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Effect of diethyl aminoethyl hexanoate on the accumulation of high-value biocompounds produced by two novel isolated microalgae

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HIGHLIGHTS

- Influence of DA-6 on growth of two newly isolated algae strains was investigated.
- Metabolin accumulation, fatty acid profiles and biodiesel properties were examined.
- Twice DA-6 dose could keep *S. quadricauda* SDEC-13 continuous rapid growth.
- DA-6 enhanced the lipid productivity of *C. ellipsoidea* SDEC-11 to 39 mg L^{-1} d^{-1}.
- DA-6 increased cell size, protein and carbohydrate production of the algae strains.

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ABSTRACT

The low productivity of microalgae has restricted scale-up application of microalgae-based biodiesel processes. Diethyl aminoethyl hexanoate (DA-6) was investigated to enhance the biomass and metabolite productivity. At a very low concentration (10^{-7} M) DA-6 made *Chlorella ellipsoidea* SDEC-11 and *Scenedesmus quadricauda* SDEC-13 obtain enlarged cell size, 114 mg L^{-1} d^{-1}, 101 mg L^{-1} d^{-1} biomass productivity and 39.13 mg L^{-1} d^{-1}, 32.69 mg L^{-1} d^{-1} lipid productivity, respectively. Biomass and lipid productivity of SDEC-11 and SDEC-13 were 100 mg L^{-1} d^{-1} and 30.05 mg L^{-1} d^{-1}, 94 mg L^{-1} d^{-1} and 28.43 mg L^{-1} d^{-1}, respectively, without DA-6. Twice hormone dose in 10^{-6} M DA-6 medium resulted in higher biomass productivity (106 mg L^{-1} d^{-1}) and longer exponential growth of SDEC-13. DA-6 also ensured the property of microalgae biodiesel to meet the EN 14214 standard. The current investigation demonstrated that DA-6 accelerated the microalgae growth and simultaneously improved the quality and quantity of lipid for biodiesel production.

1. Introduction

Fossil fuels as non-renewable energy guarantee the development of economy growth at present because they supply over 80% of the energy at a relatively low price. Nevertheless some global environmental problems caused by fossil fuel combustion break out, like global climate change (Demirbas and Demirbas, 2011; Medeiros et al., 2015). As part of endeavors to tackle the energy crisis and global warming, bioenergy sources are promising, renewable and green alternatives for sustainable and clean energy production. Algae-based biodiesel is recognized as a potential candidate due to its annual yield (>5000 gallons $acre^{-1}$), carbon neutral, non-toxic, and so on (Biodiesel 2020, 2008; U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Biomass Program, 2010; Dillschneider et al., 2013). However at present the cost of large-scale algae cultivation for biodiesel production is too high to be competitive with fossil fuels, which is a critical inhibition factor for commercial biofuel production (Wijffels and Barbosa, 2010). With the aim of reducing cost researchers have undertaken some efforts like selecting high-yield strains, culturing algae with wastewater, integrating microbial processes for multiple products and enhancing microalgae growth and lipid accumulation with chemicals or environmental factors (Çelekli and Dönmez, 2006; Cai et al., 2013; da Silva et al., 2014; Han et al., 2014; Yu et al., 2015).

Abbreviations: ABA, abscisic acid; BRs, brassinosteroids; CKs, cytokinins; DA-6, diethyl aminoethyl hexanoate; GA3, gibberellic acid-3; IAA, indole-3-acetic acid; IBA, indole-3-n-butyric acid; IPA, indole-3-n-propionic acid; KIN, kinetin; NAA, naphthalene-acetic acid; PAA, phenylacetic acid; TRIA, 1-triacontanol.

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Plant hormones (phytohormones) are a class of natural or synthetic chemical messengers that regulate growth and development in cellular activities of higher plants at very low concentrations (Voß et al., 2014). As algae are primitive eukaryotic plant cells, largely photosynthetic organisms, plant hormones may become another strategy to increase microalgae growth by controlling biochemical pathways (Tate et al., 2013). And there had been numerous studies of the effects of phytohormones on algae growth and accumulation of metabolites such as lipid, chlorophylls, carbohydrates, and proteins (Hunt et al., 2010, 2011; Tate et al., 2013; Bajguz and Piotrowska-Niczyporuk, 2014). Bajguz and Piotrowska-Niczyporuk (2014) have demonstrated the positive effects of brassinosteroids (BRs) and cytokinins (CKs) on growth, chlorophyll, monosaccharide and protein content in the green algae *Chlorella vulgaris*. Besides these natural plant hormones, the synthetic auxin naphthalene-acetic acid (NAA) solubilized in ethanol also showed superiority as a viable approach for enhancing biomass productivity in green algae, coccolithophore, diatom and cyanobacterium cultures (Hunt et al., 2011). Hunt et al. (2010) also suggested that phytohormones could prolong the exponential growth period and shorten the initial lag encountering new grow condition. Furthermore phytohormones could regulate the morphological development of specific cells in higher plants (Shibaoka, 1994) and Park et al. (2013) observed that indole-3-acetic acid (IAA), gibberellic acid-3 (GA3), kinetin (KIN), 1-triacontanol (TRIA) and abscisic acid (ABA) increased *Chlamydomonas reinhardtii* growth by 54–69% and enhanced cell size at the early stationary stage of growth significantly. There is another plant phytohormone diethyl aminoethyl hexanoate (DA-6) which has the capacity of stimulating the synthesis of chlorophyll, protein and nucleic acid and promote the photosynthetic rate and carbon and oxygen metabolism of plants (Jiang et al., 2012). This compound has been routinely used in regulating plant growth but seldom in microalgae. Salama et al. (2014) explored the effects of DA-6 on *Scenedesmus obliquus* growth, but the application of DA-6 in microalgae growth regulator was still rare. Furthermore, the impact of exogenous phytohormones on microalgae metabolic production focus on chlorophyll a and b, carotenoid and astaxanthin (Tate et al., 2013; Lu and Xu, 2015; Yu et al., 2015), and the characteristic of lipid, protein and carbohydrate accumulation of algae with DA-6 addition has not been investigated extensively. In order to enrich the understanding of the impact of DA-6 on microalgae, evaluation of effect of DA-6 on growth and lipid, protein, and carbohydrate composition of other algae should be explored.

