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Comparing growth and carotenoids production of microalgae, *Acutodesmus* sp., in autotrophic and heterotrophic environments

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Abstract

Acutodesmus sp. KKU-P2 was investigated for its growth and carotenoid production in heterotrophic conditions to provide information on the possible use of alternative substrates for its cultivation. The microalgae could completely utilize glucose and sucrose. They showed superior biomass production in sugar media over sodium acetate medium. Growth in glucose, sucrose, and xylose media was in the range of 0.38 g/L to 0.49 g/L. Growth in sodium acetate was 0.08 g/L, similar to that in autotrophic cultivation. The microalgae accumulated carotenoids at 1.57 and 1.39 mg/g_{cell} in glucose and sucrose media, compared with 1.24 mg/g_{cell} of carotenoids in autotrophic cultivation. A much lower carotenoid accumulation was evident in the xylose and sodium acetate media. On the contrary, the chlorophyll content of the microalgae in the sodium acetate medium was high and at a similar level to that in autotrophic cultivation.

Keywords: *Acutodesmus*, *Scenedesmus*, Carotenoids, Autotrophic, Heterotrophic, Microalgae, Cultivation

1. Introduction

Microalgae have been of interest as an alternative platform to produce plant-based products. Like plants, they are photosynthetic organisms with advantages over plants in many aspects. Their cultivations are independent of seasons and do not require fertile land or labor-intensive harvesting [1,2]. Since microalgae biomass contains carbohydrates, proteins, and lipids, they attract many applications such as biofuels, food, and food supplements [3,4].

Acutodesmus spp. is green microalgae that closely relate to *Scenedesmus* spp. They are considered a subgenus of the genus *Scenedesmus* [5,6]. Although most studies involving *Acutodesmus* spp. have focused on their lipid production ability, few studies reported the accumulation of carotenoids. The type of carotenoids reported in *Acutodesmus* spp. depends on the strains of the microalgae. Neoxanthin and canthaxanthin were major carotenoids in different *Acutodesmus* strains [7,8]. Lutein, zeaxanthin, and carotene were also reported [8,9]. Chlorophylls and carotenoids accumulated in the microalgae cells are antioxidants, rendering their biomass a nutraceutical product [8].

Due to their photosynthetic nature, cultivations of microalgae are often carried out in the autotrophic environment. However, many microalgae could grow in other environments, such as mixotrophic and heterotrophic environments. While the growth of most microalgae benefits from carbon sources and light provided in mixotrophic conditions, growth in heterotrophic conditions could vary according to microalgal strains [10-12]. Regarding the accumulation of carotenoids, some microalgae were reported to produce carotenoids under heterotrophic conditions, such as *Chlorella* sp. [13] and *Auxenochlorella* sp. [14,15].

In this study, we investigated the uses of sucrose, xylose, and sodium acetate in heterotrophic cultivations of *Acutodesmus* sp. KKU-P2, as there were reports of the ability of members in microalgae to grow under this condition [10,16]. The strain is newly isolated and inherits the ability to produce carotenoids rich in lutein. Autotrophic growth condition was also carried out as a benchmark for growth. This investigation would give the primary information for the possible use of alternative substrates for culturing the microalgae and future cultivation process development.

2. Materials and methods

2.1 Microalgae and cultivation medium

Acutodesmus sp. KKU-P2 (Accession number MW555785) was used in all experiments. It was isolated from a fishpond and showed an ability to produce carotenoids rich in lutein. A base medium for all cultivations in this study was Bold Basal (BB) medium [17]. The microalgae stock was kept in BB media agar slant at 4°C. Modified BB media, with 3-fold more concentrated $NaNO_3$ as a sole nitrogen source, was used as a base medium in cultivations. All media components were of analytical grades.

2.2 Microalgae cultivation in autotrophic and heterotrophic environments

The inoculum of the microalgae was prepared in two steps. The cells on the BB agar medium were transferred to 20 mL of BB broth medium. The culture was propagated at room temperature with continuous lighting at 5000 lux (cool white Light Emitting Diode (LED) light, measured at the surface facing the light source) for two days. In the second step, 10% of the first seed culture was transferred to a fresh BB medium. The incubation was under 2% CO_2 (v/v) in the airstream at 0.02 vvm for another 4 days. The cell concentration should reach $1.0\text{-}1.5\times10^7$ cell/mL.

