INVESTIGATION OF THE GROWTH CAPABILITY AND PHYCOCYANIN ACCUMULATION OF *ARTHROSPIRA PLATENSIS* UNDER VARYING NUTRITIONAL ENVIRONMENTS

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SUMMARY

Arthrospira platensis is a valuable source of diverse minerals and essential amino acids, enriched with antioxidants such as chlorophyll and phycocyanin, known to enhance health and boost the immune system. This study aimed to assess the growth capability and phycocyanin accumulation of *A. platensis* under varying environmental conditions, including different concentrations of NaCl, IAA supplementation, and their combined effects in Zarrouk medium. The results indicated that after 10 days of cultivation at 28-30°C, with a light intensity of 3500-4000 lux and pH 9, the highest fresh biomass of *A. platensis* was achieved in the medium supplemented with 1 mg/L IAA, reaching 8.45 ± 0.286 g. The highest phycocyanin accumulation occurred in the medium supplemented with 100 mM NaCl, reaching 9.81 ± 0.36 mg/g. Simultaneously, parallel investigations explored phycocyanin extraction using a 1.5% $CaCl_2$ solvent, yielding the highest concentration of 19.71 ± 2.50 mg/g with ultrasonic assistance. These findings underscore that supplementing Zarrouk medium with 100 mM NaCl and 1 mg/L IAA enhances both fresh biomass and phycocyanin content in *A. platensis*. This approach holds promise for applications in research focused on maximizing biomass production and phycocyanin yield in *A. platensis*.

Keywords: Arthrospira platensis, Fresh biomass, Phycocyanin, Pigments, Zarrouk medium.

INTRODUCTION

Arthrospira platensis, commercially known as *Spirulina platensis*, is a genus of *Cyanobacteria* characterized by photosynthetic, filamentous, helical-shaped, multicellular structures. Typically measuring between 50-500 µm in length and 3-12 µm in diameter, it thrives in alkaline environments with high temperatures and strong sunlight (Akbarnezhad *et al*., 2020*;* Wan *et al.,* 2016*).* It has high protein content (up to 70%) and significant amounts of essential fatty acids, amino acids, minerals, vitamins, and antioxidant pigments such as Chlorophyll-a (Chl-a), Carotenoids (Cart), Phycocyanin (PC) and polysaccharides. These components have potential health-promoting benefits and applications in non-communicable diseases such as diabetes mellitus, hyperlipidemia, oxidative stress-induced diseases, inflammations, allergies, hypertension, and some types of cancer (Lafarga *et al.,* 2020). The growth and accumulation of biological pigments in *A. platensis* are influenced by various factors in the culture medium, including light, high pH, salinity, temperature and nutrient composition such as Indole-3-acetic acid (IAA) and sodium chloride (NaCl). These factors impact the growth stages of *A. platensis*, altering the composition and ratio of pigments (Jasuja, 2014). This study aims to explore the effects of nutritional factors in the culture medium, specifically IAA, NaCl, and their combinations, on the growth, biomass accumulation, and pigment production (Chl-a, Cart, PC) of *A. platensis.* The research provides insights into enhancing the production of nutrient-rich foods derived from microalgae.

MATERIALS AND METHODS

Materials: *A. platensis* was obtained from the Research Institute for Aquaculture No.2 (Ho Chi Minh City, Vietnam) and stored at 4°C. Before each experiment, the microalgae were inoculated for 10-14 days in 500 mL of Zarrouk medium (Zarrouk, 1966) to achieve an optical density (OD) of approximately 1.0 at 680 nm. All containers were continuously aerated and maintained at 30 ± 2°C under continuous white fluorescent illumination at an intensity of 3500-4000 lux.

Methods

Investigation of suitable solvents for the extraction of C-Phycocyanin from A. platensis

The ultrasound-assisted method represented an advanced scientific method in research and industrial applications. This technique employed ultrasound equipment comprising a transducer and a reaction vessel to

generate high-frequency waves, effectively disrupting algae cells and liberating phycocyanin into the processing medium. This approach was characterized by its rapidity, efficiency, and preservation of the biological activity of phycocyanin, thereby ensuring optimal yield and quality of the final product. (Zhang *et al*., 2017).