Therefore the goal of this study was to construct a simple approach to achieve higher microalgae biomass and lipid productivity than general medium cultivation. This major work aims to investigate the influence of DA-6 on growth, lipid accumulation of two newly isolated algae strains *Chlorella* sp. SDEC-11 and *Scenedesmus* sp. SDEC-13 (Han et al., 2014). Besides lipid, it is necessary to detect the protein and carbohydrate content in algae influenced by DA-6 due to carbon sources synthesis pathway (Hunt et al., 2010). The effect of phytohormones on the microalgae cell size, fatty acid profile and amino acid composition was evaluated.

2. Methods

2.1. Microalgae strain

The microalgae *Chlorella ellipsoidea* (SDEC-11) and *Scenedesmus quadricauda* (SDEC-13) were obtained and cultured as description in Han et al. (2014), which are the better candidates for biofuel production than the other isolated strains in the lab, taking the lipid content and productivity into consideration. Algae inoculums were prepared from exponentially growing seed cultures.

2.2. Experimental setup

All the experiments were conducted in the vertical bubble-columns photo-bioreactor (PBR, Patent No. 2012205920571) containing 2.5 L BG11 growth medium with an initial algae biomass concentration of 0.1 g L^{-1}. In consideration that DA-6 would decompose in alkaline solution, pH value of all the media were adjusted to 6.0 prior to DA-6 addition. Stock solutions of DA-6 were prepared at 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M using distilled water. Each stock of 2.5 mL was added to 2.5 L of BG11 medium to obtain the concentrations of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M, which were used for the experiments. To test continuous impact of DA-6 on algae growth, the trail of second dose with the same concentration was performed at 10^{-6} M media of SDEC-13 when specific growth rate decreased. The cultivation in BG11 with no DA-6 addition was conducted simultaneously as control for all experiments.

All these PBRs were placed in the artificial climate chamber with the following cultivation conditions: light intensity of 60 μmol m^{-2} s^{-1} provided by daylight fluorescent tubes (Philips, 36W), temperature 25 ± 1 °C and continuous air aeration (600 mL/min) from the aerator at the bottom of PBRs. The light intensity was detected by an irradiance sensor (ZDS-10, Shanghai Cany Precision Instrument Ltd., China). All the experiments were conducted in triplicate.

2.3. Determination of the algae growth and cell size

The algae growth was measured every 24 h by biomass concentration determination (g L^{-1}, dry weight, DW) as described by Song et al. (2013). Li et al. (2014) reported that peak occurred at ex/em about 280/330 in extracellular three-dimensional excitation-emission matrix (EEM) fall just within an aromatic protein region. To detect extracellular protein secretion and observe algae growth, algae cell solution was sampled every day and filtrated by a 0.45 μm filter membrane to remove the suspensions prior to testing for EEM using a fluorescence spectrophotometer (F-4600, Hitachi, Japan) with a 1.0-cm quartz cuvette. EEM spectra were collected with emission from 250 nm to 550 nm at 5 nm increments by varying the excitation wavelength from 220 nm to 450 nm at 5 nm increments. Excitation and emission slits were both maintained at 5 nm and the scanning speed was set at 2400 nm min^{-1}.

Before algae harvest, observations of algae cell size and morphology were performed using an inverted fluorescence microscope (Ti-s, Nikon, Japan), and then statistical analysis of semi-major axis and semi-minor axis of algae cell was implemented with NIS-Elements D 4.20.00 software.

The specific growth rate (μ, d^{-1}) and biomass productivity of microalgae during the incubation were calculated according to the following formulas (Jiang et al., 2015):

$$\mu = (\mathrm{Ln}N_2 - \mathrm{Ln}N_1)/(T_2 - T_1) \quad (1)$$

$$P_b = (X_2 - X_1)/(T_2 - T_1) \quad (2)$$

where N_1 and N_2 are the dry biomass concentrations at day T_1 and T_2 respectively and X_1 and X_2 are the dry biomass concentrations at day T_1 and T_2 respectively.

2.4. Analysis of biomass components

After 15 days of algae growth, the biomass was harvested by centrifugation at 4000 rpm for 10 min, dried to constant weight at minus 50 °C in a lyophilizer (FDU-1200, EYELA, Japan), and then ground to homogeneous powder for protein, lipid and carbohydrate analysis.