In all cultivations, 10% of the inoculum was transferred to 800 mL of medium in a 1-L laboratory bottle. The cultivation was carried out at room temperature and provided with gentle mixing through a magnetic stirrer in both autotrophic and heterotrophic cultivations.

In autotrophic cultivation, 10% (v/v) of CO_2 was provided to the culture through an aquarium sand head at the flow rate of 0.02 vvm. Continuous lighting was provided at 10000 lux. BB medium was employed.

Heterotrophic cultivations were carried out in a dark environment. BB media was used, with additions of glucose, sucrose, xylose, and sodium acetate to a final concentration of 2 g/L. An airflow rate of 0.02 vvm was provided throughout the cultivation.

2.3 Carotenoids extraction

The method for carotenoid extraction was modified from Mandelli et al. [18] and Molino et al. [19]. To extract carotenoids for total carotenoids analysis, 5 mL of the culture was harvested for the biomass. Carotenoids were repeatedly extracted from the cells with acetone and the help of glass beads until a clear solution was observed. The collective acetone fraction was extracted with an equal volume of diethyl ether. Equal volumes of water and the saturated salt solution were added following the diethyl ether. After phase separation, the lower layer was discarded, the upper layer was transferred to a volumetric flask, and its volume was adjusted to 5 mL. All chemicals used in carotenoid extraction were of analytical grade.

2.4 Analytical methods

Direct cell count and dried cell weight were employed for the determination of cell concentration. Total carotenoid and chlorophyll concentrations were determined by calculation using the absorbances of the extracts at 470, 644, and 662 nm [20]. Carotenoids and chlorophyll contents were obtained by calculation using their respective concentrations divided by cell concentration at the particular time point. Glucose, xylose, and sodium acetate concentrations were determined by High Performance Liquid Chromatography (HPLC) (LC-20A, Shimadzu, Japan) equipped with an Aminex HPX-87H (Bio-Rad, USA) column and a refractive index detector (RID-6A, Shimadzu, Japan). Total sugar was used to report sucrose concentration. The phenol-sulfuric method was employed for the analysis of total sugar. Experimental data are present as average values from a duplicate experiment with error bars representing standard deviations. Statistical comparisons (*t*-test) between initial and final values of each cultivation were carried out to assist in deciding if there were significant changes between initial and final values.

3. Results

In heterotrophic conditions, four carbon sources were investigated at the concentration of 2 g/L. They are glucose, xylose, sucrose, and sodium acetate. Autotrophic cultivation of microalgae involves the use of an airstream without enrichment of carbon dioxide. The results in (Figure 1). show that *Acutodesmus* KKU-P2 could utilize glucose, sucrose, and xylose to various degrees. It could completely consume glucose and sucrose and used approximately 34% of the supplied xylose. The microalgae utilized only a minute amount of sodium acetate during the 8 days of cultivation.

In this study, the growth of the microalgae was evaluated in terms of the number of cells using cell count and the cell dry weight. Compared to the autotrophic condition, cell counts were 22% higher in the glucose medium and 20% higher in the sucrose medium. Increases in the cell dry weight were 1.55-fold in the glucose medium and 1.95-fold in the sucrose medium. A different observation is evident in the xylose medium where the number of cells was similar to that in the autotrophic condition (1.45×10^6 cell/mL and 1.54×10^6 cell/mL), but an increase in the cell dry weight was observed (0.565 g/L versus 0.21 g/L). In sodium acetate medium, the microalgae barely utilized the substrate, but growth (1.55×10^6 cell/mL and 0.206 g/L) was evident and comparable to autotrophic cultivation.

In terms of biomass yield on carbon sources, the yields on glucose and sucrose media were similar at 0.19 $g/g_{glucose}$ and 0.22 $g/g_{sucrose}$. Much higher biomass yields were observed when the microalgae were cultivated in the media with xylose and sodium acetate as carbon sources, with the yields of 0.57 g/g_{xylose} and 0.58 g/g_{NaAc}.

Acutodesmus KKU-P2 showed an ability to produce carotenoids even in heterotrophic conditions where it was cultured in a dark environment (Figure 2). In (Figure 2 (A)), high carotenoid concentrations in glucose and sucrose media were the results of high carotenoid accumulation and biomass concentration. A similar correlation also explained lower carotenoid concentration in xylose, sodium acetate, and autotrophic cultivation, where either low carotenoid content or low biomass concentration was observed.

