Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134844 2026, 25(1) 41-64

Research Article

Optimizing a seawater-based medium for large-scale cultivation of *Arthrospira platensis*: Impact on biomass, biochemical composition, and growth performance

Khaledi M.[1,2], Hesami M.J.[3], Zandi P.[4], Hafezieh M.[5*]

1 Food and Drug Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran
2 Halal Research Center of IRI, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran
3 Mohammad Javad Hessami, Department of Biotechnology, Faculty of Applied Sciences, UCSI University 56000 Cheras, KL, Malaysia
4 Faculty of Marine Sciences, Department of Marine Biology, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
5 Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
*Correspondence: jhafezieh@yahoo.com

Keywords

Arthrospira platensis,
Spirulina,
Seawater Medium,
Ionic Composition,
Pigment Enhancement,
Biochemical Analysis

Article info

Received: February 2025
Accepted: July 2025
Published: January 2026

Abstract

This study aimed to optimise the growth conditions of *Arthrospira platensis* by modifying the ionic composition of the standard Zarrouk medium using a seawater-based medium (MK1B). The optimisation process involved three stages: adjusting the ionic composition, optimizing iron concentration, and comparing different iron sources. The final mass concentrations for the control, G1, and G2 were 1.25, 1.1, and 1.15 g. L^{-1}, respectively, with no significant differences (p>0.05). The specific growth rate of the control group was significantly higher than G2 and G1. Carbohydrate content was higher in G1 and G2 compared to the control, while the protein content was lower in G1 and G2 compared to the control. In the semi-industrial phase, *A. platensis* cultivated in MK1B medium showed a productivity of 27.066 mg. L^{-1} per day and a total biomass of 0.862 g. L^{-1} after 30 days. The average chlorophyll content was 7.82 mg. g^{-1}, beta-carotene 1.9 mg. g^{-1}, protein 57.96%, lipid 9.35%, and carbohydrate 26.52%. The microbial load of *A. platensis* in MK1B medium met USP43 and ISIRI11166 safety standards, confirming its suitability for food and pigment applications. Finally, using seawater for the sustainable production of Spirulina microalgae, with potential applications in food supplements and natural pigment resources is feasible.

Introduction

Microalgae have garnered significant global attention for their ability to address pressing challenges such as food security, renewable energy production, and environmental sustainability. These photosynthetic microorganisms are capable of producing a wide array of valuable compounds. These compounds have potential applications across various industries, including food, feed, nutraceutical, pharmaceutical, cosmetic, and biofuel sectors. In addition, their rapid growth rates, ability to thrive in diverse environments, outstanding capacity of carbon dioxide sequestration and the production of a wide array of bioactive compounds make them a promising resource for the future (Dehghani *et al*., 2018; Hussian, 2018; Akbarnezhad *et al*., 2020, Barati *et al*., 2021, 2022). Among the microalgae, the cyanobacterium *Arthrospira platensis*, commonly known as Spirulina, is one of the most widely cultivated and consumed species due to its high nutritional value, easy cultivation, and environmental adaptability (Joventino *et al*., 2012; Hoseini *et al*., 2013; El-daim *et al.* 2021). *A. platensis* contains 60-70% of protein, 5-10% of lipids, 10-20% of carbohydrates, and various vitamins, minerals, and pigments, such as phycocyanin, chlorophyll, and beta-carotene (Narala *et al*., 2016; Rafa *et al*., 2021). This microalga has been repeatedly reported to have various health benefits, such as antioxidant, anti-inflammatory, immunomodulatory, antiviral, anticancer, antidiabetic, antihypertensive, and hepatoprotective effects (Syarina *et al*., 2015).

The cultivation of *A. platensis* can be performed in different types of systems, such as open ponds, closed photobioreactors, or hybrid systems, using different types of culture media, such as freshwater, seawater, or wastewater (Narala *et al*., 2016). The choice of the cultivation system and the culture media depends on several aspects, such as biomass productivity, quality, stability, cost, and environmental impact (Colla *et al*., 2007; Mehar *et al*., 2019). Among the different types of culture media, seawater-based media have some advantages over freshwater-based media, such as the availability, sustainability, and low cost of seawater, as well as the reduced risk of contamination and the improved quality of the biomass (Leema *et al*., 2010).

In regions like Iran, where freshwater resources are increasingly scarce, seawater cultivation of freshwater cyanobacteria can reduce freshwater consumption in biorefinery, potentially improving biosynthesis of amino acids and reducing water use in biorefinery processes (Iijima *et al*., 2015). Nevertheless, seawater-based media may require some modifications and optimizations to enhance the growth and the biochemical composition of *A. platensis*, such as the addition or adjustment of nutrients, including but not limited to nitrogen, phosphorus, potassium, and iron (Olguín *et al*., 1997; Leema *et al*., 2010; Dineshkumar *et al*., 2016). Recent research has demonstrated that seawater-based cultivation not only reduces reliance on freshwater resources but also contributes to environmental sustainability by minimizing the ecological footprint associated with large-scale *A. platensis*

