delrue et al., 2013).

In general, liquefaction leads to the hydrothermal conversion of solid biomass into a bio-crude, an aqueous and a gas fraction. Furthermore, also unconverted biomass remains as solid residue. The reaction is operated at high temperatures of 300–350 °C and pressures of 5–20 MPa (Chen et al., 2015). However, various studies demonstrated significant product yields even under milder conditions (e.g. Gai et al., 2015; Minowa et al., 1995). During the time course of liquefaction, the initial hydrolysis and depolymerization compete with repolymerization at a later stage (Gai et al., 2015). The fractions obtained by the hydrothermal treatment are strongly dependent on the biochemical composition of the applied biomass (Biller and Ross, 2011). Nevertheless, the study of Yu et al. (2011) revealed, that even low-lipid biomass can be attractive for bio-oil production, which is the main product of interest of the liquefaction. However, there are various other valuable components found in the product fractions, e.g. nutrients, organic acids, alkanes, alkenes, cyclic ketones and phenols and nitrogenous organic compounds (Brown et al., 2010; Biller and Ross, 2011; Pham et al., 2013). To achieve an optimal valorization of the product fractions of HTL, researchers are looking for possible applications of the aqueous phase obtained in liquefaction besides the already

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exploitable bio-oil phase. One successfully investigated approach is the nutrient recycling from the aqueous phase for microalgae cultivation (López Barreiro et al., 2015; Hognon et al., 2015; Biller et al., 2012).

The green microalga *Dunaliella salina* is an industrially used production organism of natural β-carotene. After extraction of the pigment up to 90% biomass remains unexploited in the process. The valorization of this remnant biomass can improve the overall process economics significantly. With β-carotene extraction by organic solvents, the main fraction of triglycerides in the biomass is extracted as well. Thus, conventional liquefaction of the remnant biomass seems not to be promising for lipid-based biofuel production at the first glance. As the alga has no rigid cell wall, cell constituents are easily accessible and due to the small cell size a rapid heat transfer during liquefaction is possible. This could facilitate the release of other valuable products from the remnant biomass. The present work aims for the assessment of mild HTL of extracted *D. salina* biomass. Initially, the macromolecular and elemental biomass composition was determined to identify possible liquefaction products. Under consideration of the process economics, moderate temperatures between 100 and 200 °C were investigated in the absence of a catalyst. Thereafter, the recovered glucose-containing aqueous phase was used as carbon source for mixotrophic or heterotrophic cultivations of three different microorganisms. One of the main challenges of HTL is the considerable energy consumption due to the high operation temperatures and pressures used in the process. Therefore, energy consumption and operating costs for the applied liquefaction condition of *D. salina* were calculated to finally evaluate the results.

2. Materials and methods

2.1. Origin and composition of the biomass

D. salina biomass was purchased as a carotenoid-containing dried powder from Denk Ingredients GmbH, Germany (Art. No: 967996). Prior to hydrothermal treatment, pigments were extracted to get remnant biomass. The extraction was carried out for 5 h using a Soxhlet extractor and n–hexane as extraction solvent. After the solvent has been evaporated in a rotary evaporator, the concentrated extract as well as the extracted biomass were dried overnight, respectively. The lipid content of the raw biomass was estimated from the weight of the dried, solvent free extract. The fraction of carotenoids in the biomass was measured spectroscopically using the protocol of Lichtenthaler (2001).

The carbon, hydrogen, nitrogen and sulfur (CHNS) contents in the remnant biomass were analyzed by elemental analysis (Currenta, Germany). Moisture and ash contents of the extracted *D. salina* powder were determined by weight difference of samples prior and after overnight drying at 100 °C and 450 °C, respectively. The concentration of carbohydrates was quantified based on glucose by an enzymatic test kit (R-Biopharm AG, Germany). The method of Lowry was used for protein content determination (Lowry et al., 1951).

