Bioresource Technology 102 (2011) 6487–6493

Contents lists available at ScienceDirect

Bioresource Technology

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

Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production

Dong Yan, Yue Lu, Yi-Feng Chen, Qingyu Wu *

School of Life Sciences, Tsinghua University, Beijing 100084, PR China

ARTICLE INFO

Article history:
Received 12 January 2011
Received in revised form 9 March 2011
Accepted 12 March 2011
Available online 16 March 2011

Keywords:
Waste molasses
Microalga *Chlorella protothecoides*
Oil
Biodiesel

ABSTRACT

The by-product of sugar refinery—waste molasses was explored as alternative to glucose-based medium of *Chlorella protothecoides* in this study. Enzymatic hydrolysis is required for waste molasses suitable for algal growth. Waste molasses hydrolysate was confirmed as a sole source of full nutrients to totally replace glucose-based medium in support of rapid growth and high oil yield from algae. Under optimized conditions, the maximum algal cell density, oil content, and oil yield were respectively 70.9 g/L, 57.6%, and 40.8 g/L. The scalability of the waste molasses-fed algal system was confirmed from 0.5 L flasks to 5 L fermenters. The quality of biodiesel from waste molasses-fed algae was probably comparable to that from glucose-fed ones. Economic analysis indicated the cost of oil production from waste molasses-fed algae reduced by 50%. Significant cost reduction of algal biodiesel production through fermentation engineering based on the approach is expected.

1. Introduction

Biodiesel as an alternative fuel to fossil energy has been widely used. It occupies 82% of total biofuels production in Europe (Bozbas, 2008) and the percentage is still growing up in Europe, Brazil and United States, based on great economic demands (Mata et al., 2010). Biodiesel can be produced from a wide range of feedstocks, including plant oils, non-edible oils, animal fats and other resources (Singh and Singh, 2010). At present the raw materials of biodiesel mainly come from plant oils, but the competition with the crops for farmland as well as high raw material cost limit the sustainability of biodiesel on large scale (Scarlat et al., 2008).

Microalgae have been recently proposed as one of the most efficient producers of liquid transportation biofuel. As efficient photosynthetic organisms, microalgae have unique advantages in capturing solar energy to generate reducing equivalents and converting atmospheric CO_2 to organic molecules. Microalgae also have special advantages in ability to adapt to various stressful environments, non-requirement of agricultural land and so on (Metting, 1996). Also, several microalgae such as *Chlorella protothecoides* (Wu et al., 1992), *Crypthecodium cohnii* (de Swaaf et al., 2003; de Swaaf and Sijtsma, 2003) are capable of uptaking carbohydrates (e.g., glucose) directly and transforming them to lipid (Miao and Wu, 2006). This heterotrophic metabolism allows much higher cell density and neutral lipid content (51.2 g/L and 55.2%, respectively in *C. protothecoides*), representing a promising approach for algal oil production (Miao and Wu, 2006). However, consumption of organic carbon sources such as glucose may increase the cost in biodiesel production. Some effects have been made to reduce the cost of heterotrophic algal cultivation. For examples, there are generally three approaches known to be available. First, cheap organic carbon sources like starch hydrolysates from Jerusalem asrichoke (Cheng et al., 2009b), sweet sorghum (Gao et al., 2010) or cassava (Lu et al., 2010) was utilized to displace glucose. Sunlight-driven photosynthesis was combined with heterotrophic pathways to save organic carbon inputs (Xiong et al., 2010). The scale-up of algal fermentation engineering resulted in reducing production cost (Li et al., 2007). This work further exploited the full potential of the first approach by using waste molasses to replace the entire glucose-based artificial medium for heterotrophic growth and lipid accumulation in *C. protothecoides*.

Waste molasses, a by-product in sugar refinery, is a cheap carbon stock which might be used for algal oil production. The total sugar content of the commercial waste molasses in China market is commonly higher than 48.0% (according to the standard established by the China National Light Industry Council: QB/T 2684-2005). Other nutrients include vitamins, trace elements and many other kinds of ingredients. The output of molasses in China is around 400 million tons per year and its price was maintained at relatively stable levels in recent years (http://www.ncce.biz/). But

Abbreviations: MHL, molasses hydrolysate N limited medium; MHC, molasses hydrolysate completion medium; MHD, molasses hydrolysate direct medium; GC, glucose completion medium; GL, glucose N limited medium.

* Corresponding author. Tel./fax: +86 10 62781825.
E-mail address: qingyu@tsinghua.edu.cn (Q. Wu).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.03.036

most of waste molasses is simply abandoned, generating large volumes of high strength wastewater that is of serious environmental concern. The waste molasses is strongly acidic, have dark brown color, high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (Jiménez et al., 2004; Satyawali and Balakrishnan, 2008). Even so, the abundant nutrients in waste molasses, after pretreatment or even in native forms, might be available as the raw materials for microbial industry fermentation to produce baker's yeast, amino acid and ethanol (Sirianuntapiboon and Prasertsong, 2008). On account of energy crisis, waste molasses has been tested on the feasibility of biodiesel production through yeast *Trichosporon fermentans* (Zhu et al., 2008). As mentioned before, heterotrophic culture of microalgae with rapid growth and high contents of neutral oils offers one of the most promising sources of feedstocks for biodiesel production but requires cheap carbon source. Due to waste molasses containing both organic carbons and other nutrients, in current investigation, the potential of waste molasses as an alternative to culture media for oleaginous microalga *C. protothecoides* was investigated. After optimizing culture conditions of waste molasses for the microalgae, high-density fermentation was achieved via fed-batch cultures in bioreactor. Oil extraction and conversion to biodiesel through transesterification were performed, and the composition and quality of obtained algal biodiesel were characterized. This work has revealed waste molasses as an advantageous feedstock for combination of high oil yields in *C. protothecoides* and low-cost for biodiesel production.

