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Microalgae growth on the aqueous phase from Hydrothermal Liquefaction of the same microalgae

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HIGHLIGHTS

- Detailed evaluation of the participation of AP-N compounds during growth.
- Micronutrients (e.g. Mg) must be supplied upon AP-recycling to avoid growth reduction.
- Insufficient aqueous phase dilution does not seem to be the cause of growth reduction.
- 50% N recycling was proven to be possible and it can be further improved.
- Algae showed a preference for NH_4-N with no evidence of organic-N consumption.

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ABSTRACT

Cultivation of *Desmodesmus* sp. microalgae in the recycled aqueous phase (AP) recovered after Hydrothermal Liquefaction (HTL) of the same microalgae was studied to evaluate the potential of nutrients recycling. AP dilution ratio was systematically varied, using either water or water enriched with standard medium, while keeping the same N concentration (either as total-N or as sum of ammonia and nitrate) as that in the standard medium. More than 90% of the organic compounds in the AP were indentified and quantified and potential growth inhibiting substances (e.g. phenols) were found. However, the combination of growth and analytical results showed that the lack of (macro-/micro-)nutrients, other than N and P, in the AP is the main cause of growth reduction rather than toxicity due to insufficient AP dilution, as pointed in previous investigations. Therefore, these (macro-/micro-)nutrients such as Mg should be supplied upon AP recycling. For this specific cultivation case, algae production costs related to nutrients consumption can be significantly reduced considering that a 50% N-replacement was achieved, while showing nearly identical growth as that in standard culture medium.

1. Introduction

Algae are the fastest growing photosynthetic organisms on earth and therefore an interesting resource for energy, as they are capable to store sunlight in the form of energy-rich organic compounds and do not directly compete for arable land. The productivity of algae could be 50 times higher than that of switchgrass, one of the fastest growing terrestrial plants [1]. However, the current estimated costs for algal cultivation are significantly higher; 5–10 $ kg^{-1} [2] vs. 0.025–0.1 $ kg^{-1} [3] for switchgrass. Moreover, the energy requirements associated with the production of nitrogen nutrients (e.g. nitrate) represent a significant part of the total energy inputs, comparable to the electrical requirements [4]. Also phosphorus, another essential nutrient, is a non-renewable resource and current global reserves may be depleted in 50–100 years [5]. In our research we aim to alleviate these issues for algae biorefinery concepts by recycling of these nutrients, from downstream processing back to the algae cultivation section.

In an algae biorefinery scheme, several extraction and conversion processes could be combined to co-produce high value-added products, feed/food ingredients and energy carrier products from microalgae. A promising conversion method hereto is Hydrothermal Liquefaction (HTL) to produce an algal bio-crude oil. This process circumvents the costs of energy intensive drying for complete dewatering. Several studies into the hydrothermal conversion of lignocellulosic biomass and algae have been performed in the past [6–8].

The aqueous phase (AP) obtained from HTL of microalgae contains N and P constituents that, ideally, can be recycled, aiming to reduce cultivation costs and energy production costs induced by the continuous use of fresh make-up nutrients. Furthermore, the AP contains several oxygenated hydrocarbons that may be

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assimilated when a heterotrophic/mixotrophic algae strain is used. In previous work we showed that the molecular and elemental composition of the AP can significantly vary, in particular with HTL operating temperature [9]. The largest N sources in the aqueous HTL product were always organic N-containing compounds and ammonia. Moreover, as shown in the work by Biller et al. [10], AP composition can also vary depending on the algae strain used.

The desirable situation within a biorefinery framework would be performing HTL of algae (–debris) under the adequate process conditions in which all the N in the algae is transferred to the AP in the form of NH_3/NH_4^+, while still producing enough crude bio-oil. In developing such a concept, the algae strain selected for growth might be of importance, as it must, e.g. be able to persist within a medium containing organic constituents from recycled HTL process water and preferably assimilate all the nutrients offered via this HTL aqueous phase recycling.

This idea of nutrients recycling was first proposed and tested in a pioneering investigation of Minowa and Sawayama [11]. In their study, *Chlorella vulgaris* was successfully cultivated (comparable to that in standard medium) in the recycled AP from catalytic *gasification* of the same algae when this one was diluted in nitrogen less standard medium. Recently, Jena et al. [12] and Biller et al. [10] tested similarly the ability to grow algae in diluted aqueous phase from HTL. Both stressed the importance of AP dilution in view of inhibitory effects of potential toxic compounds at high concentrations. Biller et al. [10] stated that heavy dilution of AP is required to avoid growth inhibition. Both observed that the algae in water diluted AP could not grow as fast as that in standard culture medium, even when sufficient amounts of N and P were present in the media. In this work, we also investigate the replacement/recycling of N and P containing nutrients in the growth medium. However, this was done by following a different approach. In our study we used mixtures of standard culture medium, AP and demineralized water with the same nitrogen concentration (either as total N or the sum of ammonia and nitrate) as that in standard growth medium. Consequently, the main differences between the tested media in this work were the concentrations of (macro-/micro-)nutrients and potentially toxic organic compounds.