2.2. Mild hydrothermal liquefaction of remnant biomass

A 200 mL stainless steel batch reactor (Picoclave 3, Büchi Labortechnik GmbH, Germany) was used to hydrothermally liquefy the *D. salina* biomass. Therefore, a slurry containing 6 g of the extracted biomass mixed with 100 mL bidistilled water was placed in the reactor. After sealing the reactor, the headspace was purged by nitrogen for 5 min to remove air. During an experiment the suspension was mixed with a frequency of 1800 rpm. The reactor was operated at temperatures and reaction times between 100–200 °C and 0–60 min, respectively. Once the set point of temperature was reached, the reaction time was registered. For 0 min reaction time, the heating process was directly stopped after reaching the set temperature. The time courses of temperature, pressure and stirrer frequency were recorded (Büchi log'n see bls2, Büchi Labortechnik GmbH, Germany). After cooling down, the reactor content was transferred through a preweighted filter into a separation funnel. To collect any remaining lipophilic products, the reactor and stirrer were rinsed with 60 mL n–hexane. Afterwards, the n–hexane mixture was passed through another preweighted filter into the separation funnel containing the aqueous phase. Filters were dried and oil residuals in the filter and on the solid surface were recovered by applying 30 min Soxhlet extraction using 60 mL n-hexane. Afterwards, filters were dried again and weighted to determine the yield of the solid phase. The immiscible water-hexane system in the separation funnel was intensively mixed to extract all bio-oil products into the hydrophobic phase. After that, the biphasic mixture was allowed to separate into an aqueous and a hydrophobic n-hexane phase. To recover the bio-crude the n-hexane phase was mixed with that obtained during Soxhlet extraction of the filters and evaporated at 40 °C and reduced pressure. For the quantification of the bio-crude fraction the remaining lipophilic substances were dried overnight. The yields of all product fractions were calculated based on the dry weight of the used biomass. The yield of the aqueous phase was determined by weighting two 6 mL samples of the aqueous phase after overnight drying. The yield of the gas phase was calculated as subtraction of the yields of aqueous, solid and bio-crude phase from 100%. Since the yield of the aqueous phase clearly exceeded that of the other phases, detailed investigation was done to identify which chemical compounds.

2.3. Analysis of the aqueous phase

Concentrations of glucose, fructose, sucrose, galactose and glycerol were determined in duplicates or triplicates using substrate specific enzymatic test kits (R-Biopharm AG, Germany) based on absorbance measurements at 340 nm. Nutrient concentrations were determined by ion chromatography (930 compact IC flex, Metrom, Switzerland). Therefore, concentrations of anions were measured using a Metrosep A Supp 5 column at 35 °C, an eluent containing 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ and a flow rate of 0.7 mL min^{-1}. Cations were measured using a Metrosep C6 column at 45 °C, an eluent containing 1.7 mM HNO_3 and 1.7 mM $C_7H_5NO_4$ and a flow rate of 0.9 mL min^{-1}.

2.4. Cultivation of different microorganisms on glucose obtained from the aqueous phase

Cultivation experiments were conducted using microbial representatives of highly relevant production organisms in biotechnology, which are already industrially applied for the production of a wide range of products (see Section 3.5 for more details). *Chlorella vulgaris* SAG 211.12, *Escherichia coli* MG1655 and *Saccharomyces cerevisiae* Y187 were used as model organisms for algae, bacteria and yeast, respectively. *C. vulgaris* was grown mixotrophically at a pH of 7.1 in 300 mL shaking flasks containing 100 mL BG11 medium (Stanier et al., 1971) with 0.5% glucose. The cultivations were carried out in a rotary shaking incubator as previously described in Pirwitz et al. (2015b). *E. coli* was cultivated aerobically in 500 mL shaking flasks filled with 75 mL LB medium (tryptone 1%, yeast extract 0.5%, sodium chloride 0.5%, glucose 0.5%) adjusted to a pH of 7. The cultivation occurred at 37 °C and a mixing frequency of 200 rpm. Growth experiments with *S. cerevisiae* were carried out under aerobic condition using