The microalgae suspension was centrifuged at 4000 rpm at room temperature and washed twice with distilled water. Then, 1 g of fresh sample was extracted using 1.5% $CaCl_2$ solution, 0.1 M phosphate buffer (pH 7), and distilled water with an algae ratio of 1:100 (g/mL). The tubes were then frozen at -20°C for 24 hours. After thawing, the sample was sonicated at 120 W/40 kHz for 10 minutes at 20°C using an ultrasound device (WUC-32, Jiayuanda, China), followed by centrifugation at 4000 rpm for 10 minutes, performed in triplicate. The supernatant was collected and OD values of the microalgae suspension were measured on days 3, 5, and 10.

Investigating the effects of NaCl and IAA on the fresh biomass of A. platensis

The chemical compositions of Zarrouk medium includes (g/L): $NaHCO_3$ 16.8; NaCl 1.0; $NaNO_3$ 2.5; K_2HPO_4 0.5; K_2SO_4 1.0; EDTA 0.08; $MgSO_4.7H_2O$ 0.2; $FeSO_4.7H_2O$ 0.01; $CaCl_2.2H_2O$ 0.04 and micronutrient A5 1 mL (g/mL: H_3BO_3 286; $MnSO_4.7H_2O$ 250; $ZnSO_4.7H_2O$ 22.2; $CuSO_4.5H_2O$ 7.9; $NaMoO_4.2H_2O$ 2.1.

The experiment was divided into four groups: (1) Zarrouk medium; (2) Zarrouk medium supplemented with 100 mM NaCl equivalent to 4.87 g/L NaCl; (3) Zarrouk medium supplemented with 1 mg/L IAA prepared by dissolving 10 mg of IAA in 1M KOH; (4) Zarrouk medium supplemented with 100 mM NaCl and 1 mg/L IAA. Each treatment was conducted in triplicates. All containers were continuously aerated and maintained at 30 ± 2°C under continuous fluorescent illumination at an intensity of 3500-4000 lux. Biomass samples were collected on days 5 and 10 for analysis.

Fresh algae biomass was collected by filtering through 50-µm nylon membranes on days 5 and 10 of cultivation. Each experimental group underwent triple filtration to maximize biomass recovery. Subsequently, the fresh algal biomass from each treatment was weighed and the fresh biomass was recorded.

Analysis of phycocyanin, chlorophyll a, and carotenoids content

The concentration of phycocyanin was determined using molecular absorption spectroscopy equipment (Model 752N, Jenway, England), the extracts in the solvents were measured for absorbance at wavelengths of 620 nm and 652 nm as described by Bennett and Bogorad (1973) and Silveira và đồng tác giả *(*2007). Chlorophyll a and carotenoid were measured for absorbance at wavelengths of 665, 652 and 470 nm following the method described by Xiong và đồng tác giả *(*2017).

Statistical analysis

Data were presented as mean ± standard deviation (n = 3). Statistical significance was determined using one-way ANOVA with GraphPad Prism 9.5 software, with a significance level of *p-values < 0.05*. Charts and figures were created using GraphPad Prism 9.5 software.

