



The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production



Yehu Yin^{a,b}, Changjiang Yu^a, Li Yu^a, Jinshan Zhao^c, Changjiang Sun^d, Yubin Ma^{a,*}, Gongke Zhou^a

^aKey Laboratory of Biofuels, Shandong Provincial Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, PR China

^bUniversity of Chinese Academy of Sciences, Beijing 100049, PR China

^cQingdao Institute of Animal Sciences, Qingdao 266100, PR China

^dBeijing Risun Chemical Industry Technology Research Institute Co. Ltd, Beijing 100070, PR China

HIGHLIGHTS

- The impact of light condition on duckweed biomass and starch accumulation was investigated.
- Light intensity and photoperiod had a significant effect on biomass and starch production.
- 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was the best light condition for duckweed biomass and starch accumulation.
- The results suggested high light induction was an effective method for starch accumulation.
- This study provides optimized light conditions for future industrial duckweed cultivation.

ARTICLE INFO

Article history:

Received 27 January 2015

Received in revised form 20 March 2015

Accepted 21 March 2015

Available online 27 March 2015

Keywords:

Duckweed

Lemna aequinoctialis

Light intensity

Photoperiod

Starch production

ABSTRACT

Duckweed has been considered as a valuable feedstock for bioethanol production due to its high biomass and starch production. To investigate the effects of light conditions on duckweed biomass and starch production, *Lemna aequinoctialis* 6000 was cultivated at different photoperiods (12:12, 16:8 and 24:0 h) and light intensities (20, 50, 80, 110, 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The results showed that the duckweed biomass and starch production was increased with increasing light intensity and photoperiod except at 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Considering the light cost, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was optimum light condition for starch accumulation with the highest maximum growth rate, biomass and starch production of 8.90 $\text{g m}^{-2} \text{day}^{-1}$, 233.25 g m^{-2} and 98.70 g m^{-2} , respectively. Moreover, the results suggested that high light induction was a promising method for duckweed starch accumulation. This study provides optimized light conditions for future industrial large-scale duckweed cultivation.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Nowadays, the energy crisis and environmental problems caused by the consumption of fossil fuels have attracted much more attention worldwide. The exploration for alternative energy sources has become an increasingly urgent requirement. Bio-renewable energy, especially bioethanol and biodiesel, has been considered as an important alternative energy. Bioethanol is an alcohol made by sugar fermentation and can be used as liquid fuel and gasoline mixed agent to enhance oxygen content and reduce

emissions (Sánchez and Cardona, 2008). Most ethanol fuel is produced using corn as feedstock. However, using corn for ethanol production is inevitably competing for limited cropland for food/feed production (Endo et al., 2008). Significantly, lignocellulosic biomass is of great potential source for ethanol production because the material is abundant in the world. However, conversion of lignocellulosic biomass to bioethanol is difficult and much more expensive due to the tight structure of the biomass (Sarkar et al., 2012). Therefore, there is great interest in exploring novel starch sources that do not necessarily compete for cropland and can be digested much easier than lignocellulosic biomass to make bioethanol production more sustainable. Recently, duckweed has drawn increasing attention for bioethanol production due to its high biomass and starch production (Xu et al., 2011, 2012; Cui and Cheng, 2015).

* Corresponding author at: Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, Laoshan District, Qingdao 266101, Shandong, China. Tel.: +86 532 80662731; fax: +86 532 80662778.

E-mail addresses: mayb@qibebt.ac.cn (Y. Ma), zhougk@qibebt.ac.cn (G. Zhou).

Duckweed, a small floating aquatic plant, belongs to the monocotyledonous family of Lemnaceae. The geographic ranges of duckweed span the entire globe and 37 species belonging to five genera (*Lemna*, *Landoltia*, *Spirodela*, *Wolffia*, and *Wolffiella*) have been identified so far (Appenroth et al., 2013). Duckweed grows faster than most other plants, and under ideal condition, some species can double their biomass every 16 h to 24 h (Peng et al., 2007). The duckweed starch content varies by species and growth conditions ranging from 3% to 75% of dry weight (Reid and Bielecki, 1970; Xu et al., 2012; Cui and Cheng, 2015). Duckweed starch can be readily converted to ethanol using the same protocol that used for corn starch (Ge et al., 2012). The ethanol yield of duckweed reached $6.42 \times 10^3 \text{ L ha}^{-1}$, which was about 50% higher than that of maize-based ethanol production (Xu et al., 2011). In addition to high starch content, there are lower lignin (~2% dry weight) and cellulose (~10% dry weight) content in duckweed than that in terrestrial plants (Ge et al., 2012; Zhao et al., 2014), indicating that duckweed biomass can be hydrolyzed much easier and the biomass-to-ethanol conversion process is more cost-effective (Cheng and Stomp, 2009; Ge et al., 2012). Taken together, the characteristics of rapid growth, high starch content and high digestibility make duckweed an ideal feedstock for bioethanol production.

