Bioresource Technology 126 (2012) 354–357

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Short Communication

Cultivation of a microalga *Chlorella vulgaris* using recycled aqueous phase nutrients from hydrothermal carbonization process

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HIGHLIGHTS

- Process water from hydrothermal carbonization was recycled for algae cultivation.
- Algae grew faster on process water than on BG-11 medium.
- Algae removed TN, TP and COD by 45.5–59.9%, 85.8–94.6% and 50.0–60.9%.
- Algae grown on process water had higher carbon, hydrogen and lipids content.
- Recycling process water has the potential of reducing algae production cost.

ARTICLE INFO

Article history:
Received 27 July 2012
Received in revised form 14 September 2012
Accepted 15 September 2012
Available online 24 September 2012

Keywords:
Hydrothermal carbonization
Microalgae
Nutrient recycling
Bio-oil

ABSTRACT

This study investigated the feasibility of using recovered nutrients from hydrothermal carbonization (HTC) for cultivation of microalga *Chlorella vulgaris*. Different dilution multiples of 50, 100 and 200 were applied to the recycled process water from HTC and algal growth was compared among these media and a standard growth medium BG-11. Algae achieved a biomass concentration of 0.79 g/L on 50× process water after 4 days. Algae removed total nitrogen, total phosphorus and chemical oxygen demand by 45.5–59.9%, 85.8–94.6% and 50.0–60.9%, respectively, on differently diluted process water. The fatty acid methyl ester yields for algae grown on the process water were 11.2% (50×), 11.2% (100×) and 9.7% (200×), which were significantly higher than 4.5% for BG-11. In addition, algae cultivated on process water had 18.9% higher carbon and 7.8% lower nitrogen contents than those on BG-11, indicating that they are very suitable as biofuel feedstocks.

1. Introduction

Hydrothermal conversion is a process in which biomass is converted to liquids, solids or gases in hot pressurized water. Hydrothermal technologies broadly cover chemical and physical transformations in high-temperature (200–600 °C) and high-pressure (5–40 MPa) water (Peterson et al., 2008). They have energetic advantages for wet biomass such as algae due to the elimination of energy inputs for water removal by evaporation. Many recent studies have shown that a bio-oil with a high heating value can be obtained from the hydrothermal liquefaction (HTL) of microalgae (Biller and Ross, 2011; Brown et al., 2010; Torri et al., 2012). A large amount of the process water containing carbon, nitrogen, phosphorus and minerals is produced as a co-product in this process. It is expected that these nutrients can be recycled for cultivation of microalgae to enhance the overall economic viability of algal biofuel process. There are very few reports on recycling process water from HTL for algae cultivation. Some previous studies (Biller et al., 2012; Jena et al., 2011) compared algae growth on diluted HTL process water and standard media, such as BG-11 and 3N-BBM+V. Algae grew slower and reached a lower final concentration on the diluted process water than on standard media. This is probably due to the high concentration of inhibitors, including nickel, fatty acids, phenols and other toxic compounds produced in HTL under high temperatures ranging from 250 to 400 °C. Hydrothermal carbonization (HTC) is another technology carried out in compressed water but at lower temperature (subcritical condition, ~200 °C) than HTL. HTC has been reported to produce energy densified solid fuels (Heilmann et al., 2010) and pretreat biomass for subsequent thermochemical processing, such as pyrolysis and gasification (Du et al., 2012, 2011; Hoekman et al., 2011). Our previous study utilized HTC as a pretreatment step to reduce the nitrogen content by hydrolyzing proteins in microalgae feedstock (Du et al.,

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http://dx.doi.org/10.1016/j.biortech.2012.09.062

2012). Polysaccharides and proteinaceous materials were hydrolyzed to monosaccharides, and carboxylic acids and amino acids, respectively, under HTC, and the hydrolysates were found suitable for yeast growth (Lamoolphak et al., 2006; Pourali et al., 2009). Many algae species can grow mixotrophically on substrates such as simple carboxylic acids and amino acids as the carbon and nitrogen sources (Perez-Garcia et al., 2011). In this context, the current study was carried out to evaluate the feasibility of recycling process water from HTC as the growth media for cultivation of *Chlorella vulgaris*.

2. Methods

2.1. Characteristics of process water from HTC

The process of HTC was described in details in our previous work (Du et al., 2012). Process water obtained under 200 °C/40 min was recycled for use in this study. The main characteristics of process water are listed in Table 1. Chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), ammonia (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N) and total phosphorous (TP) of the process water were determined using specific test kits on a Hach DR 5000 Spectrophotometer (Loveland, CO). Metal ion concentrations were analyzed on an inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000, Waltham, MA) by Soil Testing Laboratory at University of Minnesota, St. Paul.

