Algal Research 9 (2015) 99–106

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Cultivation of microalgae with recovered nutrients after hydrothermal liquefaction

Diego López Barreiro [a,*], Manuel Bauer [b,d], Ursel Hornung [b], Clemens Posten [c], Andrea Kruse [b,d], Wolter Prins [a]

[a] Department of Biosystems Engineering, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[b] Institute for Catalysis Research and Technology, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmoltz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany
[c] Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology (KIT), Fritz Haber Weg 2, 76131 Karlsruhe, Germany
[d] Conversion Technology and Life Cycle Assessment of Renewable Resources (440f), Institute of Agricultural Engineering, University Hohenheim, Garbenstrasse 9, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 23 February 2015
Accepted 10 March 2015
Available online xxxx

Keywords:
Microalgae cultivation
Hydrothermal liquefaction
Biofuel production
Nutrients recycling

ABSTRACT

One of the main concerns regarding the development of microalgal biofuels is the tremendous demand of nutrients that they require for growing, which places a question mark with their sustainability. In this paper, cultivation trials of *Nannochloropsis gaditana*, *Phaeodactylum tricornutum*, *Chlorella vulgaris* and *Scenedesmus almeriensis* are performed by reusing nutrients recovered from the aqueous by-product obtained during biofuel production via hydrothermal liquefaction. This provides a way to recover nutrients while simultaneously treating the wastewater generated during the production of biofuel. Direct recycling of the aqueous phase is compared to the use of an intermediate step (supercritical water gasification) to purify this stream. Also, two growth parameters (pH and percentage of substitution of nutrients from the standard medium) are studied. The results show that the response of microalgae species to the recycling of nutrients is strain-dependent: *P. tricornutum* and *S. almeriensis* were not able to grow satisfactorily in recovered aqueous by-products. On the other hand, *C. vulgaris* and *N. gaditana* could grow by replacing 75% of the nutrients from the standard medium with nutrients recovered from HTL without reducing the algae growth, compared to the standard medium.

1. Introduction

The advantages of microalgae over other sorts of biomass have been profusely mentioned in recent years [1], i.e., they grow faster than terrestrial biomass, they can be grown on non-arable land and they have a higher photosynthetic efficiency. However, their production costs are still very high when compared to other biomass sources [2], and parts of these costs are due to the need for nutrients (phosphorus, nitrogen or potassium, among others) to grow algae. Not only the costs play a key role, but also the sustainability: i.e., the scarcity of phosphorus that the world will face in the coming years [3] is becoming an urgent problem with no solution up to now.

Hydrothermal liquefaction (HTL) converts the whole microalgae biomass into biofuel while avoiding the energy-expensive step of drying the feedstock. It also leads to the production of gaseous, aqueous and solid by-products. The yields of the HTL products depend on the conditions applied (i.e., temperature, residence time). Among them, the aqueous and solid phases contain most of the nutrients present in the feedstock, which calls for a way to recover them. Various studies have examined the possibility of recovering nutrients through the aqueous phase obtained from hydrothermal processes by growing algae in it. This was typically checked against algae growth in several standard mediums, which are recipes that provide an optimized mixture of nutrients to support algae growth. Reusing the aqueous by-product after HTL would alleviate the otherwise unavoidable needs for HTL-wastewater treatment of an algae biorefinery, because of its high content in nutrients and its significant load of organic molecules.

Tsukahara et al. [4] investigated the cultivation of *Chlorella vulgaris* in 75- to 300-fold diluted aqueous phase after its low-temperature catalytic gasification, and reported that microalgae could not grow in it without the supplement of additional micronutrients. Jena et al. [5] indicated that *Chlorella minutissima* was not able to grow in dilutions of the aqueous phase after HTL just as good as it did in the standard medium BG 11. Contrary to this, Biller et al. [6] reported that recycling the aqueous phase for algae cultivation achieved higher biomass yields than the standard medium 3 N-BBM + V, at a dilution ratio of 400× for *Chlorogloeopsis fritschii* and of 200× for *C. vulgaris*. Du et al. [7] reused aqueous by-products from the hydrothermal carbonization process to cultivate *C. vulgaris* and reported that algae were growing much faster and producing more biomass than in the standard medium BG 11 at dilution ratios from 50× to 200×. Nelson et al. [8] showed that the aqueous phase obtained from HTL of *Nannochloropsis occulata* could be recycled for microbial growth of *Escherichia coli* and *Pseudomonas putida* at much lower dilution ratios than microalgae, demonstrating a higher tolerance of bacteria towards this by-product. García Alba et al.

* Corresponding author.
E-mail address: Diego.LopezBarreiro@UGent.be (D. López Barreiro).

http://dx.doi.org/10.1016/j.algal.2015.03.007
2211-9264/

[9] reported that 50% of the nutrients from the standard medium could be replaced by addition of the aqueous phase without compromising the growth of *Desmodesmus* sp. In a subsequent study [10] they demonstrated that algae were able to grow after five consecutive cycles of growth in recovered aqueous product plus HTL, although the morphology of the cells (observed by light microscopy) appeared to change throughout the process. Cherad et al. [11] grew *C. vulgaris* in the aqueous educt obtained from supercritical water gasification of macroalgae, and reported a lower growth than in the standard medium (BBM). The recovery of nutrients by means of hydrothermal carbonization was investigated by Levine et al. [12], obtaining growth yields higher than in their standard medium. According to these results, it seems that there is an opportunity for growing microalgae in the recovered aqueous product after HTL, if a certain amount of micronutrients or trace elements are also supplied to the algae culture.