Starch accumulation is the key consideration for duckweed bioethanol production. As to the influence factors for starch accumulation, nutrient starvation especially nitrogen and phosphorus, sodium, abscisic acid and other chemical growth inhibitors had been widely investigated (McLaren and Smith, 1976; Thorsteinsson and Tillberg, 1987; Janas and Osiecka, 1995; Janas et al., 1998; Cheng and Stomp, 2009; Xu et al., 2011, 2012; Cui and Cheng, 2015). On the other hand, starch is the product from the photosynthesis, light is the only source of energy for photosynthesis, and also the direct source of energy for starch accumulation (Stitt and Zeeman, 2012). However, only a few reports had been focus on the effect of light condition on duckweed starch accumulation. Cui et al. (2011) determined the starch content variation at low light intensity ($40.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$) under three photoperiod conditions (8:16, 12:12 and 16:8 h light:dark) after 6 days of cultivation. Zhao et al. (2014) measured the growth rate and starch content at 2000, 5000 and 10,000 lux after inoculation for 12 days. To the best of our knowledge, few systemic studies had been done on the effects of light conditions on duckweed starch accumulation.

Moreover, with the development of duckweed commercialization, large-scale industrial cultivation model will be popularized in duckweed cropping system. For example, Xu et al. (2012) suggested that novel cultivation reactors such as multilayer or pagoda-shaped structures would be applied in the future large-scale duckweed cultivation. In order to promote duckweed commercialization, we have established duckweed multilayer pilot reactor and 1500 m² duckweed large-scale multilayer cropping system using waste water (Unpublished results). Light condition is one of important factors for industrial cultivation model in the future, such as multilayer pilot reactors and duckweed multilayer cropping system. Therefore, it is necessary to study the effect of light condition on the duckweed biomass and starch accumulation systematically. Thus, in this study, we investigated the effects of different light intensities and different photoperiod regimes (exposure to light:dark cycles) on duckweed growth density, growth rate, biomass production, starch content and starch production. The aim of this study is to provide the possibility of improving bioethanol production through controlling light intensity and photoperiod for future industrial large-scale duckweed cultivation. Duckweed was cultivated for 39 days at six different light intensities (20, 50, 80, 110, 200 and $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and three different photoperiod cycles (12:12, 16:8 and 24:0 h light:dark).

2. Methods

2.1. Duckweed strain and culture method

Lemna aequinoctialis 6000, which had the best ability to accumulate starch, was isolated from Lixian county in Hunan province by large-scale screening of more than 100 clones of duckweed distributed in 20 provinces in China (Yu et al., 2014, 2015). *L. aequinoctialis* 6000 was pre-cultured in a large tub (60 cm × 40 cm × 10 cm) for 2 weeks on 1/2 Schenk-Hildebrandt medium (SH) (Schenk and Hildebrandt, 1972). The pH of the medium was adjusted to 5.80. The tub was placed into a 23 °C growth chamber with the photoperiod of 16:8 and light intensity of $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$ provided by wide spectrum fluorescent tubes.

The experiment was carried out using 750 mL plastic box (12.5 cm × 12.5 cm × 4.2 cm). Each box was filled with 500 mL 1/2 SH medium and inoculated with 2 g fresh duckweed to cover the entire water surface with a single layer of fronds. Duckweed was cultivated for 39 days at six different light intensities (20, 50, 80, 110, 200 and $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and three different photoperiod regimes (12:12, 16:8 and 24:0 h light:dark). The evaporated water was replenished with distilled water every day throughout the experiments. The light intensity was measured using a quantum photometer (LI-250A, LI-COR, USA). Each experiment was performed in three replicates for different light intensities and photoperiod cycles.

2.2. Measurement of duckweed growth

To investigate the duckweed growth, *L. aequinoctialis* 6000 was harvested at interval of 3 days from each box using a strainer to remove fronds from 20% of the surface area based on the previous report (Xu and Shen, 2011). The duckweed growth density was calculated based on the amount of duckweed harvested each time. To measure the wet weight, duckweed was rinsed with distilled water using a strainer. After free water stopped dripping, duckweed was blotted dry with paper towels, and then measured with a balance (Bergmann et al., 2000). To measure dry weight (DW), the fresh fronds were lyophilized using a freeze drier (ALPHA1-2LD PLUS, CHRIST, Germany) for 48 hours.

The logistic model was applied to describe the duckweed growth according to the research in other species (Ma et al., 2013):

$$B_t = \frac{B_f}{1 + \frac{B_f - B_0}{B_0} * \exp\left(-\frac{\mu_{\max} t}{B_f}\right)}$$

where B_0 and B_f are the dry weight at zero and stationary phase, t and B_t are time and the corresponding dry weight, and μ_{\max} is the maximum population growth rate, which can thus be obtained after nonlinear-fitting with the above equation using the software origin 7.0.

2.3. Duckweed starch extraction and quantification

The starch content was determined using a modified method (Smith and Zeeman, 2006). Briefly, 50 mg frozen dry duckweed was grinded into powder by a multi-tube ball mill (Tissuelyser II, Qiagen, Germany). Then, 1.5 mL 80% ethanol was added. After incubating in 70 °C for 15 min, the sample was centrifuged for 5 min (12,000g) at room temperature and then the supernatant solution was discarded. This process was repeated for twice. After evaporating ethanol, transferred the precipitate to volumetric flask and added distilled water to 10 mL, then taken 0.5 mL homogenate into 2 mL EP tube and heated in 100 °C for 10 min. After cooling, 0.5 mL 200 mM Na acetate (PH 4.8) mixed with 1 μL α -amyloglucosidase (Sigma A7095) and 1 μL α -amylase (Sigma

Download English Version:

<https://daneshyari.com/en/article/7074926>

Download Persian Version:

<https://daneshyari.com/article/7074926>

[Daneshyari.com](https://daneshyari.com)