Bioresource Technology 174 (2014) 274–280

Contents lists available at ScienceDirect

Bioresource Technology

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

Selection of microalgae for biodiesel production in a scalable outdoor photobioreactor in north China

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HIGHLIGHTS

- Eight algal species were analyzed for growth, lipid accumulation and FA profiles.
- Closed sterile culture and open culture were used for strain selection.
- Culture for strain characterization was scaling up.
- Average bio-oil productivity of 22.8 m^3 ha^{-1} yr^{-1}.
- *S. obtusus* XJ-15 showed high capacity for biofuel production in north China.

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 27 September 2014
Accepted 1 October 2014
Available online 14 October 2014

Keywords:
Desmodesmus
Scenedesmus
Photobioreactor
Scale up
Biodiesel

ABSTRACT

The aim of this study was to identify the most promising species as biodiesel feedstock for large-scale cultivation in north China. Eight species of microalgae, selected on the basis of indoor screening, were tested for lipid productivity and the suitability of their fatty acid profiles for biodiesel production under outdoor conditions. Among them, three species *Desmodesmus* sp. NMX451, *Desmodesmus* sp. T28-1 and *Scenedesmus obtusus* XJ-15 were selected for further characterization due to their possessing higher lipid productivities and favorable biodiesel properties. The best strain was *S. obtusus* XJ-15, with highest biomass productivity of 20.2 g m^{-2} d^{-1} and highest lipid content of 31.7% in a culture of 140 L. *S. obtusus* XJ-15 was further identified as the best candidate for liquid biofuel production, characterized by average areal growth rate of 23.8 g m^{-2} d^{-1} and stable lipid content of above 31.0% under a scale of 1400 L over a season.

1. Introduction

Biodiesel have recently attracted extensive interests as it is carbon-neutral and environment-friendly. Among various sources for biodiesel production, microalgae are considered as the most promising feedstock for the future of biofuel production because they have high photosynthetic efficiency, high growth rate, and can be cultivated on non-arable land (Chisti, 2007). Moreover, the growth of microalgae at the same time will contribute to Greenhouse Gas savings (Wang et al., 2008).

In spite of many advances, producing microalgal oil for biodiesel is still too expensive. In fact, the first step in an algal process is to choose the right alga with relevant properties. However, the robust algal growth and high lipid production are reversely related (Rawat et al., 2013). Certain strains of microalgae, such as *Botryococcus braunii*, have high lipid storage potential (75% by dry cell weight (DCW)) but this is accompanied by low biomass productivity (Mata et al., 2010; Rawat et al., 2013). Due to this contradiction, lipid productivity showed a combination of biomass productivity and lipid production was considered as the most important selection parameter (Griffiths and Harrison, 2009).

The appropriate oil quality, besides its yields, is also key desirable characteristic of algal-based biodiesel industry because it influences the efficiency of biodiesel conversion and its quality (Nascimento et al., 2013; Rawat et al., 2013; Talebi et al., 2013). For example, *Nannochloropsis*, as one of the most promising sources of oil feedstock for biodiesel production, has high biomass production capacity and relatively high lipid content (Rodolfi et al., 2009); however, being rich in long chain polyunsaturated fatty acids (PUFA) (Doan et al., 2011; Griffiths et al., 2012), which is not desirable for biodiesel properties (e.g., ignition quality and oxidative

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http://dx.doi.org/10.1016/j.biortech.2014.10.008
0960-8524/

stability) (Doan et al., 2011). Thus, it should be analyzed thoroughly.

In addition, to minimize costs, photoautotrophic microalgal biodiesel production must rely on freely available sunlight. Qualities generally desirable for outdoor mass culture for microalgal lipid production include resistance to contamination, tolerance to a wide range of environmental conditions (above all temperature and solar radiation changes), rapid CO_2 uptake and tolerance to shear force (Griffiths et al., 2012). Various oil-rich microalgae, particularly those belonging to the genera *Chlorella* and *Scenedesmus* have been considered as potential sources of renewable energy (Ho et al., 2010; Song et al., 2013). Especially, *Scenedesmus* have often been used for photosynthetic CO_2 reduction combined with biodiesel production (Ho et al., 2010; Toledo-Cervantes et al., 2013). In addition, *Desmodesmus*, which are identified as thermotolerant genus, have recently drawn many attentions (Pan et al., 2011). Thus, eight microalgae strains in this study belong to *Scenedesmus* or *Desmodesmus* were used to examine the capacities of oil production under outdoor conditions.

Moreover, not all the oil-rich microalgae can be cultivated under outdoor conditions in certain area. For example, oleaginous microalgae *Tetraselmis suecica* CS-187 and *Chlorella* sp. were successfully realized a long-term outdoor cultivation in Victoria, Australia, but *Dunaliella tertiolecta* CS-175 failed to scale up after many tries (Moheimani, 2012). In this study, an algal screening system was first attempted to expand culture and maintain a large scale cultivation using the selected microalgae in north China.

The experiments were all conducted in a greenhouse in Beijing, China using sunlight as energy source. The objective of this study was to determine whether the eight green algal species had the potential to accumulate lipid and select the most promising species for large scale cultivation. Biomass and lipid productivities, lipid profiles and the estimated biodiesel properties were selected as critical factors for the evaluation. The selected robust species were further examined via a cultivation of 140 L in the columns to decide the best strain for biodiesel production. Moreover, this study describes the culture of the selected strain in a larger scale of 1400 L over a period of three months.

