

Effects of light on cell growth, chlorophyll, and carotenoid contents of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry dropping medium

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ABSTRACT

Microalgae biomass and their products are invaluable bio-resources with numerous applications in food, feed, pharmacy, cosmetics, and environments. The effects of light intensities on biomass, chlorophyll-a, and total carotenoid production by *Chlorella sorokiniana* and *Ankistrodesmus falcatus* were studied in Bold's Basal Medium (BBM) and Poultry Medium (PM) as the growth media. The growth of *C. sorokiniana* and *A. falcatus* increased with increase in light intensity in PM and was inhibited at 1786 lux in *C. sorokiniana* in BBM. PM supported higher biomass production by *C. sorokiniana* than BBM while it was the reverse for *A. falcatus*. *A. falcatus* produced higher ($P \leq 0.05$) concentrations of chlorophyll-a than *C. sorokiniana* in both media. The highest carotenoid concentration of 11.84 mg/g-biomass was accumulated by *C. sorokiniana* in PM as against 7.027 mg/g-biomass obtained in BBM. On the other hand, the highest carotenoid concentration of 7.633 mg/g-biomass was accumulated by *A. falcatus* in BBM as against 4.299 mg/g-biomass obtained in PM. It is interesting to note in the present study that a cheap medium such as PM supported higher biomass and carotenoid production by *C. sorokiniana* than BBM.

1. INTRODUCTION

Food insecurity and need for functional foods have been increasing due to rapid population growth. It is especially true for the developing countries where majority of the populace live under poverty level and are thus malnourished. There is therefore an urgent need to explore alternative sources of cheap and nutritionally rich foods.

Microalgae are very good sources of proteins [1-3], carbohydrates [4], carotenoids [5-7], chlorophylls [8-10], and a variety of vitamins and pro-vitamins depending on the species and culture conditions [3,11-13]. Many species also accumulate high concentrations of various forms of lipids (both saturated and unsaturated) that can be converted to biodiesel [14] or used as functional food supplements to combat hunger and diseases [15]. Many species of microalgae therefore have great potentials as foods and food supplements.

Another important characteristic of microalgae is that they are ubiquitous with high photosynthetic efficiency and high growth rates [16-18]. Thus, their productivities can be orders of magnitude higher than those of the higher plants. They can be cultivated all the

year round and even in places that are not suitable for conventional agriculture. In comparison with other microorganisms, they can be cultivated on cheap and easily available media such as agricultural and food processing wastewater. This also means that wastewater treatment can be coupled with production of microalgae biomass and metabolites for various applications [19,20]. Metabolite production from microalgae is environmentally friendly since they use up carbon dioxide for photosynthesis and generate oxygen making their growth activities close to carbon neutral [16,21,22].

Some important species of microalgae such as *Spirulina*, *Chlorella*, *Nannochloropsis*, *Phaeodactylum*, and *Dunaliella* are already cultivated for various purposes [2,3]. However, there are still a lot of species that are yet to be evaluated. This is especially true for many African countries where research activities on microalgae are still very low.

Among the important components of microalgae are chlorophylls and carotenoids. Chlorophylls have many useful applications in food industries as colorants and nutraceuticals. They are also applied as ingredients in cosmetics and pharmaceutical preparations as anti-inflammatory, anti-mutagenic [23,24], and antimicrobial agents [25,26]. Carotenoids have several health benefits in man and animals and are used as colorants in aquaculture feed, as coloring agent in human food, and as food supplements due to their antioxidative properties. They are used in pharmaceutical

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preparations and costmetics as anti-inflammatory, anti-carcinogenic, and antimicrobial agents [6,18,27].

