Energy Conversion and Management 168 (2018) 522–528

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Combined effect of exogenous phytohormones on biomass and lipid production in *Acutodesmus obliquus* under nitrogen limitation

Nirmal Renuka, Abhishek Guldhe, Poonam Singh, Faizal Bux*

Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

ARTICLE INFO

Keywords:
Microalgae
Nitrogen stress
Biomass
Lipid
Phytohormone

ABSTRACT

The use of phytohormones in microalgal biofuel production is gaining interest due to their substantial effect on biomass and lipid enrichment under standard and nitrogen-limited conditions. In the present study, the collaborative effect of combination of selected phytohormones *viz*. zeatin (Z), indole acetic acid (IAA), and gibberellic acid (GA) was studied to optimize biomass and lipid production in microalga *Acutodesmus obliquus* under nitrogen-limitation using response surface methodology (RSM). Significantly higher biomass and lipid productivity was obtained with the supplementation of combination of Z (0.5 mg L^{-1}), IAA (1.0 mg L^{-1}), and GA (5.0 mg L^{-1}) under nitrogen-limitation with 49.07% and 77.20% increase respectively as compared to optimized nitrogen control (ON). Zeatin was found to be the most significantly influencing phytohormone followed by GA in RSM data analysis. Present study elucidated the synergistic effect of combination of exogenous phytohormones in enhancing biomass and lipid productivity under nitrogen-limitation for microalgal biofuel production.

1. Introduction

Microalgae are gaining great attention for the production of sustainable biofuels, due to their ability to proliferate under versatile environmental conditions, easy cultivation, faster growth and adequate lipid content as compared to other terrestrial biofuel crops such as jatropha, soybean, etc. [1–4]. Recently, the use of phytohormones for the biomass and lipid enrichment is one of the emerging trend in the microalgae biofuel production [5–8]. Phytohormones are the group of signalling chemicals present in extremely low concentrations in plants including algae, and are mainly divided into 5 classes' *viz*. auxins, cytokinins, gibberellins, jasmonates and brassinosteroids [9,10]. Growth hormones are accountable for many metabolic processes such as cell division, differentiation, senescence, mobilization of nutrients, etc., and regulate growth [10]. These can have effect on plant growth at very low concentrations, and therefore are used as growth regulators for plants and algae [11].

Continual research is in progress on the development of lipid and biomass enhancement strategies to increase the viability of microalgae biofuels. Ability of microalgae to alter lipid metabolism under stressed conditions has led to the development of different strategies for lipid enhancement such as salt stress, nutrient starvation, etc. [12–15]. Nitrogen (N) stress is considered to be an efficient approach due to its strong influence on lipid content and easy implementation. However, N stress causes significant losses in the biomass productivity of the microalgae cultures, which ultimately affects the lipid productivity. Therefore, there is an urge in finding the alternative solutions and development of combined strategies to overcome biomass losses in N-limited conditions [16].

The role of phytohormones in regulating the stress responses in microalgae is evidenced in the previous studies [17]. Nitrogen signalling is greatly dependent on the phytohormone level in microalgae. For example, under N-limited conditions, there is decrease in the level of cytokinins and gibberellins, and increase in the level of auxins, and abscisic acid, etc. [8,18]. However, the main mechanism of N signalling and stress response is greatly dependent on the ratio of their concentration and crosstalk [18]. Based on concentration, phytohormones can affect vital processes in microalgae in additive, synergistic and antagonistic manner. Therefore, it may be conducive to study the combined effect of phytohormones on biomass and lipid production in comparison with the individual phytohormone supplementation.

In algae, these compounds are found to have generous effect on biomass and lipid production [6,19,20]. Also recently, there are reports on the effect of individual hormones on the biomass and lipid production under N-limited conditions [8,11]. Cytokinins are the key hormones for N signalling in microalgae [18]. Exogenous supplementation of cytokinin in microalgae cultivation is reported to enhance the biomass productivity under N-limited conditions [18,21]. While, the effect of gibberellic acid on microalgae growth and lipid production for biofuel production under standard N nutrition is rarely studied [19],

* Corresponding author.
E-mail address: faizalb@dut.ac.za (F. Bux).

https://doi.org/10.1016/j.enconman.2018.05.029
Received 27 February 2018; Received in revised form 3 May 2018; Accepted 9 May 2018

and under N-limited conditions is yet to be explored. Conversely, in plants, exogenous application of gibberellic acid in agronomic plants have shown positive effect on growth and yield under reduced dose of N fertilization [22]. Moreover, auxins such as indole acetic acid, 2,4-dichlorophenoxyacetic acid (2, 4 - D), naphthalene acetic acid (NAA) etc. are reported to enhance the biomass and lipid production under standard N nutrition and N limited conditions [6,19]. There are few reports on the interactive effect of phytohormones on microalgae growth and lipid production under standard N nutrition [23,24]. However, the combined effect of hormones from different families on biomass and lipid production in microalgae under N-limited conditions is scarcely studied. Therefore, in the present study, different types of phytohormones cytokinin –zeatin, auxin – indole acetic acid and gibberellin-gibberellic acid were selected to investigate their interactive effect – individually and their combinations on growth, biomass and lipid productivity in *Acutodesmus obliquus* under N-limited conditions. *Acutodesmus obliquus* was selected based on our previous screening of microalgae for higher lipid productivity [16]. The effect of optimized concentrations of hormones on photosynthetic performance and biochemical composition of microalgae was also investigated.