Heterotrophic cultivation in glucose and sucrose had shown high carotenoid accumulation of 1.57 and 1.39 mg/g_{cell} (Figure 2 (B)). The accumulation is comparable to that in autotrophic cultivation where the carotenoid content was 1.24 mg/g_{cell}. Cultivation in xylose and sodium acetate did not show any increase in carotenoid accumulation.

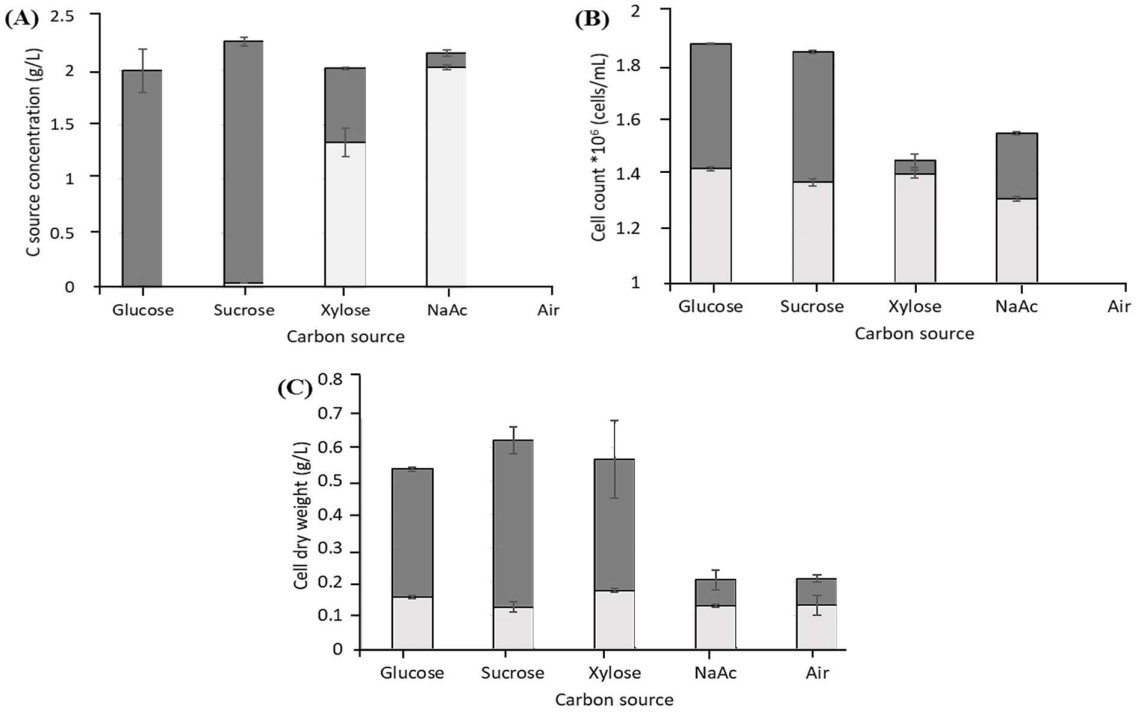

Figure 1 Changes in (A) carbon source concentrations, (B) the number of cells, and (C) cell dry weight of *Acutodesmus* sp. KKU P-2 culturing under heterotrophic and autotrophic (air) environments for 8 days. Light bars - residual concentration in (A) and initial values in (B) and (C); grey bars - concentration utilized in (A) and final values in (B) and (C); NaAc-sodium acetate.

Notwithstanding the dark environment in heterotrophic cultivation, the cultures showed increases in chlorophyll contents in all carbon sources. Marked increases in chlorophyll contents in the cell cultivated in sodium acetate and with no carbon source are evident with the final contents of 10.58 mg/g_{cell}. This chlorophyll content was as high as in the autotrophic condition where the content was 8.58 mg/g_{cell}. The microalgae cultivated in sugar media have a similar increase in chlorophyll contents during the cultivation. The final chlorophyll contents were 4.42 mg/g_{cell} in glucose, 2.21 mg/g_{cell} in glucose, and 3.46 mg/g_{cell} in xylose.