The demand for natural pigments such as chlorophylls and carotenoids has been increasing with increase in human population and the increased awareness and perceptions of the negative health implications of consuming synthetic pigments [28,29]. The world market price for carotenoid was estimated to be about 1.24 billion US dollars in 2016 and it was estimated to increase up to 1.53 billion dollars by the year 2021 [6,30]. According to the Market Research Report 2019, (Report Code 4411) the carotenoid market price is expected to go up to 2.0 billion USD by the year 2026 [31]. Algal products such as the biomass, chlorophylls, and carotenoids are indispensable components of human and animal diet [32]. Despite, the functionality of microalgae pigments such as chlorophylls and carotenoids (beta-carotene, phycocyanin, astaxanthin, and lutein) as food supplements and colorants, they are still very expensive and not affordable by many poor rural dwellers. Several species of microalgae grow freely at various locations and have the potentials to be employed in the production of useful biomass, chlorophylls, and carotenoids to provide solutions for food, health, and environmental problems of the community/rural dwellers. To make microalgae biomass and pigments cheap and affordable, there is a need to isolate local species and establish their growth on locally available nutrients. It has been estimated that the nutrients required for cultivation, maintenance, and production of metabolites from microalgae contribute up to 50% of the total production costs [33,34]. Thus, the use of agro-industrial wastes in microalgal nutrient formulation will go a long way in reducing the production cost and mitigate environmental pollution. As a means of increasing protein supply, poultry farms have been increasing steadily in both numbers and sizes, leading to generation of huge amounts of poultry manure, which when disposed untreated causes a lot of environmental pollution. The composition of poultry manure depends on the type of feed but generally it contains a lot of nitrogen (mainly in form of ammonia), potassium, and phosphorus [34] all of which are essential for the growth of microalgae. Light is also among the most important factor affecting photoautotrophic growth of microalgae. Although Nigeria and most tropic countries have abundant light throughout the year, it is important to optimize light intensity since high light intensities inhibit cell growth and metabolite formation [35].

The aim of this research work was to evaluate the influence of light intensity and poultry waste based growth medium on the production of cell biomass, total chlorophyll, and carotenoid contents by two local microalgae isolates (*Chlorella sorokiniana* and *Ankistrodesmus falcatus*).

2. MATERIALS AND METHODS

2.2. Microalgae Species

Two species of microalgae (*C. sorokiniana* and *A. falcatus*) were supplied by Prof. N.O. Nweze of the Department of Plant Science and Biotechnology University of Nigeria, Nsukka. The microalgae species were activated by cultivating in sterilized Bold's Basal Medium (BBM) and Poultry Medium (PM) for 14 days at room temperature ($27 \pm 2^\circ\text{C}$) using sun light as the light source. They were transferred and maintained in BBM and poultry agar slant cultures. The slants were stored in a refrigerator at 8°C and sub-cultured every 2 weeks into fresh liquid BBM or PM for use in the experiments.

2.2. Preparation of BBM

The BBM was prepared according to the methods of Martos [36]. The appropriate volume of the major element stock solution was dispensed into a 2000 ml round bottom flask. One milliliter each of the trace element stock solution and the diluted H_2SO_4 were added. The pH of the medium was adjusted to 6.8 with 0.1 M NaOH and the volume was made up to 1000 mL with distilled water. The medium was dispensed in 200 mL aliquots into 900 mL flat bottom jars. The jars were plugged with cotton wool, wrapped with aluminum foil and autoclaved at 121°C , 1.0 atm for 20 min.

2.3. PM

Poultry (chicken) droppings were obtained from a poultry farm at the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The chicken droppings were air dried for 24 h and 75 g was dissolved in 1 l of tap water. This was made up to 30 l and filtered with a cheese cloth to remove debris. The filtrate was left to stand for 2 days and then autoclaved at 121°C and 1.0 atm for 20 min [37].

2.4. Light Illumination

Three rechargeable 592.6 cm florescent tubes were installed inside a wooden frame and used as a source of light. The intensity of light on the culture surface was varied by varying the distance of the cultures from the florescent tubes. The light intensity was measured using a lux meter (Custom Lux Meter, Model lx-1000, China). The culture flasks were placed at distances of 3.75 cm, 7.75 cm, and 10.75 cm from the light source. These positions corresponded to 1786 lux, 1307 lux, and 702 lux, respectively.