2. Materials and methods

2.1. Microalgae strain and culture conditions

Microalga *Acutodesmus obliquus*, isolated from freshwater was used in the present study [16]. Cultures were grown under standard laboratory conditions in 1 L conical flasks containing 500 mL BG-11 medium (Supplementary Table 1) at temperature of 25 °C, photon flux of 120 μmol m^{-2} s^{-1} and photoperiod of 16:08 h light dark cycle (Supplementary Fig. 1). Culture conditions were kept constant for all sets of experiment.

2.2. Experimental design

The effect of different combinations of selected phytohormones *viz*. cytokinin- zeatin (Z), auxin-indole acetic acid (IAA) and gibberellin – gibberellic acid (GA), was studied on the growth, biomass and lipid productivity of microalga *Acutodesmus obliquus* under N limited condition in BG-11 medium by applying the Box–Behnken design (Minitab Statistical Software) of response surface methodology (RSM). The experimental design consisted of three phytohormone factors: zeatin (Z), auxin-indole acetic acid (IAA) and gibberellic acid (GA) and 3 levels of concentrations (Table 1). In the experimental design, 13 experiments with varying phytohormone combinations and three replicates at the centre points were carried out making 15 experiments in total. In addition, standard BG-11 and N limited BG-11 medium (designated as optimized N concentration or ON) without hormone supplementation were kept as positive and negative control respectively (Table 2).

The optimized N concentration i.e. 120 mg N L^{-1} (750 mg L^{-1} $NaNO_3$) was used for the N limited conditions (ON) for enhanced lipid productivity as described in our previous study [16]. Preliminary experiments were performed with individual phytohormone (IAA and GA) to evaluate their effect on biomass productivity in N limited medium. IAA and GA were supplemented at various concentrations of 0.1, 0.5, 1.0, 5.0 and 10 mg L^{-1} designated as ON-I_1, ON-I_2, ON-I_3, ON-I_4 and ON-I_5, and ON-G_1, ON-G_2, ON-G_3, ON-G_4 and ON-G_5 for IAA and GA respectively. The concentration levels for the experimental design for IAA and GA concentrations were selected based on preliminary experimental results with individual phytohormone based on higher biomass productivity. While concentration range for zeatin was selected based on findings from our previous study [21]. The biomass productivity obtained with IAA supplementation in ON-I_2, ON-I_3 and ON-I_4 were significantly higher than the other selected concentrations (supplementary Fig. 2). Thus range selected for IAA was 0.5 to 5 mg L^{-1}. Similarly, the biomass productivity obtained with GA supplementation in ON-G_2, ON-G_3 and ON-G_4 were significantly higher than the other selected concentrations (supplementary Fig. 3). Thus range selected for GA was 0.5 to 5 mg L^{-1}.

The three levels of variables for the selected factors are depicted in Table 1. All the experiments were carried out in triplicate in 1 L Erlenmeyer flasks with working volume of 500 mL. Lipid productivity was analysed as the response for the experimental design. The effect of independent factors on the dependent factors was analysed using the quadratic equation:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_2X_3 + a_{23}X_1X_3$$

where Y is the response (lipid productivity), a_0 is offset term; a_1, a_2 and a_3 are linear coefficients; a_{11}, a_{22} and a_{33} are the squared term coefficients; a_{12}, a_{13} and a_{23} are the interaction coefficients. X_1, X_2 and X_3 are Zeatin, IAA and GA respectively.

Table 1
Factors and levels for experimental design of phytohormone concentration optimization under optimized nitrogen conditions for *Acutodesmus obliquus*.

Factors	A (Zeatin concentration mg L^{-1})	B (IAA concentration mg L^{-1})	C (GA concentration mg L^{-1})
Level 1	−1 (0.05)	−1 (0.5)	−1 (0.5)
Level 2	0 (0.1)	0 (1.0)	0 (1.0)
Level 3	1 (0.5)	1 (5.0)	1 (5.0)

*IAA – Indole acetic acid; GA – Gibberellic acid.

2.3. Analytical procedures

2.3.1. Analyses of growth parameters

Photosynthetic performance of the cultures was monitored by PAM (Pulse amplitude modulated) fluorometry using a Chlorophyll Fluorometer (DUAL-PAM 100, Heinz WalzGmbh, Effeltrich, Germany). Quantum yield for PSII i.e. maximum quantum efficiency (Fv/Fm) and relative electron transport (rETR) were analysed at 3, 6, 9, 12 and 14th day of cultivation and calculated as described by White and Anandraj [25]. Biomass estimation was carried out gravimetrically. While, biomass productivity was calculated as mg L^{-1} d^{-1} at the late log phase i.e. on14th day of cultivation [16].

2.3.2. Analyses of microalgae biomass for biomolecules

Microalgal biomass was harvested using centrifugation followed by freeze drying. Dry microalgae biomass was subjected to microwave assisted solvent extraction by using chloroform: methanol (2:1 v/v) and microwave treatment for 10 min (100 °C and 1 kW) for the extraction of lipids [26]. Mixture was filtered using Whatman filter paper 1 to remove the cell debris. Organic layer was separated and oven dried at 70 °C for gravimetric analyses of lipid content and lipid productivity. Lipid productivity was calculated as mg L^{-1} d^{-1} using the equation described by [16].

$$\text{Lipid productivity } (mgL^{-1}d^{-1}) = \text{Biomass productivity } (mgL^{-1}d^{-1}) \times \frac{\text{Lipid content } (\%)}{100} \quad (1)$$

Extracted lipids were subjected to simultaneous esterification and transesterification reaction for biodiesel conversion using sulfuric acid and methanol as a catalyst and acyl acceptor respectively. The reaction