2. Methods

2.1. Cell strains and cultivation

The initial *C. protothecoides* strain (without strain No.) was obtained in 1990 from Dr. Neil Grant (William Paterson University of New Jersey), which originally came from Culture Collection of algae at the University of Texas (Austin, TX). After cultivating and screening for high growth rate and oil content in Algae Bioenergy Laboratory at Tsinghua University, Beijing, China, the strain (CGMCC No. 2578) was collected by China General Microbiological Culture Collection Center (CGMCC) and renamed as *C. protothecoides sp.* 0710. The basal medium and culture methods were described before (Xiong et al., 2008). To prepare the heterotrophic medium, 30 g/L glucose and 2 g/L yeast extract were added to the basal medium and glycine was reduced to 0.1 g/L. The heterotrophic microalgal cells were incubated at 28 °C in flasks and shaking at 220 rpm.

2.2. Hydrolysis of waste molasses

Waste molasses was obtained from the Xianggui Sugar Refinery (the Guangxi Province, China). The brix and pH are 88.92°BX and 5.4, respectively. Waste molasses contains 36.24% of sucrose and 32.07% of reducing sugar. It was diluted with distilled water at a ratio of 1:1.5 (v/v). The diluted waste molasses was divided into two aliquots. One was untreated as a control. The other was boiled for 20 min to remove precipitations by centrifugation at 6000 rpm for 2 min. The supernatant was saved for enzymatic hydrolysis by MAXINVERT® 200000MG β-invertase (DSM Food Sp. Seclin, France). The mixtures of molasses and invertase at a ratio of 2500:1 (w/w) were incubated at 60 °C for 24 h to generate molasses hydrolysate with more reducing sugar. As a control, acid-hydrolysis was designed to determine enzymatic hydrolysis rate of sucrose: equivalent molasses hydrolysate in 0.36 M HCl for 20 min at 80 °C and then neutralized with NaOH. Sucrose could be completely hydrolyzed into glucose and fructose by this acid-hydrolyzed method.

2.3. Medium preparation and optimization of culture conditions in 500 ml flasks

The optimization was performed in 500 ml conical flasks containing 200 ml of culture media. In order to compare the effects of molasses hydrolysate, glucose, nitrogen source and other medium components on heterotrophic growth and oil accumulation of alga, five kinds of media, including glucose completion medium (GC), glucose N limited medium (GL), molasses hydrolysate completion medium (MHC), molasses hydrolysate N limited medium (MHL) and molasses hydrolysate direct medium (MHD) were prepared respectively (in Table 1). The reducing sugar concentrations in all the media were adjusted to 30 g/L. The pH in all the media was adjusted to pH 6.3. After sterilization, heterotrophic microalgae were inoculated at a ratio of 1:20 (v/v, that is, 5%) to the medium and incubated at 28 °C with continuous shaking at 220 rpm for 5 days.

To investigate the effects of initial reducing sugar concentrations on biomass growth, the MHL media supplemented with 10, 30, 50, 70, or 90 g/L reducing sugar were compared.

2.4. Cultivation in 5 L fermenters

Algal cells were harvested and transferred to two 5-L fermenters each of which contained 2.5 L molasses hydrolysate N limited medium or 2.5 L molasses hydrolysate direct medium respectively. Concentrated molasses hydrolysate was batch-fed to keep organic carbon source about 30 g/L. KOH solution (10 g/L) was batch-fed to keep the pH value above 6.3; dissolved oxygen (DO) concentration was controlled within a range of 20–50% air saturation by adjusting agitation speed and airflow rate. Aeration rate and the agitation speed were variable and initially set at 240 l/h (1:1 vvm) and 300 rpm. Temperature was maintained at 28 °C.

2.5. Oil extraction and biodiesel preparation by transesterification

Cells were collected by centrifugation then freeze dried in a high vacuum overnight. Oils were extracted by the Soxhlet extraction with *n*-hexane as a standard solvent (Schäfer, 1998). The

Table 1
The ingredients of various media. The base medium components were maintained constant in all media except the MHD which only contained molasses hydrolysate.

Ingredient	Various media				
	GC	GL	MHC	MHL	MHD
KH_2PO_4 (g/L)	0.7	0.7	0.7	0.7	0
K_2HPO_4 (g/L)	0.3	0.3	0.3	0.3	0
$MgSO_4 \cdot H_2O$ (g/L)	0.3	0.3	0.3	0.3	0
$FeSO_4 \cdot 7H_2O$ (mg/L)	3	3	3	3	0
Vitamin B_1 (mg/L)	0.01	0.01	0.01	0.01	0
A_5 trace mineral solution (mg/L)	1	1	1	1	0
Glycine (g/L)	0.1	0	0.1	0	0
YE[a] (g/L)	2	0	2	0	0
Glucose (g/L)	30	30	0	0	0
Reducing sugar in molasses hydrolysate (g/L)	0	0	30	30	30

Base medium = KH_2PO_4 + K_2HPO_4 + $MgSO_4 \cdot 7H_2O$ + $FeSO_4 \cdot 7H_2O$ + Vitamin B_1 + A_5 trace mineral solution.
GC (glucose completion medium) = base medium + glucose + glycine + YE.
GL (glucose N limited medium) = base medium + glucose.
MHL (molasses hydrolysate N limited medium) = molasses hydrolysate + base medium.
MHC (molasses hydrolysate completion medium) = molasses hydrolysate + base medium + glycine + YE.
MHD (molasses hydrolysate direct medium) = molasses hydrolysate is the sole component in the medium.
[a] YE = yeast extract.