Bioresource Technology 247 (2018) 561–567

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhanced growth and fatty acid accumulation of microalgae *Scenedesmus* sp. LX1 by two types of auxin

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ARTICLE INFO

Keywords:
Microalgae
Scenedesmus sp.
Auxin
Growth
Fatty acid methyl esters

ABSTRACT

Microalgae are potential candidates for the production of valuable products, such as renewable biodiesel, health products and pigments. However, low biomass productivity has restricted their large-scale applications. In this study, the effects of two auxins (one natural type of indole-3-acetic acid (IAA) and the other synthetic type of 2,4-dichlorophenoxyacetic acid (2,4-D)) on the growth and fatty acid methyl esters (FAMEs) production of a freshwater microalgae *Scenedesmus* sp. LX1 were investigated. Both auxins showed a "low dosage-promotion and high dosage-inhibition" effect on the growth and FAMEs accumulation. The optimum dosage of IAA and 2,4-D were 1 mg L^{-1} and 0.1 mg L^{-1}, respectively. Moreover, the IAA could increase the monounsaturated fatty acid content. The auxins may promote the growth by enhancing the photosynthetic activity through increasing chlorophyll contents. Therefore, auxin significantly enhanced microalgal growth and FAMEs accumulation, and has a potential for application in developing efficient microalgal cultivation.

1. Introduction

Microalgae are photosynthetic organisms which can rapidly accumulate valuable biocomponents, including lipid/fatty acids, proteins and polysaccharides (Chisti, 2006). Therefore, microalgae biomass has a wide range of applications. The lipids/fatty acids are the feedstock of biodiesel and nutrient substances (Behrens and Kyle, 1996; Groom et al., 2008), the polysaccharide can be used for feed and the protein is a well-balanced protein source for animal and human (FAO/WHO, 1973). Meanwhile, the process of microalgae culture can absorb CO_2 and reclaim wastewater. But the large-scale production of microalgal biomass is mainly constricted by its high cost. Thus, it is necessary to achieve efficient microalgae production and then impel its commercial feasibility (Gouveia and Oliveira, 2009).

The process of microalgae-based production includes mainly three stages: microalgae biomass production, harvesting and processing (conversion) (Ferrell and Sarisky-Reed, 2010; Harun et al., 2010). Among these stages, microalgae cultivation is the fundamental, yet expensive step, which determines the quantity and quality of the microalgae-based produced. Therefore, with the aim of reducing cost, it's better to optimize microalgae cultivation to achieve high biomass production with quality biocomponents accumulation.

In the last decades, lots of efforts have been employed in this field, including selecting high-yield microalgae strains, developing efficient bioreactor, culturing microalgae with wastewater and heterotrophic culture (Cai et al., 2013; Çelekli and Dönmez, 2006; Da Silva et al., 2014; Han et al., 2014; Yu et al., 2015a). Some good results have been obtained through these efforts, while there is still a gap for the large-scale microalgae biomass production from the viewpoint of economy. Therefore, it is necessary to further improve the production efficiency of microalgae.

Auxin is one class of plant hormones, including nature auxins (such as indole-3-acetic acid (IAA)) and synthetic auxins (such as 2,4-dichlorophenoxyacetic acid (2,4-D)). Auxin could stimulate cell division, growth, leaf formation, ethylene biosynthesis, root development and fruit setting of the plant (Finet and Jaillais, 2012). Auxin have been widely used in agriculture and forestry, which have profound effects on plant growth and development even at a very low dosage (Rubio et al., 2009; Zhao, 2010). Meanwhile, from the evolutionary viewpoint, microalgae belong to the original plant cell. Therefore, auxin may also

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http://dx.doi.org/10.1016/j.biortech.2017.09.079
Received 6 July 2017; Received in revised form 9 September 2017; Accepted 11 September 2017
Available online 14 September 2017
0960-8524/

stimulate the growth of microalgae, which would be a feasible strategy to improve biomass productivity and lipid production of microalgae (Tate et al., 2013). However, the auxin has been routinely used in regulating plant growth but seldom in microalgae. Bajguz and Piotrowska-Niczyporuk (2014) showed that 0.1 μM indole acetic acid (IAA), 0.1 μM indole butyric acid (IBA), and 1 μM naphthalene acetic acid (NAA) induced an increase in the highest cell density by 53%, 46% and 24% after 72 h growth of *Chlorella vulgaris*, respectively. Ozioko et al. (2015) found that IAA (15 mg L^{-1}) and IBA (15 mg L^{-1}) significantly enhanced the dry cell weight and cell number of *C. sorokiniana*. Liu et al. (2016) added indole-3-propionic acid (IPA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) to enhance the microalgal biomass production and showing satisfactory results. Yu et al. (2017) showed that 20 mg L^{-1} of mixed phytohormones led to an increase in microalgal biomass concentration and lipid content of 59.3% and 200% for *Scenedesmus* sp. SDEC-8 and 76.6% and 180% for *Chlorella sorokiniana* SDEC-18. The growth of *Scenedesmus obliquus* was improved by IAA, with the maximum growth at 10^{-5} M of 17.7 × 10^6 cells mL^{-1} and total fatty acid of 97.9 mg g-DCW^{-1}, and enhanced the growth by 1.9-fold compared to the control (9.5 × 10^6 cells mL^{-1}) (Salama et al., 2014).

