Algal Research 35 (2018) 471–478

Contents lists available at ScienceDirect

Algal Research

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

Effects of L-amino acids as organic nitrogen source on the growth rate, biochemical composition and polyphenol content of *Spirulina platensis*

G. Shanthi[a], M. Premalatha[a,*], N. Anantharaman[b]

[a] Algal Research and Biotechnology Laboratory, Department of Energy & Environment, National Institute of Technology, Tiruchirappalli, Tamil Nadu 620 015, India
[b] Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, Tamil Nadu 620 015, India

ARTICLE INFO

Keywords:
Spirulina platensis
L-Amino acids
FT-IR
Biochemical composition
Polyphenol

ABSTRACT

Nitrogen plays on important role in enhancing the biochemical composition during photosynthesis. The form of nitrogen source either organic or inorganic further has an impact on the growth and biochemical composition of microalgae. The present work focuses on the influence of various L-amino acids on the growth rate, biochemical composition and polyphenol content of *Spirulina platensis*. Addition of 0.1 g L^{-1} of L-Arginine, L-Asparagine and L-Glutamine exerted the biomass productivity of 175.8 ± 0.005, 153.0 ± 0.001 and 144.2 ± 0.003 mg L^{-1} day^{-1} respectively while it was 114.0 ± 0.003 mg L^{-1} day^{-1} for conventional medium. The yield of protein could be increased by 29% under 0.1 g L^{-1} of L-Arginine. Addition of 0.05 g L^{-1} of L-Asparagine increased the carbohydrate and lipid content by 260% and 242% compared with inorganic nitrogen. The in-vitro digestibility of the feed protein is in the order of L-Arginine > L-Asparagine = Nitrate > L-Glutamine. The polyphenol content was 5.0 ± 0.5 4.0 ± 0.2 and 3.2 ± 0.4 mg g^{-1} for L-Asparagine, L-Glutamine and L-Arginine respectively which is higher than that of inorganic nitrogen (2.4 ± 0.2 mg g^{-1}). The results indicate that the L-amino acids could be utilized by the *S. platensis* to improve the overall biochemical composition and the resulting biomass may have potential application for food and biofuel production.

1. Introduction

Dissolved nitrogen in the rivers causes the toxic effect to the aquatic system by cultural eutrophication. It has been estimated that, about 12–33% of the anthropogenic nitrogen in the streams worldwide, is derived from the discharge of industrial wastewater particularly, municipal wastewater effluent and strom water runoff. The remaining is due to the agriculture and fertilizer runoff [1,2,3]. The nitrogen discharge in the wastewater could be in the form of organic and inorganic. The concentration of inorganic form of nitrogen in the wastewater has efficiently been reduced > 95% by nitrification-denitrification systems. But there is a lack of information about the removal of organic nitrogen in the wastewater and wastewater effluent. The discharge of dissolved organic nitrogen (DON) from the conventional wastewater treatment plant comprises approximately 65% of the dissolved nitrogen in the effluent. When the wastewater gets highly treated with secondary treatment such as nitrification-denitrification processes, the dissolved organic nitrogen accounts for up to 80% of the dissolved nitrogen in the effluent [4,5]. Hence, the most efficient and economically viable technologies are required to address the dissolved organic nitrogen in the raw wastewater or wastewater effluent before letting out into the environment.

Microalgae are the potential candidate for treating wastewater, due to the higher assimilating capability of nitrogen, phosphorous and micropollutant during the growth [6]. The harvested biomass could directly be utilized for biofuel production. *Spirulina platensis,* filamentous cyanobacterium (blue green algae) is a promising source of protein supplement and one of the most widely studied algal species for wastewater treatment [7–11]. *S. platensis* has been extensively cultivated in tropical countries like India, due to the sustainability of the species at intense solar radiation and temperature (40 °C) [12,13].