It is worth noting that the color of the cultures changed during heterotrophic cultivation. Initially, the cultures appeared light green in color due to the color of the inoculum. The color of the culture changed or faded as the cultivation progressed. Cultures in glucose and sucrose media appeared yellowish-green color, while the colors of the cultures in xylose and sodium acetate media faded to a much lighter color towards being white.

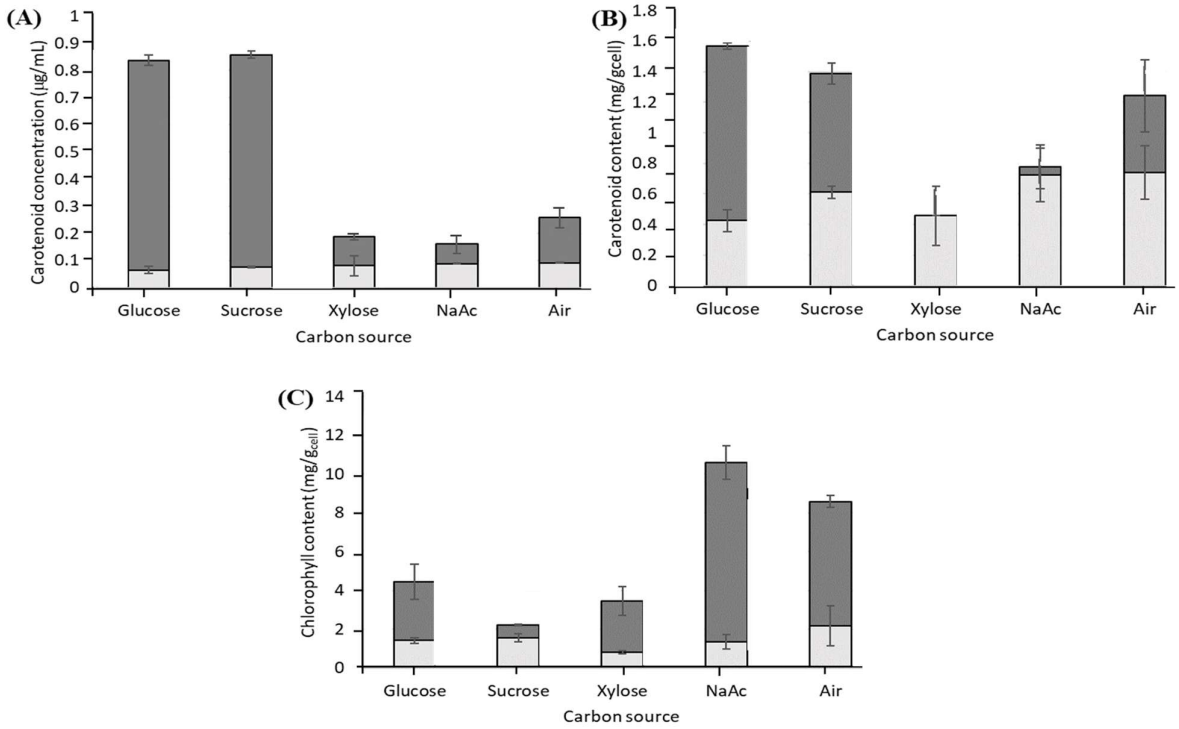

Figure 2 (A) Carotenoid concentrations, (B) carotenoid content, and (C) chlorophyll content of *Acutodesmus* sp. KKU P-2 culturing under heterotrophic and autotrophic (air) environments for 8 days. Light bars - initial values; grey bars - final values; NaAc - sodium acetate.

4. Discussions

Microalgae generally generate energy in the form of adenosine triphosphate (ATP) through photosynthesis. In heterotrophic cultivation where there is no light, microalgae use organic carbon sources as their energy source without photosynthesis [21]. Glucose has been reported as the most suitable carbon source for heterotrophic cultivation. In the study of *Chlorella vulgaris*, glucose resulted in the highest biomass compared to the growth on sucrose and xylose media, where the cell weights were 50% and 70% lower than that obtained from glucose [22]. In addition, a study on *Chlorella* sp. HS2 also demonstrated that growth in glucose was superior to that in sucrose by at least 2-fold [23]. These results are different from ours, where all sugars (glucose, sucrose, and xylose) showed a similar cell dry weight. There are several *Scenedesmus* spp. (the paraphyletic subgenus) that grew under heterotrophic conditions using glucose and sucrose media [24-26].