This paper aims at assessing the recoverability of nutrients within an algae biorefinery. For this reason, aqueous phase was obtained after HTL (HTL-AP) of the freshwater species *Scenedesmus almeriensis* and the marine *Nannochloropsis gaditana* at 350 °C and 15 min, to assess the recycling of nutrients from both environments. The HTL-AP was re-used directly for growth, but was also subjected to an intermediate supercritical water gasification (SCWG) [13] step to produce a new aqueous by-product (SCWG-AP). This intermediate step aimed at reducing the load of organic compounds in the HTL-AP which can be harmful for algae cultivation. It also degrades nitrogen-containing organic molecules to ammonium, thus increasing the bioavailability of nitrogen for algae cultivation. A scheme of the recycling routes tested is presented in Fig. 1.

Both aqueous by-products were separated from the other products without the use of any organic solvent that could extract some of their organic compounds. This constitutes a major difference between our work and most of the previous studies mentioned in this section. At the scale of a real algae biorefinery the use of big quantities of organic solvents must be avoided for economical, health and environmental reasons.

HTL-AP and SCWG-AP were used to compare their performances in the algae growth of four species: the freshwater *C. vulgaris* and *S. almeriensis*; and the marine *N. gaditana* and *Phaeodactylum tricornutum*. Nitrogen (in the form of nitrate or ammonium) was taken as nutrient of reference, because the supply of this particular nutrient is one of the main issues (economic and energetic) when culturing algae [14]. The algae have been fed with fractions (25% and 50%) of the nutrients from the standard medium, and a complimentary amount of HTL-AP or SCWG-AP, until the requirements of nitrogen from the standard medium were fulfilled.

2. Materials and methods

2.1. Thermochemical conversion

2.1.1. Microalgae strains

The marine species *N. gaditana* (CCAP 849/5) and the freshwater species *S. almeriensis* (CCAP 276/24) were obtained in a freeze dry state from the cultivation facility in Las Palmerillas (University of Almería, Spain). These same strains were used in a previous study from our group, providing a detailed explanation about their characterization and composition [15]. Table 1 summarizes its main characteristics.

Fig. 1. Schematic representation of the recycling routes used in this research.

2.1.2. Hydrothermal liquefaction and supercritical water gasification

The algal biomass was subjected to hydrothermal liquefaction at 350 °C for 15 min in microautoclaves made of stainless steel 1.4571 with a volume of 10 mL. 70% of their volume was filled with water containing 10 wt.% of algal dry matter. The headspace was flushed with nitrogen, and subsequently pressurized up to 2 MPa, before tightly closing them. They were then placed in a GC-oven and the temperature was adjusted to 350 °C. It took about 18 min to reach the desired temperature inside the reactor, which was then maintained for 15 min.

For the supercritical water gasification experiments, Inconel® 625 autoclaves with a volume of 10 mL were used. A temperature of 450 °C and a pressure of 30 MPa were employed, at a residence time of 30 min. These conditions were selected as a compromise between sufficient degradation of carbon and nitrogen-containing molecules in the HTL-AP and the need to minimize the leaching of metals from the reactor walls that might hinder the algae growth, after a series of screening experiments (data not shown). The composition of the metal alloys used for manufacturing the stainless steel 1.4571 and Inconel® 625 microautoclaves shows the presence of several metals (i.e., chromium, nickel) [17] that, if leached during the hydrothermal step, could damage the algae growth when recycling the aqueous by-product. A pre-calculated quantity of HTL-AP of 1.49 mL was loaded to reach the desired 30 MPa at the selected temperature, according to the steam tables [16]. The headspace was flushed with helium, and then 0.5 MPa were loaded, in order to be able to collect gas samples for analysis.

Once the hydrothermal reaction (HTL or SCWG) was completed, the autoclaves were submerged in an ice bath for fast quenching. All the experiments were repeated until enough HTL-AP or SCWG-AP was obtained for the cultivation trials.

2.1.3. Product separation and analysis

When the autoclaves achieved room temperature, they were opened to release the gas. A gas sample was analyzed in a gas chromatograph (HP 6890). The content of the autoclaves was poured out and filtered using a Whatman nylon membrane (47 mm, 0.45 μm pore size). Only the aqueous by-products passed through the filter. The solids were retained in the filter cake, and most of the oil remained stuck to the reactor walls. No organic solvent was used to separate the aqueous phase from the other product phases.

The concentration of inorganic matter in the aqueous by-products was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) on a 720/725-ES emission spectrometer with a CCD detector from Agilent Technologies. The plasma was generated with a 40 MHz quartz-controlled generator where argon is the carrier gas. Ion chromatography was used for the determination of cations (Metrohm device with a Metrosep C3 250 × 4 mm column, eluent with 3 mM HNO_3 and 10% acetone and a conductivity detector), anions (Metrohm device with a Metrosep Dual2 75 × 4.6 mm column, eluent with 190.5 mg·L^{-1} Na_2CO_3, 168.5 mg·L^{-1} $NaHCO_3$ and 150 mL·L^{-1} of acetone and a conductivity detector), organic acids (Merck Hitachi device with a Bio Rad Aminex HPX 87H 300 × 7.8 mm column, eluent with 4 mM H_2SO_4 and an UV L7400 and a RI L-7490 detectors) and phenols (VWR Hitachi device with a Penomenex Kinetex 2.6u PFP100A 150 × 4.6 mm column, eluent with 80/20 phosphate buffer (pH 2)/methanol and a DAD L2455 detector). The total carbon (TC), total inorganic carbon (TIC) and total nitrogen (TN) were measured by a Dimatec® 2000 instrument. Total organic carbon (TOC) is calculated by a differential method, subtracting TIC from TC. The device measures these parameters based on chemical catalytic oxidations of the elements containing them, measuring them by IR spectroscopy. Samples were measured at several dilution rates and, when possible, with various detectors to assess the reproducibility of the results. These and previous experiments with these devices showed that the analytical error in this concentration range is below 5% relative.