RESULTS AND DISCUSSION

Selecting the appropriate solvent for extracting phycocyanin from A. platensis

Fresh *A. platensis* biomass were extracted using various solvents, including 1.5% $CaCl_2$ solution, 0.1 M phosphate buffer (pH 7), and distilled water at a ratio of 1 g algae per 100 mL of each solvent. After three consecutive extraction cycles, 1.5% $CaCl_2$ solution exhibited the highest phycocyanin yield, with mean values of 14.38 ± 3.31 mg/g, 4.28 ± 1.52 mg/g and 2.15 ± 0.55 mg/g, respectively. Phycocyanin productivity from distilled water was significantly lower compared to 1.5% $CaCl_2$, with average values of 2.97 ± 0.77 mg/g, 10.68 ± 1.68 mg/g, and 1.01 ± 0.57 mg/g. Yield from phosphate buffer solution was also lower than from 1.5% $CaCl_2$, with average values of 0.92 ± 0.18 mg/g, 9.78 ± 0.11 mg/g, and 3.08 ± 1.05 mg/g. Statistical analysis showed no significant difference among the three solvents after three extraction cycles ($p < 0.05$) (Figure 1). However, the total phycocyanin yield obtained from 1.5% $CaCl_2$ was higher than from distilled water and phosphate buffer (Figure 2) and this difference was statistically significant ($p < 0.05$). This finding aligned with İlter và đồng tác giả (2018), who investigated the extraction of phycocyanin using various solvents. They found that 1.5% $CaCl_2$ solvent yielded the highest amount of phycocyanin when using frozen algal biomass and a ratio of 1 g algae: 100 mL solvent.

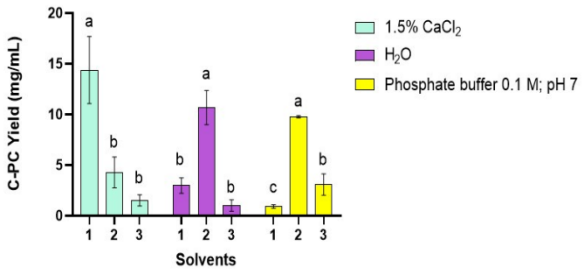

Figure 1. Phycocyanin yields after three cycles of extraction

In the same extraction cycle, columns topped by different letters are significantly different from each other ($p < 0.05$).

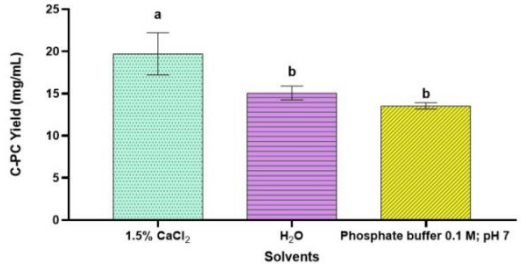

Figure 2. Total phycocyanin yields after three cycles of extraction

Columns topped by different letters are significantly different from each other ($p < 0.05$).

Efficiency of 1.5% $CaCl_2$ in Phycocyanin Extraction from A. platensis Using Ultrasound-Assisted Method

This study demonstrates that 1.5% $CaCl_2$ solution is the most effective solvent for extracting phycocyanin from *A. platensis* using ultrasound-assisted extraction. This effectiveness is attributed to $CaCl_2$'s appropriate ion strength, which facilitates phycocyanin dissolution without compromising the algae cell structure. In contrast, while distilled water effectively disrupts algae cells, it can also degrade phycocyanin, leading to lower extraction yields.

Phosphate buffer solution, though gentler on algal cells compared to distilled water, may form complexes with phycocyanin, thereby reducing extraction efficiency. In a study by İlter và đồng tác giả (2018), it was found that 1.5% $CaCl_2$ does not disrupt the cell structure of *A. platensis* algae (Figure 3). The efficacy of 1.5% $CaCl_2$ is likely due to its specific ion strength, capable of dissolving sodium-calcium channels without compromising the overall cell structure. In contrast, distilled water results in a deep blue color due to complete cell disruption by ultrasound, releasing all synthesized pigments within the cells. Despite its higher ion density, phosphate buffer solution (pH 7) causes less cell disruption compared to distilled water (Figure 3) (İlter *et al.*, 2018). Therefore, 1.5% $CaCl_2$ solution was selected as the preferred extraction solvent for achieving the highest phycocyanin content compared to the other two solvents. Subsequently, the 1.5% $CaCl_2$ solution was chosen for further experiments at a ratio of 1 g algae per 100 mL solvent using the ultrasound-assisted extraction method.

