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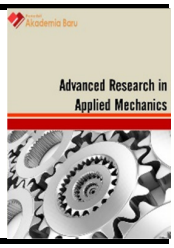

Comparison Accumulation of Total Carotenoid in Green Microalgae Under Normal and Stress Induction Growth Condition

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ABSTRACT

Carotenoids are a group of isoprenoid-derived compounds usually synthesized in all photosynthetic organisms that play roles in photosynthesis including photosynthetic bacteria, plants and microalgae. The structure of carotenoids that make up from conjugated double bond making them as strong antioxidant than other organisms that has been widely applied in nutraceuticals and pharmaceuticals industry. Recently, production of carotenoids by microalgae has been extensively studied as most potential for carotenoid production. In this study, the preliminary study on growth characteristics and amount of total carotenoid by other fast growing green microalgae were reported. The freshwater green microalgae Acutodesmus sp., Chlorella sp. and Coelastrum sp. were compared in term of their growth and total carotenoid accumulation under normal growth condition and stress induction growth condition to study whether the microalgae cultures can produce biomass and accumulate carotenoids simultaneously and yet to fasten the production of carotenoids. Total carotenoid was identified and quantified using spectrophotometric method based on absorption coefficients. The outcomes of the experiment indicated that all these microalgae accumulated significance amounts of total carotenoid under normal and stress induction growth condition.

1. Introduction

Carotenoids are natural pigments which are a group of isoprenoid-derived compounds made up of conjugated double bond, often coloured in yellow, orange and red. They are synthesized in all photosynthetic organisms and in some non-photosynthetic organisms [1]. Production of carotenoids from natural sources mainly found in photosynthetic bacteria, crustacean-by products, yeast, plants and microalgae. Human and animals could not synthesize carotenoids but able to obtain essential carotenoids from their diets [2]. Production of carotenoids by microalgae has been

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intensively studied nowadays with commercial interest including ß-carotene, zeaxanthin, lutein, canthaxanthin and astaxanthin [3]. There are two groups of carotenoids; carotenes and xanthophyll. Carotene are hydrocarbon that contain only carbon with no oxygen atom. Example of carotene are ß-carotene and Lycopene. Xanthopyll are oxygenated derivatives with the presence of hydroxyl or carbonyl substitution on one or both end group. Example of xanthophyll are astaxanthin, lutein, and zeaxanthin [4]. Carotenoids has high value in the market because it has antioxidant ability. Antioxidant is very important for human body as it can neutralize and remove free radicals. As a result, it can prevent and stop the damage caused by oxidants which can leads to cancer and disease. In spite of its antioxidant ability, carotenoids have other special biological functions in nature. In microalgae, it functions as accessory pigments to protect photosynthesis components from excess exposure to irradiance. Some carotenoids able to act as vitamin A precursors [5].

Generally, there are two proposed strategies for commercial application in production of carotenoids from microalgae which are production of biomass and carotenoids in separates stages while the other approach is for continuous culture under stress condition so that the biomass and carotenoids production occur simultaneously. There are 2 stages involved in production of carotenoids from microalgae; first stage (green stage) and second stage (orange stage). The first stage is carried out under optimum conditions to achieve high growth rate and high cellular densities. Once the desires cellular density is achieved, the microalgae will be exposed to stress conditions in the second stage in order to induce and increase carotenoids accumulation [6].

Three strains of freshwater green microalgae including Acutodesmus sp., Chlorella sp. and Coelastrum sp. have been tested in this study for potential production of carotenoids. The aim of this study was to screen and compare in respect of their cell growth and total carotenoid accumulation under normal and stress induction growth condition.

2. Materials and Methods

2.1 Microalgae Strains and Culture Conditions

In this study, three freshwater green microalgae were selected for experimental purpose. The green microalgae *Acutodesmus* sp. and *Coelastrum* sp. were isolated from Ulu Langat, Kuala Selangor and identified using 18S rDNA analysis. Meanwhile, green microalgae *Chlorella* sp. were obtained from the culture collection of Algae from Carolina Biological Supply Company.The freshwater microalgae strains (*Acutodesmus* sp., *Coelastrum* sp. and *Chlorella* sp.) were cultured in AF-6 and BG-11 medium. The media were prepared according to the media recipe available in the Microbial Culture Collection National Institute for Environmental Studies (NIES-collection) and Culture Collection of Algae at The University of Texas at Austin (UTEX).

2.2 Comparison of Normal Growth Condition and Stress Induction Growth Condition towards Biomass and Total Carotenoid Accumulation

There are two set of cultures with different conditions in order to compare the biomass and carotenoid production by three different species of freshwater microalgae. The 15 days cultures grown in a medium were used as 10% inoculum to inoculate into each flask. The first set of cultures with three different green microalgae species involved two stages production were cultured in normal growth condition under normal photon flux densities (70 µmol photon m^{-2} $s^{-1)}$ and labelled as 2-phase. While another set of cultures is an approach for continuous culture under stress conditions were grown under continuous higher photon flux densities (PFD) with 250 µmol photon m^{-2} s^{-1} to induce the carotenoid accumulation simultaneously and it is labelled as 1-phase. The aim

of this study was to compare the growth and total carotenoid production under normal and stress induction growth condition.