production. Recent research demonstrates the potential of seawater-based cultivation for *Arthrospira platensis* (Spirulina) production, offering environmental and economic benefits. While seawater cultivation may reduce biomass productivity compared to freshwater (Villaró *et al*., 2023), it can enhance the production of valuable compounds such as essential amino acids, carotenoids, and specific fatty acids (Wu *et al*., 2021; Villaró *et al*., 2023). In addition to seawater, other sustainable water sources have been explored for Spirulina cultivation, demonstrating high biomass productivity and efficient nutrient removal (Leca *et al*., 2023). These findings highlight the potential for more sustainable large-scale production of Spirulina using alternative water sources. Studies have shown that *A. platensis* can be successfully adapted to seawater environments through the careful adjustment of nutrient levels and optimization of growth conditions, resulting in biomass production that is competitive with traditional methods while maintaining product quality. Furthermore, seawater cultivation systems have the potential to decrease the dependency on synthetic fertilizers by utilizing the natural mineral content inherent in seawater. This approach is well-aligned with broader sustainability objectives, offering a means to further reduce the environmental impact of algae production (Berden Zrimec *et al*., 2024). These developments hold significant promise for enhancing the economic viability of microalgae-based industries, particularly in regions where the scarcity of freshwater poses a substantial challenge to growth.

In the present investigation, optimization of the seawater-based culture media for the cultivation of *A. platensis* by testing different formulations and concentrations of nutrients was studied. In addition, the performance and the quality of the optimized media were compared to a conventional freshwater-based media, the Zarrouk medium. Moreover, an evaluation of the feasibility and cost-effectiveness of the optimized medium was conducted through pilot-scale cultivation. To the best of our knowledge, this is the first study to optimize seawater-based media and compare them to freshwater-based media for *A. platensis* cultivation in Iran.

This approach aligns with broader efforts to enhance water-use efficiency and sustainability in resource-limited environments and the findings of this study hold potential significance, offering valuable insights and guidance for the advancement and enhancement of the *A. platensis* industry in Iran and analogous regions characterized by comparable climatic and environmental conditions. Nevertheless, despite the promising potential of seawater-based cultivation, research on optimizing such systems for *A. platensis* remains limited, particularly in regions like Iran. This study addresses this critical knowledge gap by systematically optimizing seawater-based media and comparing them to traditional freshwater systems, offering new insights that could transform microalgae cultivation practices in Iran and similar regions.

Materials and methods

Microalgal strain and inoculum preparation

The microalgae strain, *A. platensis* (abdf2224), was obtained from the Algae Bank of Iran, Fars Province, Shiraz and cultured in Zarrouk medium under optimum conditions, including 30±1 °C, 14:10 light/dark cycle, constant aeration (2vvm), and controlled light intensity (135 $\mu mol\ photon\ m^{-2}S^{-1}$). The inoculum was prepared by centrifuging a sample of the microalgae culture at 4000 rpm for 10 min, washing the pellet twice with physiological saline (8.5 g. L^{-1} NaCl) (Soni *et al*., 2019), and resuspending the cells in a known volume of saline. All groups were inoculated with 10 % v. v^{-1} (mean cell concentration 0.025 g. L^{-1} dry weight) exponentially growing inoculum under aseptic conditions (Raoof *et al*., 2006).

Measurement of growth indices

The dry weight of the cells was measured as a growth characteristic. For this purpose, 15 mL of the microalgae sample after centrifugation at 4000 rpm for 10 minutes and washing twice with distilled water (to remove salts) was suspended with the help of distilled water and poured into a pre-weighed foil container. It was oven-dried for 24 hours at 60-70°C and weighed again. Through the difference of these two weights, the cell dry weight was calculated in g. L^{-1} (Madkour *et al.*, 2012). A relationship between OD680 and biomass (g. L^{-1}) of dry microalgae was obtained:

$$\mathrm{Xd}\left(\frac{\mathrm{g}}{\mathrm{L}}\right) = 0.890\ \mathrm{OD} - 0.0481 \quad R^2 = 0.9964$$

Growth induction of *A. platensis* was evaluated using the following equations, where Xm and Xi are the biomass concentrations at times t2 and t1, respectively. Production efficiency was calculated according to Danesi *et al.* (2011):

$$\mathrm{P(g/L/day)} = \frac{\mathrm{Xm-Xi}}{\mathrm{t2-t1}}$$

The maximum specific growth rate (μm) was calculated according to the method of Madkour *et al.* (2012):

$$\mu\mathrm{m(div/day)} = \frac{\ln(\mathrm{Xm-Xi})}{\mathrm{t2-t1}}$$

Productivity was calculated following the method described by Richmond (2013):

$$PV\ (\mathrm{g/L/day}) = V * X * \mu$$

Where PV is the productivity in volume, V is the culture volume (liter), X is the biomass production in 1 L of culture medium (g. L^{-1}), and μ is the maximum specific growth rate (μ_{max} day^{-1}).

Chlorophyll and carotenoids analyses

The pigment extraction procedure was adapted from the method described by Teresa Papalia *et al*. (2019) with minor modifications. Briefly, 0.1 g of the specimen was mixed with 10 mL of HPLC-grade acetone (80% v/v) in glass tubes. The mixture was homogenized using a vortex mixer for 1 minute and then left to extract in the dark at 4°C for 24 hours to ensure complete pigment release. The extract was centrifuged at 4,500 ×g for 15 minutes at 4°C to separate the supernatant from the biomass residue. The supernatant was filtered through a 0.45 μm PTFE membrane filter to remove any cellular debris.