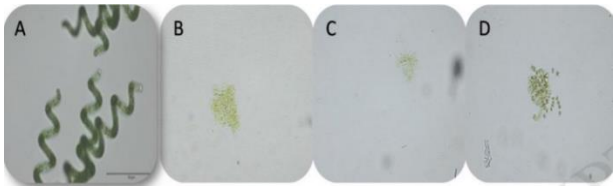

Figure 3. Microscopic images of *A. platensis* biomass extracted with three different solvents

(A): non-extracted A. platensis, (B): A. platensis extracted with sodium phosphate buffer (pH 7.4), (C): A. platensis extracted with distilled water, and (D): A. platensis extracted with 1.5% $CaCl_2$ solution (İlter *et al.*, 2018).

Investigating the effects of NaCl and IAA on fresh biomass of A. platensis

The standard cultivation medium: In the standard Zarrouk medium, *A. platensis* showed consistent growth, yielding 4.309 ± 0.1028 g and 7.596 ± 0.245 g of fresh biomass on days 5 and 10, respectively (Figure 4). After 5 days, biomass was higher without IAA supplementation compared to 1 mg/L IAA (4.309 ± 0.1028 g versus 2.236 ± 0.139 g), indicating stable growth without IAA.

The impacts of NaCl: *A. platensis* could tolerate up to 100 mM (equivalent to 5.8‰) NaCl without significant biomass reduction. Over 10 days, the fresh biomass obtained in the 100 mM NaCl treatment were 4.42 ± 0.418 g and 6.652 ± 0.461 g on days 5 and 10 (Figure 4), showing statistical significance ($p < 0.05$). Biomass was lower in the treatment containing only 100 mM NaCl compared to that in the treatment with both 100 mM NaCl and 1 mg/L IAA (6.652 ± 0.461 g *versus* 8.193 ± 0.173 g). This study highlighted that while NaCl supports stable growth, optimal biomass production requires IAA supplementation. Similarly, the research by Yu *et al.* (2024) also found that the salinity of the culture environment significantly improved the content and yield of phycocyanin. Evidence of this was that when adding 100 mM NaCl, the highest biomass content (1.89 g/L) and phycocyanin yield (30.61 mg/L) were achieved after 10 days of cultivation.

The impacts of IAA: The addition of 1 mg/L IAA had contrasting effects on *A. platensis* growth. By day 5, the algae exhibited reduced growth with a fresh biomass of 2.236 ± 0.139 g (Figure 4), which was significantly lower

compared to the nutrient-unamended control ($p < 0.05$). However, by day 10, biomass in the IAA-supplemented medium had increased substantially to 8.45 ± 0.286 g (Figure 4), surpassing the control biomass of 7.596 ± 0.245 g. The cost-effective nature of IAA supplementation proved beneficial in enhancing *A. platensis* biomass, which could be advantageous for future biomass production studies.

Combined effect of NaCl and IAA: Simultaneous supplementation of 100 mM NaCl and 1 mg/L IAA had notable effects on *A. platensis* growth. After 5 days, the algae reached their highest fresh biomass in the nutrient-unamended control supplemented with both NaCl and IAA, achieving 5.314 ± 0.242 g (Figure 4) with significant differences observed ($p < 0.05$). By day 10, biomass in this combined supplement condition further increased to 8.193 ± 0.173 g, demonstrating superior growth compared to the control supplemented with NaCl alone (6.652 ± 0.461 g). The synergistic effects of these supplements significantly boosted biomass production, highlighting potential applications in future research.