During the exponential growth phase which is after 15 days of growth, all the microalgae cultures were harvested and transfer to conditions which promote the accumulation and production of carotenoid. All the cultures were exposed to combine stresses condition under mixotrophy medium with addition of 100 mM sodium acetate as carbon source and nitrogen deprivation to induce production of carotenoid. The 2-phase cultures were exposed to continuous higher photon flux densities (PFD) with 250 µmol photon m^{-2} s^{-1}. The biomass yield and carotenoid accumulation were investigated before and after the induction of stress.

2.3 Extraction of Chlorophylls and Total Carotenoids

10 mL of all microalgae cultures were centrifuged at 4000 rpm for 5 min at 4 °C. The pellet obtained were extracted with 5 mL of extractant solvent (acetone) and vortex vigorously for few minutes. Then, the mixture was centrifuged at 4000 rpm for 15 min. The absorbance of supernatant was used for determination of chlorophyll a, chlorophyll b and total carotenoid [7].

The accumulation of total carotenoids and chlorophyll were identified and quantified using spectrophotometric method based on absorption coefficients. The absorbance of extracted chlorophyll was read at 663 nm and 645 nm, while the extracted total carotenoid using acetone was analyzed spectrophotometrically by measuring absorbance at 470 nm using Lichtenthaler equations [8].

3. Results and Discussion

All the microalgae studied were grown and compared in term of biomass yield and total carotenoid accumulation under normal and stress induction growth condition. This study was carried out to identify whether the microalgae culture able to yield biomass and accumulate carotenoid simultaneously and at the same time to fasten the production of carotenoid.

The results obtained for *Acutodesmus* sp. in Figure 1(a) and Table 1 shows that the biomass in normal growth condition (2-phase) was higher than biomass under stress induction growth condition (1-phase) during the first and second stage. However, the amount of total carotenoid (1.29±0.05 mg/L) production in 1-phase culture was slightly higher compared to total carotenoid (1.17±0.18 mg/L) in 2-phase culture during the first stage. This is maybe due to continuous exposure to high light intensity for 1-phase culture during the first stage that lead to production of higher carotenoid. Intriguingly, the total carotenoid (2.75±0.13 mg/L) for 2-phase culture surpassed slightly than total carotenoid (2.58±0.19 mg/L) in 1-phase culture during second stage. Besides that, the cells grown under continuous stress induction (1-phase) also turned to yellow colour before second stage indicating early of carotenoid formation.

In the first stage, the biomass yield of 2-phase culture in *Chlorella* sp. was higher than the biomass yield of 1-phase culture under continuous exposing to high light as shown in Figure 1 (b). However, high light induction and nitrogen deprivation during the second stage caused slower growth of *Chlorella* sp. in 2-phase culture. Total carotenoid content in 1-phase culture for both stages was found to be in range 4.01±0.69 mg/L which is higher than total carotenoid in 2-phase culture within range 3.34±0.06 mg/L.