Pigment quantification was performed using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) at room temperature (23±2°C). Absorbance measurements were recorded at specific wavelengths: 662 nm and 645 nm for chlorophyll-a, and 450 nm for beta-carotene (Mohebi Najafabadi, and

Naeimpoor., 2023). All measurements were conducted in quartz cuvettes with a 1 cm path length, using 80% acetone as a blank. The concentrations of chlorophyll-a and beta-carotene were calculated using the equations established by Lichtenthaler and Buschmann (2001).

Biochemical analysis

Biochemical analyses were performed on samples collected during the late exponential growth phase. Culture aliquots were filtered through 0.45 µm GF/C filters to capture biomass and then the harvested biomass was freeze-dried and preserved for analysis. Total protein content was quantified by homogenizing filters in 10% v. v^{-1} trichloroacetic acid to precipitate proteins (Dortch *et al*., 1984), followed by colorimetric determination using a modified Lowry assay after re-dissolving the precipitated proteins in 1 M NaOH (Lowry *et al*., 1951; Madkour *et al*., 2012). Carbohydrate content was measured by the phenol-sulfuric acid method described by Dubois *et al.* (1956). The Sulfo-Phospho-Vanillin (SPV) colorimetric method described by Mishra *et al.* (2014) was utilized for the quantification of total cellular lipids. This analytical approach allowed characterization of the protein, carbohydrate, and lipid fractions composing the *A. platensis* biomass grown under the experimental cultivation conditions.

Seawater preparation

Seawater was collected from the shore of the Persian Gulf Biotechnology Park on Qeshm Island, Iran. The seawater had a salinity of 39.23 ppt and a pH of 8.01. To conduct pretreatment, the seawater underwent the following procedural steps:

1. Pre-filtration through a felt bag filter to remove large particles and debris.
2. Coagulation with iron chloride (1.7 mg. L^{-1}) to precipitate heavy metals and suspend solids (Johnson *et al*., 2008; Abushaban *et al*., 2021), followed by 24-hour sedimentation to allow coagulated matter to settle.
3. Post-filtration through a second felt bag filter to remove any remaining particles.
4. Disinfection with active chlorine (1 mg. L^{-1}) for 2 hours, then de-chlorination with sodium thiosulfate to remove residual chlorine (confirmed by chlorine test) (Abushaban *et al*., 2021).
5. Hardness reduction by precipitating calcium and magnesium as their respective carbonates and sulfates upon the addition of calculated amounts of sodium carbonate (2.821 g) and sodium sulfate. Sodium carbonate was preferred due to its lower solubility product (Table 1).
6. Final sterile filtration through a two-stage 1 µm polypropylene pre-filter and 0.22 µm polytetrafluoroethylene (PTFE) filter.

These pretreatment steps effectively removed contaminants and reduced the hardness of the seawater, rendering it suitable for the cultivation of the microalga *A. platensis* medium development.

Designing the cultivation environment

The cultivation medium was optimized in three stages based on seawater composition: (1) adjusting the ionic composition, (2) optimizing iron

concentration, and (3) comparing iron sources.

Stage 1 involved comparing the ionic composition of Zarrouk medium (standard medium) and seawater, then excluding ions present in sufficient quantities in seawater from the formulation (Table 2).

Table 1: Analysis of Persian Gulf Water (used for cultivating Spirulina microalgae).

ION	Before Hardness Removal (g/L)	After Hardness Removal (g/L)
Na	7.906	13.266
CO2	0.154	10.422
Cl	20.885	20.885
Mg	1.268	0.0224
SO4	2.466	2.466
Ca	0.478	0.0204
K	0.543	0.543

Table 2: Ion Concentrations in Seawater, Zarrouk Medium, and Modified Zarrouk Medium.

ION	Zarrouk Medium (g/L)	Before Hardness Removal (g/L)	After Hardness Removal (g/L)	Modified Zarrouk Medium (g/L)
Na	5.672648	7.906000	13.266000	0
CO2	8.801267	0.154000	10.422000	0
Cl	0.626596	20.885000	20.885000	0
Mg	0.019722	1.268000	0.019000	0
SO4	0.632900	2.466000	2.466000	0
EDTA	0.069538	-	-	0.069538
Ca	0.010904	0.478000	0.010000	0
NO3	1.823787	-	-	1.823787
K	0.673183	0.543000	0.543000	0.130183
PO4	0.272593	-	-	0.272593
Fe	0.002009	0.000010	0.000010	0.002009

Alternative nitrogen sources (NH_3^-, NH_4^+, NO_3^-) were tested to optimize ionic ratios and prevent precipitation. The pH was adjusted to 9. The new formulations were designated MK, MI, MH, and MZ (Table 3) and evaluated for *A. platensis* growth to select the best medium. The second step was to investigate the effect of different iron concentrations on the growth of *A. platensis*. Desired media for *A. platensis* generally have low iron concentration (0.002 g. L^{-1} Fe for Zarrouk and Hiri and 0.0002 g. L^{-1} Fe for Jordan media). Therefore, concentrations of 0.002, 0.003, 0.005 and 0.007 g. L^{-1} Fe was used to investigate the growth process under different iron ion concentrations. The microalgae growth media consisted of three groups with varying iron (Fe) concentrations: MK contained 0.002 g. L^{-1} Fe, MK1 contained 0.003 g. L^{-1} Fe, MK2 contained 0.005 g. L^{-1} Fe, and MK3 contained 0.007 g. L^{-1} Fe.