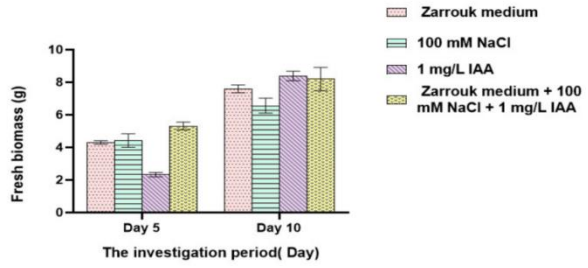

Figure 4. Fresh biomass accumulation of *A. platensis* over cultivation time

Analysis of phycocyanin, chlorophyll-a and carotenoid contents

Phycocyanin content: On days 5 and 10 of cultivation (Figure 5), *the* phycocyanin productivity in the treatment supplemented with 100 mM NaCl were 5.72 ± 0.06 mg/g and 9.81 ± 0.36 mg/g, respectively. In contrast, the treatment supplemented with 1 mg/L IAA exhibited the lowest phycocyanin accumulation of 4.69 ± 0.427 mg/g on day 5 and 6.813 ± 0.505 mg/g on day 10. Additionally, phycocyanin accumulation in the treatment supplemented with both 100 mM NaCl and 1 mg/L IAA showed relatively stable growth, with values of 5.91 ± 0.16 mg/g and 8.98 ± 0.233 mg/g on days 5 and 10, respectively. Rangkuti và đồng tác giả (2023) also found that a salinity range of 5-10‰ was the most optimal for increasing phycocyanin accumulation in algae, which was similar to the salt concentration used in the current study.

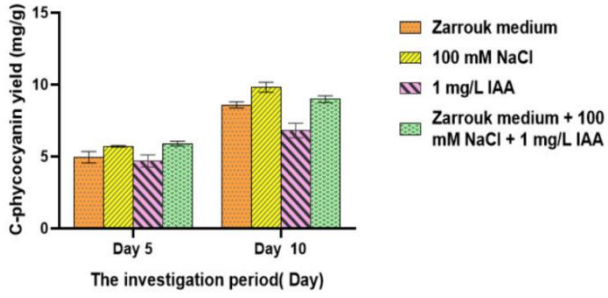

Figure 5. Phycocyanin productivity in *A. platensis* over 10 days of cultivation

Chlorophyll-a and Carotenoids Contents: The study indicates that supplementation with 100 mM NaCl significantly enhances phycocyanin accumulation in *A. platensis*. Conversely, supplementation with 1 mg/L IAA does not increase phycocyanin content in the algae. Furthermore, phycocyanin productivity also shows a significant increase in the medium supplemented with both 100 mM NaCl and 1 mg/L IAA. This suggests that the NaCl concentration used in this study (100 mM) did not exert any negative influences on the accumulation of phycocyanin in the algae. Therefore, this approach could be promising for maximizing the phycocyanin production in *A. platensis*. The study observed changes in the pigment compositions of *A. platensis* under different nutrient environments (100 mM NaCl, 1 mg/L IAA, and both factors). After 10 days of cultivation, chlorophyll-a content in the medium supplemented with 100 mM NaCl and 1 mg/L IAA significantly decreased compared to the nutrient-unamended control. This suggests that supplementation with 100 mM NaCl and 1 mg/L IAA appeared to inhibit the biosynthesis of chlorophyll-a and carotenoids by *A. platensis*. Additionally, after 10 days of cultivation, carotenoid content in the nutrient-unamended control was higher than in environments supplemented with nutrients (100 mM NaCl, 1 mg/L IAA, and both factors). This indicates that nutrient supplementation, including 100 mM NaCl and 1 mg/L IAA, may influence carotenoid synthesis differently or potentially inhibit its accumulation compared to the standard Zarrouk medium.

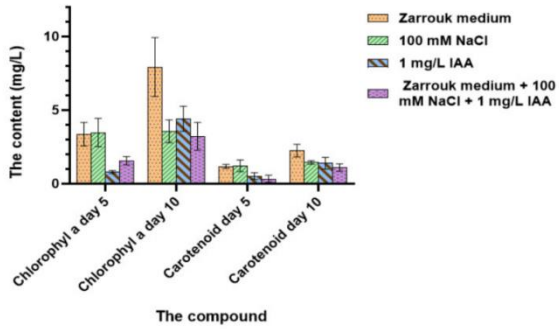

Figure 6. Chlorophyll-a and carotenoids in *A. platensis* over 10 days of cultivation