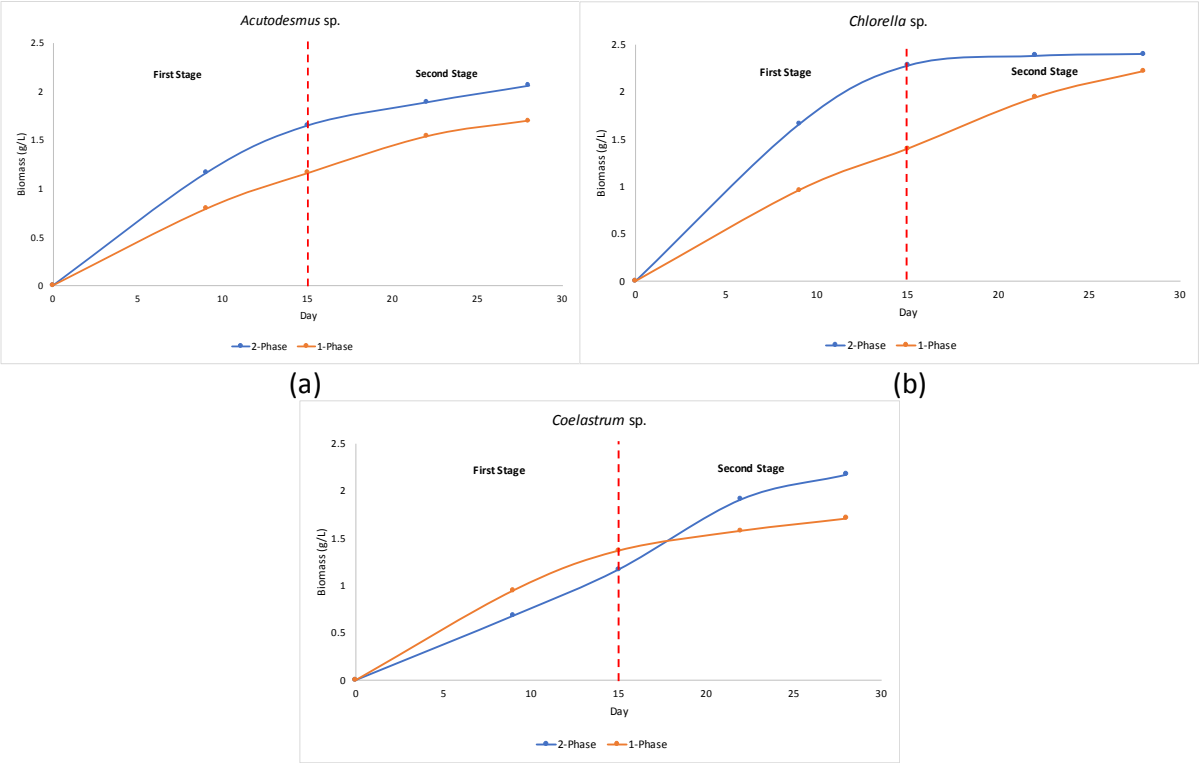

Fig.1. Comparison of biomass yield under normal growth condition (2-phase) and stress induction growth condition (1-phase) during first and second stage by (a) *Acutodesmus* sp., (b) *Chlorella* sp. and (c) *Coelastrum* sp.

Table 1
Comparison of total carotenoid production under normal growth condition (2-phase) and stress induction growth condition (1-phase) during first and second stage by (a) *Acutodesmus* sp., (b) *Chlorella* sp. and (c) *Coelastrum* sp.

Microalgae sp.	First Stage		Second Stage	
	Total carotenoid (mg/L)		Total carotenoid (mg/L)	
	2-Phase	1-phase	2-Phase	1-Phase
Acutodesmus sp.	1.17±0.18	1.29±0.05	2.75±0.13	2.58±0.19
Chlorella sp.	0.60±0.04	2.02±0.27	3.34±0.06	4.01±0.69
Coelastrum sp.	2.56±0.26	3.97±0.31	7.05±0.30	5.74±0.17

The pattern of biomass production in *Coelastrum* sp. was quite different from other microalgae strains. Unlike other species of microalgae, the biomass obtained in 1-phase culture was slightly higher than 2-phase culture under continuous high light intensity during the first stage of production. Total carotenoid was found to be 3.97±0.31 mg/L in 1-phase culture during first stage higher than total carotenoid (2.56±0.26 mg/L) in 2-phase culture. Moreover, the colour of the cell

grown under continuous stress induction in 1-phase culture also changes to red-orange colour during the first stage which depict the faster accumulation of carotenoid. Nevertheless, the biomass yield of 2-phase culture was greatly increased after exposing to stress induction in the second stage as depicted in Figure 1 (c). *Coelastrum* sp. accumulated highest carotenoid (7.05±0.30 mg/L) after the second stage of production in 2-phase culture. This might be due to the higher biomass formation in 2-phase culture that lead to the production of higher carotenoid accumulation after stress induction.

3.1 Comparison of Three Strains of Green Microalgae Studied

Spectrophotometrically analysis of total carotenoid accumulation under combine stresses condition showed varied amount of carotenoid accumulation. The 15 days old cultures of all four green microalgae species were exposed to conditions which promote production and accumulation of large amount of carotenoid which were continuous higher photon flux densities (PFD) with 250 µmol photon m^{-2} s^{-1} and treated with sodium acetate (100 mM) and nitrogen starvation during second stage. During this time, total carotenoid accumulation was higher compared to first stage for both normal (2-phase) and stress induction growth condition (1-phase).

Of the three green microalgae studied, *Chlorella* sp. exhibited the highest biomass yield compared to other microalgae species. Besides that, the total carotenoid production was highest in *Coelastrum* sp. after exposing the culture to combine stresses in second stage production. During the first stage, the accumulation of total carotenoid in *Coelastrum* sp. was higher under stress induction growth condition (1-phase) compared to normal growth condition (2-phase). However, as the cultures were transfer to second stage with combine stresses condition the amount of carotenoid was greatly increased and highest under normal growth condition (2-phase). This might be because the higher biomass yield in normal growth condition (2-phase) lead to accumulation of higher carotenoid.

Therefore, in terms of productivities and efficiency yield of biomass and accumulation carotenoid, showed that 2-phase culture perform better than 1-phase culture after induction of stress. The difference amount of carotenoid accumulation by different species of microalgae studied might be attributed to the distinct activities of the enzymes that responsible for carotenoid synthesis.

4. Conclusion

The present study has shown the potential of three different species of freshwater green microalgae in production of total carotenoid. Among the three freshwater green microalgae species studied, *Coelastrum* sp. showed the highest accumulation of carotenoid under combine stresses. Moreover, the biomass yield of *Coelastrum* sp. could rise up to 2.17 g/L biomass comparable to microalgae species with the fastest growth of *Chlorella* sp. which was 2.4 g/L. It was also discovered from this study that *Coelastrum* sp. accumulated significant amount of carotenoid under normal growth condition (2-phase) compared to stress induction growth condition (1-phase). Therefore, on relative efficiency, the 2-phase culture performs better than 1-phase culture.

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