In the third step, MK1 medium containing $FeSO_4$ $6H_2O$ (0.003 g. L^{-1} Fe) and EDTA (50 mg. L^{-1}), which was coded as MK1A was compared to a MK1B medium that contained commercial Fe-EDTA chelate fertilizer (13% FeNa-EDTA $3H_2O$) as the iron source. *A. platensis* growth in each medium was evaluated to select the final formulation. The details of the media composition and the growth parameters are shown in Tables 3 and 4, respectively. The final medium (MK1B) was selected based

on the highest biomass productivity and yield.

Microalgae cultivation and comparison of culture media

The purpose of this experiment was to evaluate the performance and the quality of the MK1B medium, which was optimized based on seawater and alternative sources of nitrogen, phosphorus and iron, for the cultivation of *A. platensis*.

Table 3: Ion concentration of Zarrouk's medium (standard medium) and the revised media (MK, MI, MH, and MZ)

ION	Concentration (g. L^{-1})				
	Zarrouk	MK	MI	MH	MZ
Na	5.673	-	-	-	-
CO_2	8.801	10.422	10.526	10.422	-
Cl	0.627	-	-	-	-
Mg	0.020	-	-	-	-
SO4	0.633	-	-	-	-
EDTA	0.070	0.050	0.050	0.050	0.070
Ca	0.011	-	-	-	-
NO_3	1.824	1.227	-	0.912	1.824
K	0.673	0.818	-	0.880	0.130
PO_4	0.273	0.165	0.042	0.136	0.273
Fe	0.002	0.002	0.002	0.002	0.002
Mn	0.001	-	-	-	-
Zn	0.000	-	-	-	-
MoO_4	0.000	-	-	-	-
Cu	0.000	-	-	-	-
NH_4	-	0.089	-	-	-
NH_3	-	-	0.080	-	-
Water	DW	SW	SW	SW	SW

Table 4: Growth components of Zarrouk's medium (standard medium) and the revised media in three stages (Mean±SD, n=3).

Stage	Treatment	Specific growth rate μ (day^{-1})	Px (g. L^{-1}. day^{-1})	PV
Stage 1	Zarrouk	0.1760±0.03	0.1056 ±0.02	0.1332±0.02
	MK	0.1568±0.02	0.0979±0.01	0.1201±0.02
	MH	0.1446±0.04	0.0636±0.01	0.1097±0.03
	IR25	0.1297±0.04	0.0520±0.009	0.0857±0.04
	MZ	0.1232±0.01	0.0453±0.01	0.0732±0.03
Stage 2	MK	0.1585±0.03	0.0846±0.01	0.1304±0.02
	MK1	0.1606±0.02	0.0884±0.008	0.1371±0.04
	MK2	0.1495±0.03	0.0699±0.003	0.1052±0.05
	MK3	0.1474±0.04	0.0670±0.01	0.1003±0.04
Stage 3	MK1A	0.1621±0.04	0.0916±0.04	0.1426±0.04
	MK1B	0.1634±0.03	0.0943±0.02	0.1473±0.01

The MK1B medium was compared with the Zarrouk medium, which is a conventional freshwater-based medium widely used for *A. platensis* cultivation (Raoof *et al.*, 2006; Soni *et al.*, 2019). The comparative analysis encompassed a thorough examination of biomass productivity, lipid content, protein content, carbohydrate content, chlorophyll concentration, and carotenoid levels. Since one of the goals of this study is to reduce freshwater consumption, the experiment was conducted in three different groups, specifically to reduce freshwater use. The microalgae inoculum was prepared from a pre-culture in Zarrouk medium and added to each flask at a concentration of 0.025 g. L^{-1} dry weight. The control group (C) used Zarrouk medium prepared with distilled water. The first experimental group (G1) used MK1B medium prepared with seawater. The second experimental group (G2) used MK1B medium prepared with coastal well water, which had a lower salinity than seawater (salinity of 28.32 and a pH of 8.3). The coastal well water was obtained from an artificial well located in the *Zist Darya Parvaran Anahita* Company, Persian Gulf Bio-Technology Park, Qeshm Island. The preparation process of coastal well water was like the preparation process of seawater. The experiment lasted for 25 days, and samples were taken every 3 days to measure the growth parameters and the biochemical composition of the microalgae.

Briefly, the optimization of *A. platensis* semi-industrial culture medium was conducted in three stages in this study. In the first stage, optimization of ionic composition was conducted and five media formulations, including Zarrouk, MK, MH, IR25 and MZ using different sources of nitrogen (NH_3^-, NH_4^+, NO_3^-), phosphorus (KH_2PO_4, $(NH_4)_2HPO_4$), and medium water (freshwater, seawater) were examined. In the next stage, MK medium was used as the base medium, and optimization of iron concentration was accomplished, where different iron concentrations (0.002, 0.003, 0.005, and 0.007 g. L^{-1} Fe) were tested and coded as MK, MK1, MK2, and MK3. Finally, in the third stage, comparison of iron sources was carried out.