2. Methods

2.1. Organisms and culture

The eight microalgal species (*Desmodesmus abundans* T12, *Desmodesmus* sp. T28-1, *Desmodesmus* sp. NMX451, *Desmodesmus intermedius* HB12-2, *Desmodesmus obtusus* XJ-36, *Scenedesmus pectinatus var* XJ-1, *Scenedesmus obtusus* XJ-15, *Scenedesmus obtusus* XJ-19) used in this study, gifted by prof. Xu Xudong of Institute of Hydrobiology in China, was selected after a laboratory screening. Among these, *D. abundans* T12 and *Desmodesmus* sp. T28-1 were isolated from one of the largest Chinese freshwater lake (Lake Taihu). *Desmodesmus* sp. NMX451 and *S. obtusus* XJ-19 were isolated from Inner Mongolia of China. The rest of the species were isolated from reservoirs or ponds in Hubei province in China. Stock cultures for all the strains were grown in modified BG-11 medium containing 300 mg $NaNO_3$, 30 mg K_2HPO_4, 36 mg $CaCl_2{\cdot}2H_2O$, 6 mg ammonium citrate monohydrate, 6 mg ammonium ferric citrate, 1 mg EDTA, 2.86 μg H_3BO_3, 1.81 μg $MnCl_2{\cdot}4H_2O$, 0.222 μg $ZnSO_4{\cdot}7H_2O$, 0.39 μg $NaMoO_4{\cdot}5H_2O$, 0.079 μg $CuSO_4{\cdot}5H_2O$, 0.050 μg $CoCl_2{\cdot}6H_2O$ in 1 L sterile distilled water.

2.2. Experimental setup

All experiments were conducted in bioreactors (5 L flask bioreactor or 140 L airlift bag columns) as described previously by Xia et al. (2013, 2014) in the green house in Beijing, China (40°22′N, 116°20′E). For strain selection, the cultures were grown in 5 L Erlenmeyer flask (0.37 m height × 0.22 m in diameter) with 3 L medium. BG-11 was prepared from tap water which was filtered through 1 μm polypropylene filters (FTW) or autoclaved distilled water (ADW) and then added with nutrient solutions, respectively for open culture and closed culture. The closed culture was sealed with cotton plug and aerated continuously with sterile filtered air, while open culture sealed with filter paper and aerated with air without any treatment. The closed one kept sterile operation during the whole period of cultivation while the open one did not. To ensure well cell mixing, a 5 cm magnetic stir bar (mixing at 150 rpm) was placed at the middle of the bioreactor chamber for stirring. CO_2 in air (0.03–5%, v/v) was supplied to each bioreactor using an air compressor through the pipage during the daytime. The following large scale trials using the selected microalgae were carried out in 140 L bioreactor, which composed with two connected 70 L hanging column bags (1.80 m height × 0.22 m in diameter). CO_2 in air (0.03–5%, v/v) was supplied to each bioreactor using an air compressor through the pipage during the daytime. For all the trials, the initial cell concentration was 0.1 g L^{-1}, and culture media was BG-11 with nitrogen source of only 0.2 g L^{-1} urea because urea is significantly less expensive and exhibited more favorable effect on algal growth in outdoor cultures (Xia et al., 2013, 2014). The average day time high and night time low air temperatures during the tested period were 39 °C and 23 °C.

2.3. Analytical procedures

2.3.1. Biomass measurement

The biomass of 500 mL culture cells was harvested by centrifugation, then the wet cell mass was frozen overnight at −70 °C and freeze-dried at −54 °C under a vacuum (Alpha 1-2 LD plus, Christ). The biomass concentration (BC, mg L^{-1}) of the tested microalgae was determined by measuring optical density of 680 nm (OD_{680}) via an ultraviolet photospectrometer and using the following equations:

$$\text{BC } (D.abundans \text{ T12}) = 320 \times OD_{680} \ (R^2 = 0.996) \quad (1)$$

$$\text{BC } (Desmodesmus \text{ sp. T28-1}) = 322 \times OD_{680} \ (R^2 = 0.999) \quad (2)$$

$$\text{BC } (Desmodesmus \text{ sp. NMX451}) = 230 \times OD_{680} \ (R^2 = 0.998) \quad (3)$$

$$\text{BC } (D.intermedius \text{ HB12-2}) = 213 \times OD_{680} \ (R^2 = 0.998) \quad (4)$$

$$\text{BC } (D.obtusus \text{ XJ} - 36) = 201 \times OD_{680} \ (R^2 = 0.997) \quad (5)$$

$$\text{BC } (S.pectinatus \ var \text{ XJ-1}) = 277 \times OD_{680} \ (R^2 = 0.998) \quad (6)$$

$$\text{BC } (S.obtusus \text{ XJ-15}) = 229 \times OD_{680} \ (R^2 = 0.996) \quad (7)$$

$$\text{BC } (S.obtusus \text{ XJ-19}) = 368 \times OD_{680} \ (R^2 = 0.999) \quad (8)$$

The volumetric biomass productivity (VBP, mg L^{-1} d^{-1}) was calculated according to the Eq. (9):

$$\text{VBP} = (B_2 - B_1)/T \quad (9)$$

where B_2 and B_1 represents the dry weight biomass density at the time T (days) and at the start of the experiment, respectively.

The areal biomass productivity (ABP, g m^{-2} d^{-1}) was calculated according to the Eq. (10). The calculation was based on VBP and the floor area (required for columns and additional area required for operational convenience such as empty space between reactors as well as ground area to avoid shading effect), which is 0.26 m^{-2} for each column.