Recently, *S. platensis* has been considered as a potential feedstock for biodiesel production, even though the lipid content is < 15% [14,15]. It is due to the availability of lipid is in the form of structural lipids. In addition to the protein, it is capable of accumulating a wide range of essential nutrients and bioactive functional constituents such as polyunsaturated fatty acids (gamma-linolenic acid), essential amino acids, enzymes, vitamins (especially B_{12}), minerals (especially iron), pigments (β-carotene, chlorophyll *a*, phycocyanin) and polyphenols. Nowadays, *S. platensis* biomass has attracted the attention for its ability to produce the antioxidant compounds such as polyphenolics [16] used for the treatment of various human diseases [17]. These compounds play a

* Corresponding author.
E-mail address: latha@nitt.edu (M. Premalatha).

https://doi.org/10.1016/j.algal.2018.09.014
Received 27 January 2018; Received in revised form 11 September 2018; Accepted 20 September 2018

vital role in preventing the formation of free radicals, in particular, the reactive oxygen (ROS) and nitrogen (RON) species [18]. The polyphenol accumulation in cyanobacteria has also been directly related to the nitrogen source and its concentration [19].

The growth and biochemical composition of cyanobacteria biomass are affected by many factors including nutrient availability, salinity, pH and environmental parameters such as temperature and light. In particular, the biochemical composition varies with reference to the type and quantity of nitrogen used in the medium. Nitrogen is an essential component in the biosynthesis of protein [20]. Various types of industrial effluents which are rich in inorganic nitrogen, in the form of nitrate, nitrite, and ammonium [8–11] were effectively utilized for their growth. In such cases, *S. platensis* efficiently reduced the inorganic load.

The organic nitrogen in the wastewater is also available in the form of free amino acids [21]. The researchers have focused on the effect of various amino acids as sole nitrogen source on the growth and biochemical composition of microalgae to utilize the amino acid-rich industrial waste effluents [22–25]. It has been effectively assimilated by providing a stimulatory effect to achieve the maximum biomass yield in microalgae. They also concluded that organic form of nitrogen is considered more favorable for the growth and lipid production than inorganic form [26]. Nitrogen assimilation rate and carbon to nitrogen ratio (C/N) of the biomass also depend on the chemical form of nitrogen used in the cultivation medium [27].

The effect of amino acids on the growth and biochemical composition of microalgae have extensively been studied [22–25] whereas only the limited number of investigations were reported on the growth of cyanobacteria *S. platensis* [28]. The uptake of organic nitrogen source in the form of L-amino acid from the wastewater effluent varies from species to species. Hence, there is an urgent need to evaluate the growth, biochemical composition, and the antioxidant potential which is highly recommended for the biopharmaceutical application in response to the various organic nitrogen supply. The present work aims to assess the influence of eighteen L-amino acids as organic nitrogen source on the growth rate, biochemical composition and polyphenol content of *S. platensis*.

2. Material and methods

2.1. Spirulina strain and culture conditions

The cyanobacterium *S. platensis* used in this study was obtained from the Gandhi gram rural University, Dindugal, Tamil Nadu and grown in Zarrouk medium with the following composition (g L^{-1}): 16.8 $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$,1 g K_2SO_4,1 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2$, 0.01 g $FeSO4{\cdot}7H_2O$, 0.08 g Na_2EDTA, and 1 ml of A_5 micronutrient solution containing (g L^{-1}): 2.8 g H_3BO_3, 1.8 g $MnCl_2{\cdot}4H_2O$, 0.220 g $ZnSO_4{\cdot}7H_2O$, 0.080 g $CuSO_4{\cdot}5H_2O$ and 0.02 g $(NH_4)_6$ $Mo_7O_{24}{\cdot}4H_2O$. The temperature was maintained at 30 ± 2 °C, and illumination was provided by 40 W cool white fluorescent lights with the light intensity of 2500 lx under 16 h:8 h (light: dark) cycle. Nitrogen source was changed as an organic nitrogen source in the form of different amino acids, instead of $NaNO_3$ in the Zarrouk medium.