Morphological studies

Cell morphology was evaluated using light microscopy during the exponential growth phase for cultures grown in Zarrouk medium, seawater-based MK1B medium, and coastal well water-based MK1B medium. Slides were examined under 100X magnification using a compound light microscope.

Semi-industrial cultivation

Based on optimal growth in the coastal well water-based MK1B medium at laboratory scale, semi-industrial cultivation of *A. platensis* was performed as follows:

The plastic tubs (200 L) were used as the culture vessels. Before each run, the tanks were cleaned by washing with detergent, alkaline hypochlorite solution (5 g. L^{-1} NaOH, 0.5 g. L^{-1} NaOCl), 0.1 M HCl, and filtered sterile water to remove contamination (Bamba *et al*., 2014). The tanks were filled to 120 L (total volume) with coastal well water and sterile MK1B medium. Mixing the medium was conducted using aeration (2 vvm) and the media temperature was adjusted to 30 °C.

Inoculum was prepared by a semi-continuous subculture of *A. platensis*, with a regular exchange of 2.5 L culture volume with fresh medium to reach a density of 1.1 g. L^{-1} dry biomass. The inoculum was added to the tanks to provide biomass density (50±3 mg. L^{-1}) and the measured inoculum concentration according to the following formula. An equivalent volume of tank medium was removed before inoculum addition.

$$V = \left(\frac{(120 \times 50)}{Xm}\right)$$

Where, V is inoculum volume (L), 120 is tank volume (L), 50 is initial biomass density (mg. L^{-1}), and Xm is inoculum biomass (g. L^{-1}). Cultivation conditions were 31°C/27°C Day/night temperature, 250 $\mu mol\ photon\ m^{-2}S^{-1}$ illumination with 14h: 7h light: dark cycle, and intermittent stirring. Evaporative water loss (approx. 0.5 L. Day^{-1}) was replaced with sterile distilled water equilibrated to tank temperature. Growth was monitored by regular measurement of biomass concentration.

Microbial load analysis

The microalgae harvested from the semi-industrial stage, after drying, were sent to the microbial laboratory of *Behesht Ain* Laboratory Complex (partner of the reference laboratory diagnosis and applied studies center of the country's veterinary organization) located in Tehran for microbial load analysis. Microbial tests were performed to measure *Escherichia coli*, coliforms sp., *salmonella* sp., fungi and *Staphylococcus aureus* according to USP43 and ISIRI11166 standards.

Statistical analysis

The data were expressed as mean ± standard deviation (SD) of three replicates. The statistical analysis was performed using GraphPad Prism software (version 9). One-way analysis of variance (ANOVA) and Tukey's test were used to compare the differences among the groups. A p-value of less than 0.05 was considered statistically significant.

Results

The objective of this test was to select and evaluate an optimized seawater-based medium for cultivating the microalga *A. platensis* through modifying the ionic composition of the standard Zarrouk medium and using alternative sources of nitrogen, phosphorus and iron suitable for growth in seawater. In the First stage, five media formulations using different sources of nitrogen phosphorus and medium water were examined and the highest specific growth rate (0.1760± 0.03 day^{-1}), biomass productivity (Px=0.1056±0.02 g. L^{-1}. day^{-1}) and volumetric productivity (PV=0.1332±0.02 g. L^{-1}) were achieved in the standard Zarrouk medium followed by MK medium.

In the secound stage, the iron concentration was optimized, resulting in the highest growth rate (0.1606±0.02 day^{-1}), Px (0.0884±0.02 g L^{-1} day^{-1}), and PV (0.1371±0.04 g L^{-1}) when using the MK1 medium supplemented with 0.003 g L^{-1} Fe. In stage 3, the effect of the iron source was evaluated, where the use of a commercial Fe-EDTA chelate fertilizer produced comparable results, yielding a growth rate of 0.1621±0.04 day^{-1}, Px of 0.0916±0.04 g L^{-1} day^{-1}, and PV of

0.1426±0.04 g L^{-1} in the MK1B medium (Table 4).

Morphological studies were conducted to investigate the effect of the MK1B medium on the growth and morphology of *A. platensis* compared with Zarrouk medium. Zarrouk medium supports optimal growth of *A. platensis*, however, it is not economically feasible for large-scale cultivation. The MK1B medium was expected to allow comparable growth since it contains similar ingredients; however, the use of seawater and coastal well water rather than fresh water posed potential challenges.

In this study, cells cultured in Zarrouk medium exhibited tighter coil morphology compared to those in MK1B medium prepared with seawater or coastal well water (Fig. 1). In addition, trichomes were slightly less coiled in seawater-based MK1B compared to coastal well water. However, overall cell size, number of coils, and general spiral structure were similar across all three culture media. This suggests that the MK1B medium maintained standard growth and morphology despite the use of saline water sources. The comparable morphology indicates the suitability of the MK1B medium for large-scale cultivation of *A. platensis* using seawater or coastal well water.

Figure 1: Effect of different culture media on the coil shape and size of *A. platensis* cells (magnification 100X).