2.5. Pre-culture of the Microalgae Species

Twenty milliliters (20 mL) of the stock microalgae culture (section 2.1) were used to inoculate 200 mL of BBM or PM in 900 ml glass jars to activate the microalga cells before the main experiments. The cultures were incubated at room temperature ($27 \pm 2^\circ\text{C}$) and cultivated for 14 days with intermittent manual shaking twice daily to re-suspend the cell sediments and facilitate oxygen and carbon dioxide transfer.

2.6. Effects of Light Intensities on the Growth, Chlorophyll-a, and Total Carotenoid Production by *C. sorokiniana* and *A. falcatus* in Bold's Basal and Poultry Media

Thirty six glass jars each with a total volume of 900 mL (section 2.5) were used for the experiment. BBM or PM (250 mL) was dispensed into each jar, plugged with cotton wool and a sampling tube was inserted into each jar. They were autoclaved at 121°C (1.0 atm) for 15 min. After cooling, 18 of them were inoculated with 50 ml of *C. sorokiniana* culture with cell concentration of 2×10^7 cells/ml while the other 18 jars were inoculated with *A. falcatus* culture with the same cell concentration. For each species of microalgae, three jars were incubated at 702 lux, three at 1307 lux while the other three were incubated at 1786 lux. The cultivation was done inside the wooden box at room temperature ($27 \pm 2^\circ\text{C}$) for 15 days. Sample (0.5 mL each) was withdrawn every 3 days to measure biomass, chlorophyll, and carotenoid concentrations.

2.7. Analytical Methods

2.7.1. Measurement of cell growth

Cell growth was measured by counting the cells and measuring the optical densities. Hemocytometer was used to count the cell number

while UV visible spectrophotometer (Shimadzu Model UV-1200, Japan) was used to measure the optical density (OD) at a wavelength of 750 nm.

2.7.2. Measurement of chlorophyll and carotenoid concentrations

2.7.2.1. Extraction

Chlorophyll and carotenoids were extracted from wet biomass following a modified method of Becker [38]. Five milliliters (5 ml) of culture broth were centrifuged at 3000 rpm for 15 min and the supernatant was discarded. The pellet was washed twice by re-suspending in 5 ml of distilled water and centrifuging at 3000 rpm for 10 min. The supernatant was discarded and 3 ml of 90% methanol was added and vortexed for 5 min to mix. The mixture was incubated in a water bath at 60°C for 60 min. The mixture was then made up to 5 ml with 90% methanol and centrifuged at 3000 rpm for another 15 min. The supernatant was collected in a clean test tube and used for absorbance reading.

2.7.2.2. Measurement of the absorbance

The extract was diluted with distilled water and the absorbance measured at 470, 650, 655, and 750 nm using a UV visible spectrophotometer (Shimadzu Model UV-1200, Japan).

2.7.2.3. Calculation of the chlorophyll and carotenoid contents

Chlorophyll-a and total carotenoid contents were calculated from the following equations:

$$\text{Chlorophyll a; Chl-a } (\mu\text{g/ml}) = 16.72 A_{660} - 9.16 A_{650}$$

$$\text{Chlorophyll b; Chl-b } (\mu\text{g/ml}) = 34.09 A_{650} - 15.28 A_{660}$$

$$\text{Carotenoid; carotenoids } (\mu\text{g/ml}) = \frac{(1000A_{470} - 1.63 \text{ chl a} - 104.9 \text{ chl b})}{221}$$

Where A_{660} , A_{650} , and A_{470} represent absorbance at 660 nm, 650 nm, and 470 nm, respectively, Branisa et al. [39].

2.8. Statistical Analysis

The experiments were performed in triplicates and the data generated were analyzed by one way analysis of variance. Where there was a significant difference, the means were separated using least significant difference.