2.2. Experimental design

Batch experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of nitrogen- starved cells with the initial cell dry weight of 0.08 g L^{-1}. Then, 0.05 g L^{-1} and 0.1 g L^{-1} of eighteen different L-amino acids such as lysine (Lys), arginine (Arg), proline (Pro), threonine (Thr),tyrosine (Tyr),glutamic acid (Glu), tryptophan (Trp), aspartic acid (Asp), glutamine (Gln),alanine (Ala), glycine (Gly), asparagine (Asn), leucine (Leu), histidine (His), serine (Ser), valine (Val), cysteine(Cys), methionine (Met) were introduced into each flask respectively and incubated for a period of 7 days. Nitrogen starved cells were used for inoculum and were prepared by introducing 5% (v/v) of exponentially growing strain with the biomass concentration of 0.7 g L^{-1} into the 4 L of nitrogen free Zarrouk medium. The culture was maintained at nitrogen starved condition for 24 h before inoculation. All culture samples were manually shaken for three times a day to make sure that the nutrients and light are supplied equally to all the cells. The culture grown in Zarrouk medium without nitrogen source served as the Control 1 and with nitrate as Control 2. Batch experiments were run in triplicate.

2.3. Growth measurement

The optical density of the samples was measured daily using UV/VIS spectrophotometer (Spectroquant®Pharo300, Merck) at 560 nm. The cells were centrifuged at 5000 rpm for 15 min to measure the final biomass concentration, on the sixth day. Thereafter, they were washed with deionized water to remove the medium salts. Then the cells were transferred into the pre-weighed Eppendorf tubes and dried at 80 °C until the weight became stable to determine the dry cell weight. The specific growth rate (μ) and biomass productivity (P_b) of the cells were calculated by the following Formula:

$$\mu\ (\text{day}^{-1}) = \frac{(\ln N_2 - ln\, N_1)}{(t_2 - t_1)} \quad (1)$$

$$\text{Pb (mg L} - \text{1 day} - \text{1)} = \frac{N_{2-N_1}}{t_{2-t_1}} \quad (2)$$

where N_2 and N_1 are the biomass concentrations (g $L-^1$) at the times t_2 and t_1(days) respectively.

2.4. Determination of biochemical composition

2.4.1. Estimation of protein content

The crude protein content and protein yield were determined from the following Formula [29]:

$$\text{Crude protein content } (C_P,\%) = \text{Total nitrogen} \times 6.25 \quad (3)$$

$$\text{Protein yield (g } L^{-1}) = B \times Cp \quad (4)$$

where total nitrogen N is obtained from Elemental (CHNS) analysis (CHN series II 2400, Perkin Elmer, USA) and the instrumental run was duplicated. B is the dry cell weight of the biomass (g L^{-1}).

The nitrogen assimilation rate was calculated by multiplying the nitrogen content by the specific growth rate (μ).

$$\text{Nitrogen assimilation rate } (\text{day}^{-1}) = \text{nitrogen content} \times \mu \quad (5)$$

The elemental analysis also was used to calculate the cellular stoichiometry of carbon to nitrogen (C/N) ratio [27].

2.4.2. Determination of protein secondary structure

The secondary structural components of protein in the biomass were quantitatively determined by Fourier Transform Infrared (FT-IR) Spectroscopy (Perkin Elmer-Spectrum Two). The amide I region (1600–1700 cm^{-1}) was selected to quantify the secondary structural components. The following band assignment in the frequency range of 1645–1662, 1613–1637, 1662–1682 and 1637–1645 cm^{-1} were assigned to the secondary structure components of α-helix, β-sheet, β-turn and random coil respectively. The peak area corresponding to each component was determined with the baseline corrected deconvoluted spectra. The ratio of the peak area of α-helix to β-sheet was used to directly relate with the in vitro digestibility of the protein [30,31]. The higher ratio of α-helix to β-sheet represents the increase in in-vitro digestibility of the feed protein.

2.4.3. Determination of carbohydrate and lipid content

The carbohydrate and lipid content were determined using the FT-IR spectroscopic method. The absorbance spectra were collected