Previous studies mainly focused on the effects of auxin on the growth of microalgae but little on the fatty acid methyl esters (FAMEs) accumulation, especially its composition. Meanwhile, the underlying mechanism of effects of auxin on microalgae was not well clarified. Moreover, there was a lack of researches about the comparisons of different effects of natural auxin and artificial synthetic auxin on the growth and FAMEs accumulation of microalgae.

The aim of this study was to investigate the influences of natural auxin (IAA) and artificial synthetic auxin (2,4-D) on the growth, FAMEs accumulation (biodiesel precursors), photosynthetic activity and the morphological characteristics of *Scenedesmus* sp. LX1. *Scenedesmus* sp. LX1a green microalgae with high-lipid yield, which can survive in low-nutrient environment, such as secondary effluent (Li et al., 2010b). Besides, the underlying mechanism was clarified by detecting photosynthetic activity and photosynthetic pigments content of *Scenedesmus* sp. LX1. The obtained results could be important for achieving the high microalgae biomass production with better quality lipids/fatty acids. It will further reduce the cost and advance the large-scale utilization of microalgal biomass production.

2. Materials and methods

2.1. Strain and materials

Scenedesmus sp. LX1 (Collection No. CGMCC 3036 in China General Microbiological Culture Collection Center) was previously isolated from tap water. The auxin (i.e. IAA and 2,4-D) were obtained from Sigma (USA).

2.2. Culture conditions and medium

The effects of auxin addition on the microalgal growth was examined with batch experiments. Two types of auxin, one natural type of IAA, and the other synthetic type of 2,4-D, were applied at dosages of 0.01, 0.1, 1 and 10 mg L^{-1}. The microalgae were grown in the modified mBG11 medium (Yu et al., 2015b), which was supplemented with auxin at appropriate dosages for the experimental groups, while the same amount of solvent without auxin was added to the control. There were three replicates for each culture condition. Cultivation was performed at 25 °C and the light intensity was 6000 Lux, with light/dark period of 14 h/10 h. During the experiment, samples were taken in sterile environment and were analyzed immediately.

2.3. Analytical methods

2.3.1. Microalgae growth property

The microalgal density was determined by measuring the optical density of the microalgal culture at 680 nm (OD_{680}). The relationship between the microalgal density (D, cells mL^{-1}) and the OD_{680} was expressed as follows (Eq. (1)):

$$D = 21.13 \times OD_{680}, \quad R^2 = 0.98 \tag{1}$$

The logistic model was used to describe the microalgal growth (Li et al., 2010a).

$$N = \frac{K}{1 + e^{a-rt}} \tag{2}$$

$$R_{\max} = \frac{rK}{4} \tag{3}$$

where N (cells mL^{-1}) is the algal density at time t (d), K (cells mL^{-1}) is the carrying capacity (the maximum algal density reached in the culture), a is a constant in the logistic model which indicates the relative position from the origin, r (d^{-1}) is the intrinsic growth rate, and $R_{\max}$ (cells mL^{-1} d^{-1}) is the maximum population growth rate.

2.3.2. Fatty acid methyl esters analysis

Fatty acids were analyzed using a modified method of Brandl et al., (1988) 20 mg of freeze-dried microalgal cells were added to a solution with 1 mL chloroform, 0.85 mL methanol and 0.15 mL concentrated sulfuric acid. The contents were saponified for 2.5 h at 100 °C. After the saponification, the macroalgal samples were cooled down to room temperature, and then 0.5 mL water was added and vortexed for 1 min. Separation was achieved by centrifuging at 2000*g* for 5 min. Then the bottom organic phases were removed with a new tube and 200 mg (anhydrous $NaSO_4$) was added. After shaking tubes, the liquid was transferred to another tube using a hypodermic disposable polypropylene syringe and subsequently filtered using 0.2 μm polyvinylidene fluoride (PVDF) syringe microfilters before transferring to a GC vial. Then the FAMEs in the extracted liquid was analyzed by GC-MS (QP2010, SHIMADZU) equipped with FID and HP-5MS capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness) with a temperature programming from 80 to 280 °C at an increasing rate of 6 °C min^{-1}. Supelco 37 Component FAME Mix (Sigma-Aldrich, USA) was used as the standard substance to identify the fatty acid methyl esters.

2.3.3. Photosynthesis activity

The photosynthesis oxygen evolution characteristic of *Scenedesmus* sp. LX1 under different auxin dosages was determined via Liquid-Phase Oxygen Measurement System (Hansatech Ltd., Chlorolab 2) (Wu et al., 2015).

The *P-I* Curve was used to describe the photosynthetic oxygen elution rate and Illumination intensity.

$$P = P_m \times \tanh\left(\frac{a \times I}{P_m}\right) + R_d \tag{4}$$

$$I_k = \frac{P_m}{a} \tag{5}$$

$$I_c = \frac{R_d}{a} \tag{6}$$

where I (μmol m^{-2} s^{-1}) is photosynthetic photon flux density, P (μmol O_2 g $cell^{-1}$ min^{-1}) is the rate of photosynthetic oxygen evolution, P_m (μmol O_2 g $cell^{-1}$ min^{-1}) is the maximum photosynthetic rate, a (10^{-3} μmol O_2 m^2 g $cell^{-1}$) is a constant in the model, R_d (μmol O_2 g $cell^{-1}$ min^{-1}) is the dark respiration rate. I_k (μmol m^{-2} s^{-1}) is the light saturation point, and I_c (μmol m^{-2} s^{-1}) is the light compensation point.