2.2. Algal strain and culture conditions

Algal strain was a wild-type *C. vulgaris* isolated from local freshwater and its selection procedure was described in details in our previous study (Li et al., 2011). The algal strain was enriched in 100 ml BG-11 medium (Stanier et al., 1971) with 2 g/L glucose to obtain enough starting cultures for this study. Since the nutrient levels were too high for microalgae to survive, the process water was diluted to 50×, 100× and 200× times' volume with distilled water. BG-11 medium was used as the control to evaluate the growth efficiency of *C. vulgaris* on diluted process water. Algae were inoculated with the starting dry weight of 0.15 g/L in 250 mL Erlenmeyer flasks containing 150 mL autoclaved medium. The flasks were placed on a shaker with 100 rpm rotation speed. All cultures were kept at 25 ± 2 °C under continuous cool-white fluorescent light illumination at 100 μmol m^{-2} s^{-1}. Each growth condition was carried out in triplicates and a fourth culture was used to supplement the medium after sampling.

2.3. Algae growth and chemical analysis

Algal growth was determined daily by measuring total volatile suspended solids (TVSS) using 4 mL algae suspension collected from each flask. The biomass productivity was calculated from the equation:

$$P(\mathrm{gL^{-1}day^{-1}}) = (\mathrm{TVSS} - \mathrm{TVSS_0})/t$$

where t (day) was the time between the two measurements, TVSS and $TVSS_0$ were the concentration of biomass at day t (growth curve leveling off) and day 0, respectively. A one-way analysis of variance (ANOVA, at 0.05 significance level) and the least significant difference method (LSD) was carried out for the statistical analysis of algal growth on the four different media.

A volume of 8 mL algae suspension was collected daily from each flask for nutrient analysis. The samples were centrifuged at 7000 rpm for 10 min and supernatants were collected and diluted to suitable concentrations for analysis. TN, TP and COD were measured using test kits on a Hach DR 5000 Spectrophotometer.

Algae harvested at the end of the 5-day batch culture were analyzed for their C, H and N contents with an Exeter Analytical CE-440 Elemental Analyzer (Chelmsford, MA). Fatty acid content and composition were analyzed using acid-catalyzed *in-situ* transesterification method (Indarti et al., 2005). Dried algae (ca. 0.05 g) were weighed in 25-ml screw-top glass tubes, and 10 ml of a mixture of methanol, concentrated sulfuric acid and chloroform (volume ratio 4.25:0.75:5) was added. The glass tubes were sealed and placed into a 90 °C water bath for 90 min. Upon cooling, the tubes were shaken and centrifuged at 7000 rpm for 5 min after adding 3 ml of distilled water into the reaction mixture. The chloroform layer containing fatty acid methyl esters (FAME) was carefully collected and subjected to gas chromatography–mass spectrometry (GC–MS). An Agilent 7890–5975C GC–MS with a HP-5MS (30 m × 0.25 m × 0.25 μm) capillary column was used for FAME analysis. The carrier gas was helium at a flow rate of 1.2 mL/min. The oven temperature was initially 80 °C for 1 min, then increased to 290 °C at a rate of 4 °C/min, and held at 290 °C for 5 min. The injector and detector were maintained at constant temperature of 250 and 230 °C, respectively. Compounds were identified with the National Institute of Standards and Technology (NIST) mass spectral data library and quantified with external standard calibrations of C14−C22 FAME standards (Sigma–Aldrich).

Table 1
Characteristics of process water from HTC.

Parameters	Concentration (mg/L)	Metals	Concentration (mg/L)
COD	134800 ± 2287	K	775.45
TOC	45700 ± 1513	Mg	4.025
TN	9650 ± 1582	Mn	0.01
Ammonia	1343 ± 75	Fe	3.085
Nitrate	211 ± 20	Na	8966
Nitrite	3.63 ± 0.73	B	1.855
TP	343 ± 43	Ni	0.005
		Cr	0.115

*Each data point indicates the mean ± standard deviation for three independent measurements; data for metal analysis were the average value of duplicate measurements.

3. Results and discussion

3.1. Algal growth

Fig. 1 shows the growth curves for the four different media. The *C. vulgaris* could survive in all media as evidenced by the increase of biomass concentration. The biomass productivities were 0.013, 0.160, 0.092 and 0.054 g L^{-1} d^{-1} for BG-11, 50×, 100× and 200 × process water, respectively. Algae had significant higher productivities and biomass concentrations on the three dilutions of process water than BG-11 medium. Different from HTL carried out at high temperatures with many growth inhibitors produced, polysaccharides and proteins were mainly hydrolyzed to mono-sugars and amino acids in HTC. These mono-sugars and amino acids provided adequate carbon and nitrogen nutrients which can be readily used by algae. However, algae need to sequester CO_2 as the sole carbon source when grown on inorganic BG-11 medium photoautotrophically. Many reports showed that mixotrophic growth can result in higher biomass production than phototrophic growth (Bhatnagar et al., 2011; Zhou et al., 2012). Among the three dilutions of the process water, both biomass productivity and final biomass concentration were in the following order: 50× > 100× > 200×. This indicates that algae can endure the higher concentration of potential growth inhibitors in the more concentrated process water. It is noticed that algae grew rapidly in the first 4 days and then decreased significantly on the fifth day on the 50 × process water.