The effect of different media and water sources on the growth and biochemical composition of *A. platensis* was investigated. Table 5 shows the cell productivity (Px), maximum specific growth rate (μm) and final biomass concentration (g.L^{-1}) of *A. platensis* grown in control medium (Zarrouk) and MK medium with different water sources (coastal well water and seawater). The statistical analysis revealed that there was no significant difference (p>0.05) in the specific growth rate and cell productivity among the treatments. However, the final biomass concentration was significantly higher (p<0.05) in the control medium than in the MK medium with either water source (Fig. 2).

The protein, carbohydrate, and lipid content (% dry weight) of *A. platensis*, grown in the media (as detailed in Table 3) and water sources (as outlined in Table 4), is presented in Table 7. Additionally, Table 6 shows the pigment content (mg. g^{-1} dry

weight) of *A. platensis* cultivated under the same conditions. The statistical analysis showed that there was a significant difference (p<0.05) in the chlorophyll and beta-carotene content among the treatments (Fig. 3). The control medium had the highest chlorophyll and beta-carotene content, followed by the MK 0medium with seawater and then the MK medium with coastal well water. The beta-carotene/chlorophyll ratio was also significantly different (p<0.05) among the treatments, with the MK medium with coastal well water having the highest ratio, followed by the MK medium with seawater and then the control medium.

Table 5: Cell productivity (Px), maximum specific growth rate (µm) and final biomass concentration (g.L^{-1}) of *A. platensis* grown in control medium (Zarrouk) and MK with different water media (water from coastal wells and seawater). (Mean±SD, n=3).

Media	Treatment	Specific growth rate µ (day^{-1})	Px (g. L^{-1}. day^{-1})	biomass production (g. L^{-1})
Zarrouk	Control	0.156± 0.001	0.049± 0.002	1.252 ± 0.04
MK1B	G1	0.1513± 0.003	0.043±0.007	1.104± 0.07
	G2	0.1531± 0.002	0.045± 0.001	1.156± 0.05

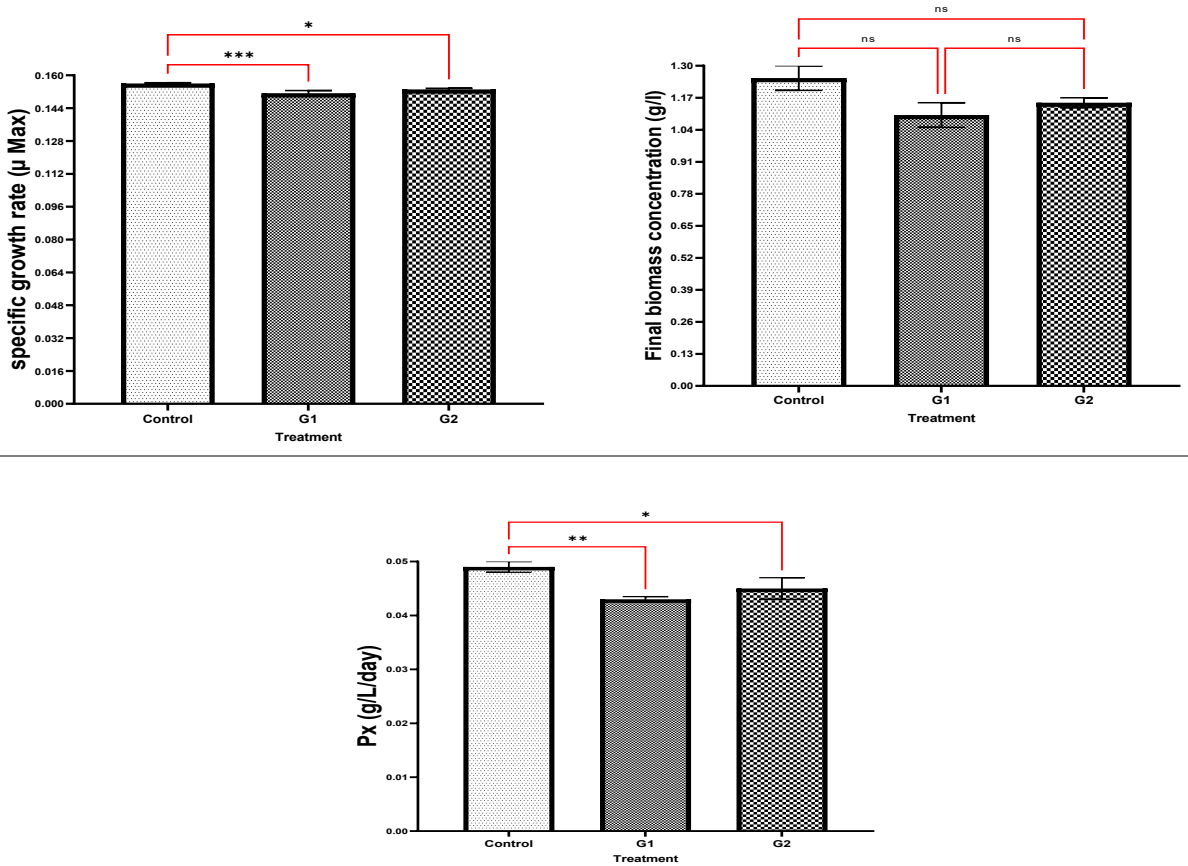

Figure 2: Statistical Analysis of Cell Productivity (Px), Maximum Specific Growth Rate (µm), and Final Biomass Concentration (g/L) of S. platensis in Zarrouk Control Medium and MK Medium with Varied Water Sources (G1 and G2) (Mean±SD, n=3; *p<0.05, **p<0.01, ***p<0.001).