3. RESULTS

3.1. Growth in BBM

As shown in Figure 1, the growth of *C. sorokiniana* and *A. falcatus* in BBM increased with increase in the light intensity. This result agrees with the work of Metsoviti et al. [40], who reported an increase in the growth rate of *Chlorella vulgaris* with increase in light intensity when cultivated both indoors and outdoors. However, *A. falcatus* exhibited higher growth in BBM than *C. sorokiniana* with an OD of 1.2 after 15 days at 1786 lux as against OD of 0.85 obtained for *C. sorokiniana* at the same light intensity and cultivation period. At a lower light intensities of 1307 and 702 lux, *C. sorokiniana* exhibited higher growth rate than *Ankistrodesmus* with optical densities of 0.8 and 0.65 against 0.7 and 0.4 for *Ankistrodesmus*, respectively. This result implied that at higher light intensity, the growth of *C. sorokiniana* was inhibited. This agrees with the findings of Nzayisenga et al. [41], who reported that a light intensity of about $150 \mu\text{Em}^{-2}\text{s}^{-1}$ was the optimal for biomass production by *C. vulgaris* and *Scenedesmus obliquus* and

that increasing the intensity to $300 \mu\text{Em}^{-2}\text{s}^{-1}$ was inhibitory to their growth. This result shows that the intensity of light required by the two microalgae differs and that *Ankistrodesmus* has higher light saturation intensity than *Chlorella*.

3.2. Growth in PM

The growth of the two species of microalgae in PM took a similar pattern with the growth in BBM. Their growth increased with increase in the light intensity. However, PM favored the growth of *Chlorella* more than *Ankistrodesmus*. The optical densities were 1.45, 1.33, and 1.12 after 15 days of culturing *C. sorokiniana* at 1786, 1307, and 702 lux, respectively. The highest OD obtained in *A. falcatus* was 1.0 as shown in Figure 2. This might be due to the differences in the composition of the two media. PM is known to contain higher nitrogen and other elements than BBM [36,37]. This result agrees with the work of Kumar et al. [42], who reported that nutrient from poultry excrete gave the highest cell growth of their isolate of *Chlorella sorokiniana* among the four animal wastes they tested. In case of *Ankistrodesmus*, there are no reports in literature on the effect of media or light intensity on the growth. A comparison of the growth of the two species in PM and BBM is shown in Table 1. At a light intensity of 1307 lux, the growth of both *Ankistrodesmus* and *Chlorella* in PM was significantly higher ($P < 0.01$) than in BBM, showing that at that light intensity,

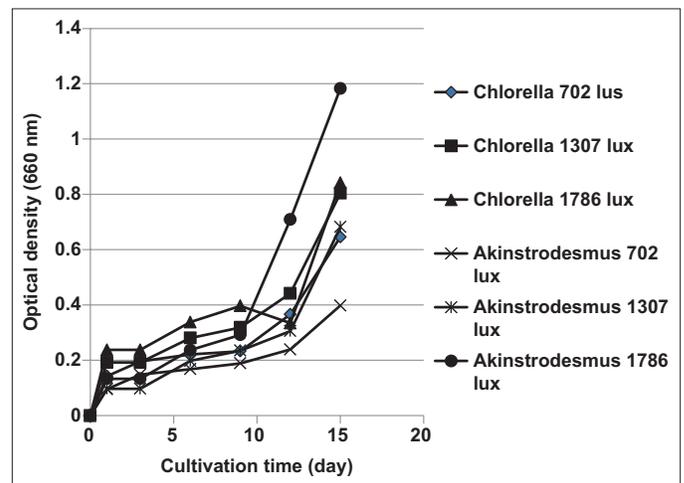


Figure 1: Effect of light intensity on the growth of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in Bold's Basal medium.