Acutodesmus sp. KKU-P2 could fully utilize glucose and sucrose, although with small growth. There is a possibility to increase the growth of the microalgae by increasing the initial sugar concentrations. However, this microalgal strain showed an indication of limitation in xylose utilization in the heterotrophic condition as xylose utilization stopped at the final reported concentration after one day of the cultivation (data not shown). A study on *Scenedesmus quadricauda* in 4 g/L xylose showed that low biomass at ~0.4 g/L was obtained, with only a minute amount of xylose uptake [27]. Similar observations of small growth on xylose were also reported in *Chlorella* sp. [22,28].

Although *Acutodesmus* sp. KKU-P2 used sugar during the cultivation, it did not show obvious utilization of sodium acetate. Since there was no reference to the growth of *Acutodesmus* sp. on acetate media, we compared

the result with those reported on its paraphyletic subgenus *Scenedesmus* spp. The results on the growth of this microalgae strain on sodium acetate contradict some reports on the other *Scenedesmus* spp. A small growth of approximately 0.15 g/L on 5 g/L sodium acetate was reported on *Scenedesmus bujugus* [29]. In addition, small but higher biomass of ~0.5 g/L was obtained from *Scenedesmus obliquus* where ~3 g/L of sodium acetate was consumed [30]. Although sodium acetate seems to be a choice for heterotrophic cultivation, its utilization and growth support appear to be quite limited also in many other microalgae strains. Approximately 0.28 g/L of *Micractinium inermum* biomass was reported with 1.2 g/L of sodium acetate [31]. *Haematococcus pluvilis* grew better in a medium containing 1.6 g/L sodium acetate, in which the biomass reached 1.0-1.1 g/L [32]. *Chlorella* sp. HS2 had a small growth (~0.3 g/L) over the range of sodium acetate of 1-20 g/L with slight decreases in cell growth as the sodium acetate concentration increased [23]. Similarly, *Chlorella vulgaris* 31 was able to produce 0.424 g/L and 0.621 g/L cells in the media with 2 g/L and 10 g/L sodium acetate [22]. Substrate inhibition on growth was reported in *Chlamydomonas reinhardtii* at a sodium acetate concentration of higher than 0.4 g/L [33]. The strong and progressive inhibition of sodium acetate on growth could explain the low and insignificant changes exhibited in *Acutodesmus* ap. KKU-P2 and in other microalgae. The capability of sodium acetate uptake and growth support also widely varied among the same genera of microalgae.

It is worth noting that most of the reported growth on heterotrophic cultivation resulted in a low cell dry weight, mostly around 1 g/L or less, which is most likely due to low sugar concentration used or poor conversion to biomass [24,25]. However, several researchers have reported high cell weights ranging from 4.3 g/L to 19.6 g/L on glucose media [13,15,26]. Cell weights also showed an increasing trend with higher glucose concentration [22,23,26] but not for sucrose and xylose media [22].

Although there was no light during the heterotrophic cultivation of *Acutodesmus* sp. KKU-P2, the microalgae still produced the photosynthetic pigments (carotenoids and chlorophylls. Many studies have reported the same observations [25-27,34]. Higher chlorophyll content in the sodium acetate culture of the microalgae as compared to those in sugars was also observed in *Chlorella vulgaris* [22], *Chlamydomonas globosa* [24], and *Micractinium inermum* [31]. A study on *Scenedesmus* sp. has suggested that it maintains an intact and functional photosynthetic apparatus even in growth in the absence of light, possibly through a light-independent photosynthetic carbon reduction (PCR) cycle to synthesize chlorophylls [25]. Therefore, the microalgae could resume their photosynthesis ability once transferred to light.

5. Conclusion

Acutodesmus sp. KKU-P2 has shown an ability to grow and accumulate pigments under heterotrophic conditions. Glucose and sucrose could well support the microalgal growth with an accumulation of carotenoids. Chlorophyll accumulation was pronounced when cultivated in a sodium acetate medium. Based on the results from this study, heterotrophic conditions on sugar substrates could be an option for the cultivation of the microalgae while maintaining pigment accumulation ability.

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