The statistical analysis indicated that there was a significant difference (p<0.05) in the protein, carbohydrate and lipid content among the treatments (Fig. 4).

Table 6: pigment content (mg. g^{-1} dry weight) of *A. platensis* MK medium in seawater, coastal well water and control medium (Zarrouk) after 25 days of cultivation (Mean±SD, n=3).

Treatment	Pigment content (mg/g dry weight)		b-carotene/ chlorophylls ratio
	chlorophylls (mg. g^{-1})	Beta-carotene (mg. g^{-1})	
Control	15.31± 1.14	3.25± 0.04	0.2106± 0.004
G1	7.49± 0.14	2.40± 0.08	0.3291± 0.004
G2	9.12± 0.50	2.68± 0.01	0.2919± 0.002

Table 7: protein, carbohydrate and lipid content (% dry weight) of *A. platensis* in Zarrouk culture medium (control), MK1B medium in seawater, coastal well water (Mean±SD, n=3).

Media	Treatment	Protein (%)	Carbohydrate (%)	Lipid (%)
Zarrouk	Control	69.32 ± 0.75	14.27 ± 0.75	10.42 ± 0.60
MK1B	G1	58.71 ± 3.25	27.03 ± 0.75	9.17 ± 0.55
	G2	65.68 ± 2.30	22.15 ± 0.75	11.32 ± 0.12

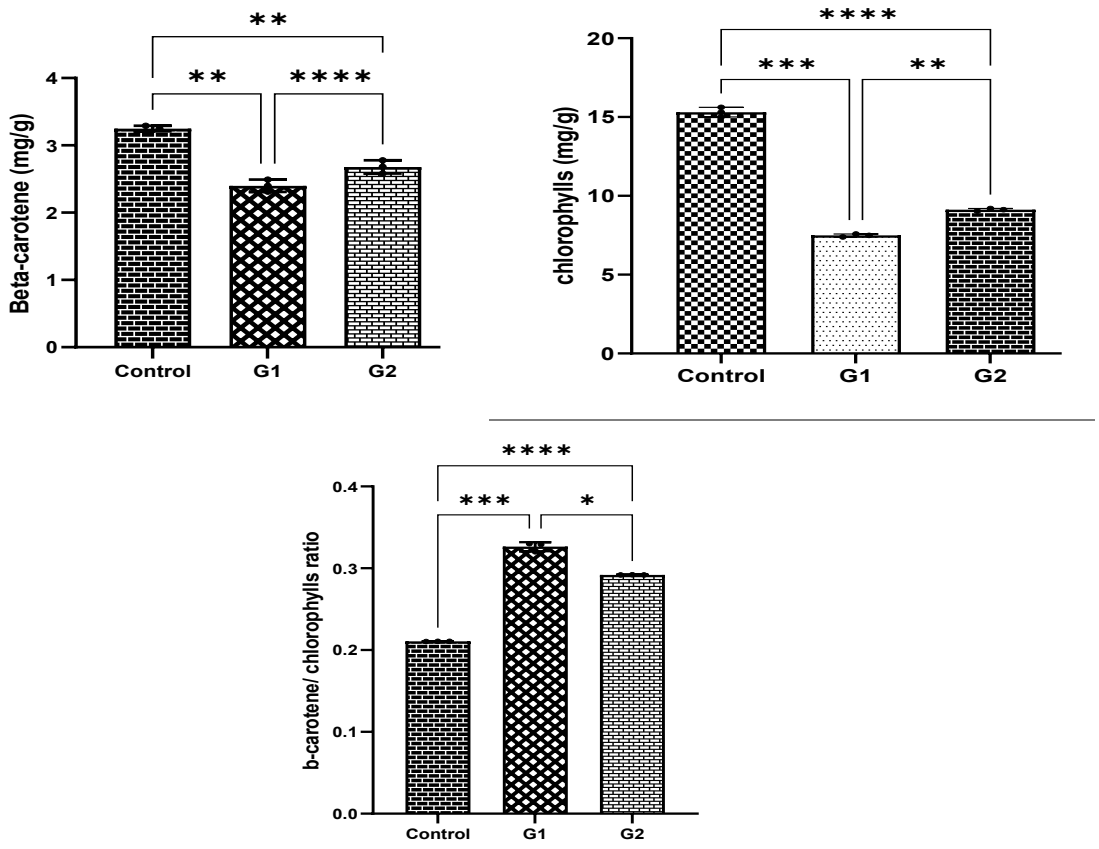

Figure 3: Comparative Analysis of Pigment Content (mg/g Dry Weight) in S. platensis Cultivated in MK1B Medium with seawater, Coastal Well Water, and Zarrouk Control Medium Over a 30-Day Period **(Mean±SD, n=3; *p<0.05, **p<0.01, ***p<0.001).**