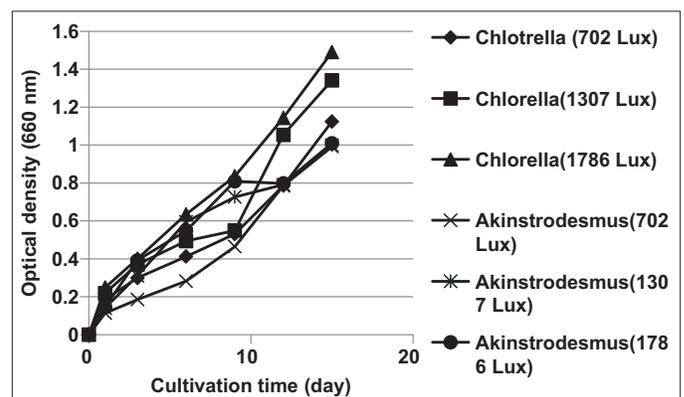


Figure 2: Effect of light intensity on the growth of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry manure.

nutrients was limiting in BBM. However, at higher light intensity of 1786 lux, there was no significant increase in the growth of both *Ankistrodesmus* and *Chlorella* in PM, but there was significant increase in BBM implying that light was already saturating in PM but still limiting in BBM. On the whole, the effects of light intensity on the growth of the two species in PM were not significant, probably due to the turbidity of the medium which affects light absorption.

3.3. Chlorophyll-a Production in BBM

The results of the effects of light intensities on chlorophyll-a production by *C. sorokiniana* and *A. falcatus* in BBM are shown in Figure 3. For the two microalgae cultivated in BBM, biomass harvested after 15 days of growth had higher chlorophyll-a content than the one harvested on the 12th-day irrespective of the light intensity. In the case of *C. sorokiniana*, the highest light intensity (1786 Lux) gave the highest chlorophyll-a content. However, chlorophyll-a contents decreased with increase in light intensity in the case of *A. falcatus*. Hence, the highest chlorophyll-a content in *A. falcatus* was obtained on day 15 at 702 lux which was the lowest light intensity employed in this study. Although light is important for chlorophyll synthesis, very high light intensities inhibit chloroplast development [43,44]. On the whole, the chlorophyll-a contents of *A. falcatus* were significantly higher ($P < 0.05$) than those of *C. sorokiniana* under all the light intensities investigated.

3.4. Chlorophyll-a Production in PM

The results of chlorophyll-a contents of *C. sorokiniana* and *A. falcatus* in PM are shown in Figure 4. When the two microalgae were cultivated in PM, light intensity did not have any significant effects on the chlorophyll-a contents of *C. sorokiniana* but harvesting the

Table 1: Comparison of the growth of *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in BBM and PM under different light intensities.

	Optical density (660 nm)			
	<i>Chlorella sorokiniana</i>		<i>Ankistrodesmus falcatus</i>	
	BBM	PM	BBM	PM
702	0.658±006	1.123±0024	0.404±001	0.991±002
1307	0.804±008	1.333±001	0.682±001	1.0±001
1786	1.2±0112	1.48±001	1.002±0112	1.002±001

BBM: Bold's Basal medium, PM: Poultry medium

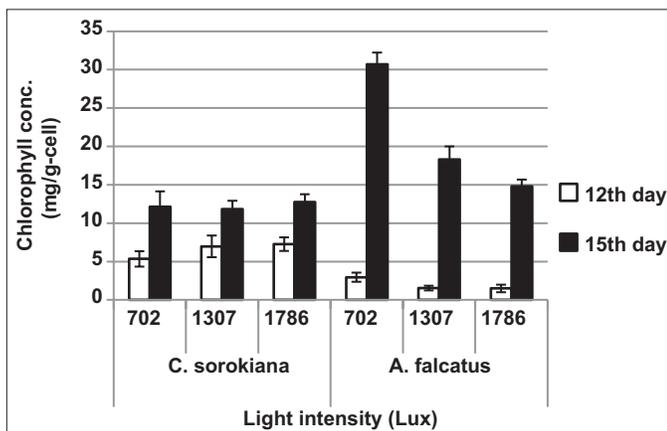


Figure 3: Effect of light intensity on chlorophyll-a production by *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in Bold's Basal medium.