Numerous studies highlighted that phycocyanin was used in food as a dietary supplement and as a natural colorant. It was also employed in the cosmetics and biotechnology industries as a biochemical marker, including as a fluorescent probe for cellular analysis and immunological tests. The role of phycocyanin in cancer was demonstrated by its ability to inhibit tumor cell growth, suppress the cell cycle, and induce apoptosis and autophagy in these cells (Braune *et al.*, 2021; Fernandes *et al.*, 2023). The biosynthesis of pigments in *A. platensis* was complex and dependent on environmental culture conditions, making it crucial to research various nutritional supplements to enhance pigments production.

CONCLUSION

In summary, cultivating *A. platensis* under varied nutrient environments (100 mM NaCl, 1 mg/L IAA, and both) revealed distinct outcomes. Supplementation with 1 mg/L IAA enhanced growth, with the highest fresh biomass of 8.45 ± 0.286 g after 10 days. Chlorophyll-a and carotenoid levels were 7.93 ± 2.004 and 2.25 ± 0.43 µg/mL in the control. The highest accumulation of phycocyanin (9.81 ± 0.36 mg/g) was observed with 100 mM NaCl treatment. These findings indicate that while 1 mg/L IAA enhances biomass production without affecting phycocyanin levels, 100 mM NaCl consistently enhances both phycocyanin yield and biomass. To optimize nutrient conditions for maximizing both biomass and phycocyanin content, simultaneous supplementation of 100 mM NaCl and 1 mg/L IAA is recommended. This approach demonstrates the potential for scalable algae production while maintaining high phycocyanin levels.

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KHẢO SÁT KHẢ NĂNG SINH TRƯỞNG VÀ TÍCH LŨY PHYCOCYANIN CỦA VI TẢO *ARTHROSPIRA PLATENSIS* KHI NUÔI CẤY TRONG CÁC ĐIỀU KIỆN MÔI TRƯỜNG DINH DƯỠNG KHÁC NHAU

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TÓM TẮT

Arthrospira platensis là nguồn cung cấp các khoáng chất và acid amin thiết yếu, cùng với các chất chống oxy hóa như chlorophyll và phycocyanin, có lợi cho sức khỏe và hỗ trợ tăng cường hệ miễn dịch. Nghiên cứu được thực hiện nhằm đánh giá khả năng sinh trưởng và tích lũy phycocyanin của *A. platensis* dưới các điều kiện môi trường nuôi cấy khác nhau, bao gồm thay đổi nồng độ NaCl, IAA, và khảo sát ảnh hưởng kết hợp của các yếu tố này khi bổ sung vào môi trường Zarrouk. Kết quả cho thấy sau 10 ngày nuôi cấy ở 28-30°C, với cường độ ánh sáng 3500-4000 lux và pH 9, lượng sinh khối tươi cao nhất của *A. platensis* đạt được trong môi trường được bổ sung 1 mg/L IAA, đạt 8,45 ± 0,286 g. Hàm lượng phycocyanin tích lũy cao nhất trong môi trường nuôi cấy bổ sung 100 mM NaCl, đạt 9,81 ± 0,36 mg/g. Đồng thời, kết quả từ đề tài cũng cho thấy khi sử dụng dung môi $CaCl_2$ 1,5% với sự hỗ trợ của thiết bị siêu âm ly trích được phycocyanin cao nhất là 19,71 ± 2,50 mg/g. Từ đó, có thể kết luận rằng, việc bổ sung vào môi trường Zarrouk 100 mM NaCl và 1 mg/L IAA không chỉ giúp tảo tăng sinh khối tươi mà còn tăng tích lũy phycocyanin. Phương pháp này có tiềm năng lớn trong việc nghiên cứu tối ưu hóa sản xuất sinh khối và năng suất phycocyanin từ *Arthrospira platensis.*

*Từ khóa: Arthrospira platensi*s, sinh khối tươi, Phycocyanin, sắc tố, môi trường Zarrouk.

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