2. Experimental

2.1. Algae feedstock

The freshwater microalgae used, *Desmodesmus* sp., was provided by Ingrepro B.V. (The Netherlands), where industrial cultivation is performed in raceway ponds and at high pH to maintain monoculture conditions. The batch obtained was centrifuged and maintained in the dark, cold and in COMBO (*CB*) growth medium [13] from which KCl, $Na_2SiO_3{\cdot}9H_2O$, animal trace elements and later $NaHCO_3$ (since CO_2 gas was used as carbon source) were excluded. The constructed growth setup was inoculated with that maintained culture. The proximate and ultimate analyses and biochemical composition of this type of microalgae can be found in our previous work [9].

2.2. Hydrothermal Liquefaction (HTL) and aqueous phase (AP) recovery

From a batch of concentrated *Desmodesmus* sp. solution (7.66 wt% of algae dry ash free), several batch HTL tests (using 20 g of feedstock each time) were performed using a 45 ml stainless steel autoclave, heated by immersion in a hot fluidized sand bed. These experiments were carried out with an inert atmosphere at 300 °C for 5 min reaction time (excluding 6–7 min of heating time). After these 5 min of reaction time, fast quenching (~1–2 min) was performed by inserting the autoclave in a water bath. After gas analysis, a subsequent product collection and separation procedure was carried out to obtain the other three products: oil (crude from dichloromethane soluble fraction), water soluble organics (aqueous phase AP), and solid residue. A detailed explanation of the setup, products separation procedures and typical mass balance closure can be found elsewhere [9]. In the present study, however, additional detailed analyses on the obtained *aqueous phase* fractions were performed.

Nutrients concentration, *Ni and COD (in mg L^{-1})*: These compounds were quantified with standardised tests (HACH LANGE) and measured with a spectrophotometer (DR 5000, HACH Corporation). The compounds quantified were: total nitrogen (TN, LCK 238), nitrate (NO_3-N, LCK 339), ammonia (NH_4-N, LCK 305), phosphate (PO_4-P, LCK 349), Ni (LCK 337), and COD (chemical oxygen demand, LCK 414). Organic nitrogen (org-N) was calculated as: TN–(NO_3-N)–(NH_4-N)–(NO_2-N). Nitrite was below the detection limit for all the aqueous phases analysed. In the labelled "NH_4-N" test, the total nitrogen coming from both NH_3 and NH_4^+ is determined. Both, ammonia and ammonium coexist in equilibrium in water depending on pH.

Total Organic Carbon, *TOC (in mg L^{-1})*, was measured with a Shimadzu TOC/TIC analyser.

Na, K, Ca, S and Mg nutrients were determined by means of Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Varian Vista MPX) and *Cl* by Ion Chromatography (IC, Dionex ICS 2100). Analyses were performed in duplicate.

AP organic compounds: Analyses of volatile compounds (acetic acid, ethanol and acetone) in AP were performed by acidifying the medium to pH = 2 with potassium bisulphate prior to 10 min SPME (Solid Phase Microextraction, polyacrilate coating) immersion sampling and direct GC–MS (equipped with MDN-5S) and GC–FID (equipped with NUKOL™ capillary column) for acetic acid analysis. Quantitative analyses were done by calibration with solutions of known concentrations of pure standards. The non-volatile fraction of water soluble organics was quantified by evaporation of water, derivatization and GC–MS analysis. Derivatization and analysis were performed following a published procedure [14] which was slightly modified in order to obtain complete silylation of cyclic dipeptides and polyhydroxylated compounds.

2.3. Cultivation system

A batch growth setup was designed, constructed and calibrated (Fig. 1). The total reactor volume was distributed over 10 glass reactors (1–10 in Fig. 1a), each having a volume of 2 L. Fluorescent artificial lighting (Philips MASTER TL-D Graphica 58W/9651SL) was used as light source in continuous mode. Light intensities and wavelength spectra inside the reactor compartment and individual reactors were determined using a USB4000 spectrophotometer (Ocean Optics). As a significant drop in received light was measured for reactors 1 and 10, located at both ends of the reactor compartment (see Fig. 1a), these two reactors were not used.

The temperature inside the reactor compartment was kept constant with max. ±1 °C difference throughout the reactor compartment by air circulation (blue[1] arrow in Fig. 1a). For mixing, oxygen stripping and carbon dioxide supply inside the reactors, aeration enriched with pure CO_2 was used. This gas mixture was saturated with water through two saturators (in Fig. 1a, vessels A and B containing demineralized water) to minimize evaporation losses. A gas pressure equalizer ensured equal inlet pressure for all connected

[1] For interpretation of colour in Fig. 1, the reader is referred to the web version of this article.