biomass after 12 days of growth gave higher chlorophyll-a contents than harvesting after 15 days. In the case of *A. falcatus*, the highest chlorophyll-a content was obtained at the lowest light intensity (702 lux) from biomass harvested on day 15. As in the case of BB medium, increase in light intensity led to a reduction in the chlorophyll-a contents in *A. falcatus*. In other words, high light intensity inhibited chlorophyll-a synthesis in *A. falcatus* irrespective of the medium. As in the case of BBM, chlorophyll-a contents of *A. falcatus* were relatively higher than those of *C. sorokiniana* but chlorophyll-a contents of *C. sorokiniana* cultivated in PM were higher than those cultivated in BBM.

3.5. Carotenoid Production in BBM

The results of carotenoid production by *C. sorokiniana* and *A. falcatus* in BBM are shown in Figure 5. The carotenoid contents of the two microalgae harvested after 9 days of cultivation were very low. Day 12 was the optimum period for harvesting *Chlorella* biomass for carotenoid extraction in this study and the optimum light intensity was 1307 lux. This result is similar to the work of Jalal *et al.* [45], who reported that the carotenoid contents of the tropical marine microalgae *Isochrysis* sp. of biomass harvested from a 10-day-old culture grown at 1200 lux was the optimum. On the other hand, the highest carotenoid

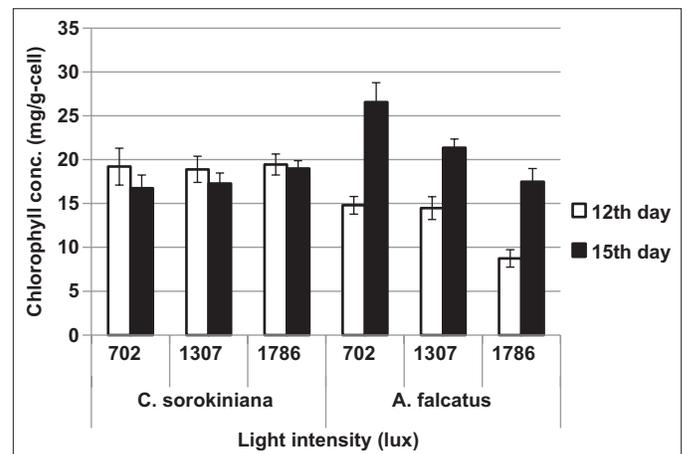


Figure 4: Effect of light intensity on chlorophyll-a production by *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry medium.

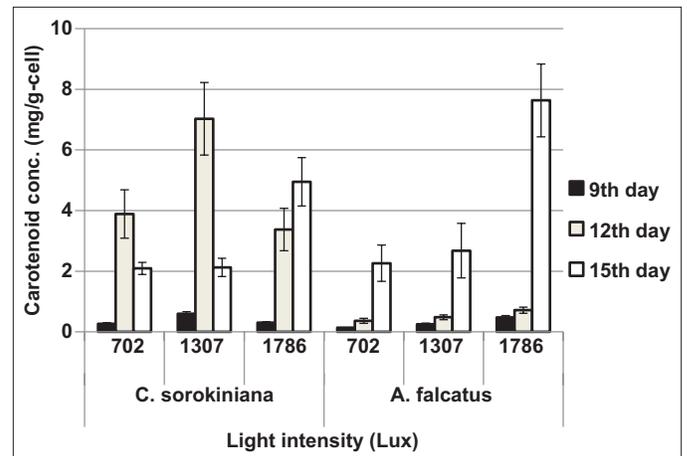


Figure 5: Effect of light intensity on carotenoid production by *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in Bold's Basal medium.

content was obtained from *A. falcatus* biomass cultivated at 1786 lux and harvested on the 15th day. This shows that *C. sorokiniana* requires less cultivation time and a lower light intensity for carotenoid production than *A. falcatus* when grown in BBM. This result is also in line with that of Raman and Mohamad [46], who reported that cultivation of *C. sorokiniana* indoors favored astaxanthin production than outdoors. Cordero *et al.* [5] also reported that carotenoid production by *C. sorokiniana* was favored by a light intensity below 690 micromole/m/s. It is important to note that except for the highest light intensity (1786 lux), carotenoid contents of *C. sorokiniana* biomass were higher than those of *Ankistrodesmus* while chlorophyll-a contents of *A. falcatus* were higher than those of *C. sorokiniana*.

3.6. Carotenoid Production in PM

The results of carotenoid production by *C. sorokiniana* and *A. falcatus* in PM at different light intensities are shown in Figure 6. Carotenoid production by *C. sorokiniana* increased with increase in light intensity for the cells harvested on the 12th day. However, the highest carotenoid content was obtained from biomass cultivated at 1786 lux and harvested after 15 days. This was the opposite of what was obtained in BBM culture and might be due to the turbidity of PM that shielded the alga from high light intensity. For the *A. falcatus*, the highest carotenoid was obtained after 12 days of cultivation at a light intensity of 1307 lux. This result is different from the results obtained in BBM. These results suggest that the length of time required for cultivating microalgae for carotenoid production varies from one microalga strain to another and it is also affected by the type of medium used. In PM, the carotenoid contents of *C. sorokiniana* were more than 4 times higher than those of *A. falcatus* under all the light intensities tested. Furthermore, PM favored carotenoid accumulation in *C. sorokiniana* more than BBM.

Although several growth media are employed in cultivation of microalgae, the choice of the medium to be used depends on the cost, target product(s), accessibility, and the choice of individual laboratory based on experience. BBM is widely used to cultivate fresh water microalgae because it contains the nutrients required for microalgae growth in the appropriate proportions [47]. In addition to the defined synthetic media, different agro-industrial wastes are also employed in microalgae cultivation to reduce cost. Such agricultural wastes include poultry droppings [48,49], cow dungs, palm oil, and rubber industrial effluents [50]. It is interesting to note that in this study, PM supported better cell growth as well as chlorophyll-a and carotenoid accumulation than BBM. This is very significant considering that BBM is expensive

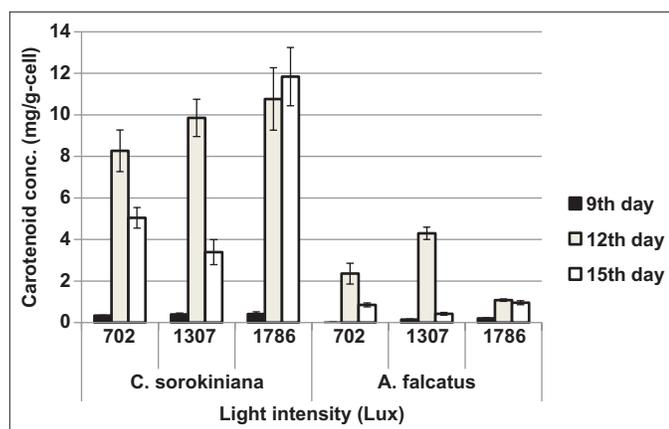


Figure 6: Effect of light intensity on carotenoid production by *Chlorella sorokiniana* and *Ankistrodesmus falcatus* in poultry medium.

and not easily available in many developing countries. The use of PM will drastically reduce the cost of cultivating microalgae in developing countries and thus facilitate establishment of cottage microalgae cultivating industries in rural communities.

4. AUTHORS' CONTRIBUTIONS

Professor Nkechinyere O. Nweze conceptualized the research idea, provided the microalgae strains used in the study, and supervised the experiments; Jane Chizie Ogbonna carried out the literature review and performed the experiments; Dr. Christiana N. Ogbonna co-supervised the work and drafted the manuscript. All the authors read and agreed on the final version of the manuscript.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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