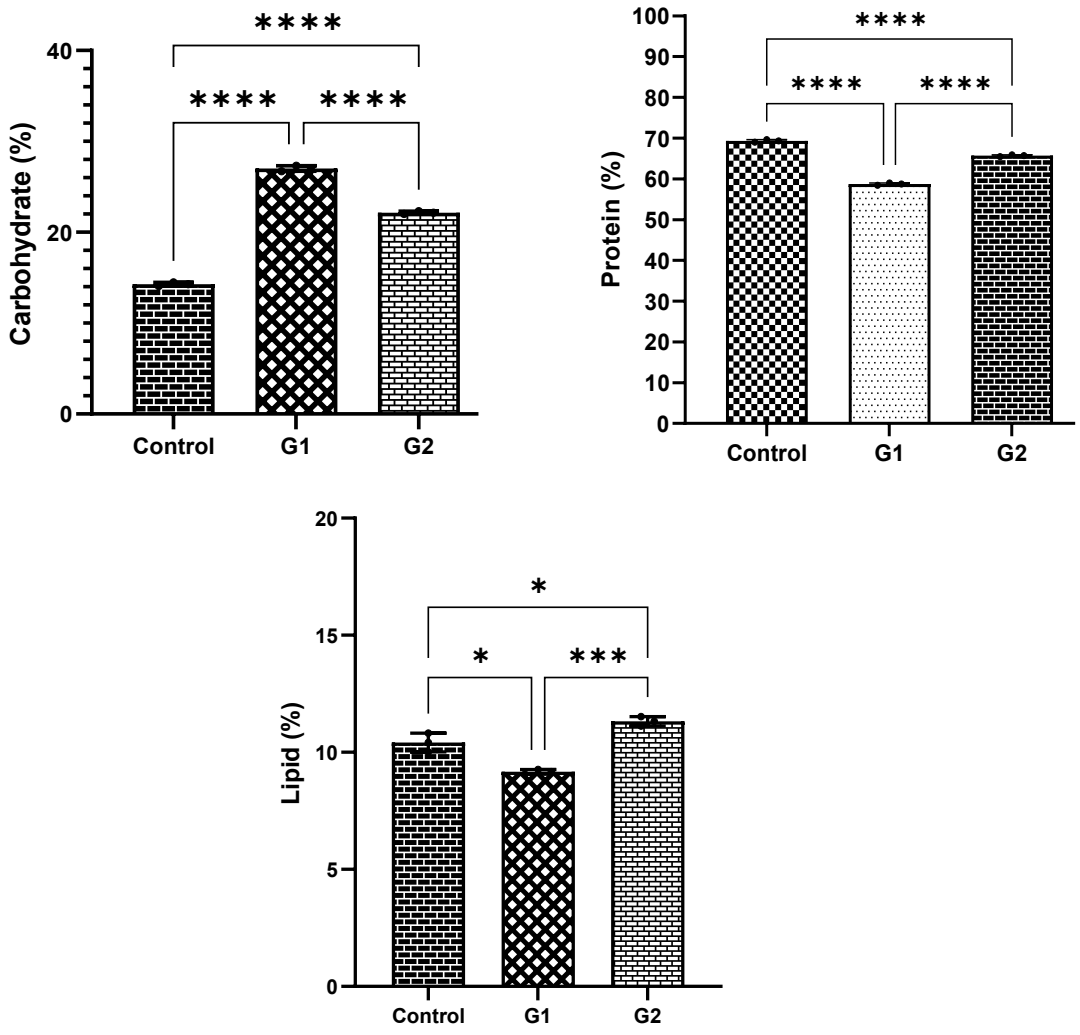

Figure 4: Statistical Comparison of Protein, Carbohydrate, and Lipid Content (% Dry Weight) in S. platensis Cultured in Zarrouk Control Medium and MK1B Medium with seawater and Coastal Well Water (Mean±SD, n=3; *p<0.05, **p<0.01, ***p<0.001).

The control medium had the highest protein and lipid content, followed by the MK medium with seawater and then the MK medium with coastal well water. The MK medium with coastal well water had the highest carbohydrate content, followed by the MK medium with seawater and then the control medium.

Table 6 illustrates the growth parameters, pigment content and biochemical composition of *A. platensis* grown in MK1B culture medium in three cycles with the same culture conditions. The results indicate that the growth rate and final biomass concentration of *A. platensis* were consistent across the three cycles, with an average of 0.115 day^{-1} and 0.863 g. L^{-1}, respectively. The protein content of *A. platensis* was also stable, ranging from 57.14% to 58.68%. The carbohydrate and lipid contents varied slightly among the cycles, with the lowest values in cycle 2 (25.21% and 9.83%, respectively) and the highest values in cycle 1 (27.43% and

8.96%, respectively). The chlorophylls and beta-carotene contents also showed some variation, with the highest values in cycle 2 (8.96 mg. g^{-1} and 2.04 mg. g^{-1}, respectively) and the lowest values in cycle 3 (6.83 mg. g^{-1} and 1.75 mg. g^{-1}, respectively). To further validate consistency between cycles (Fig. 5 and Table 8), the non-parametric Kruskal-Wallis test was applied for all growth and compositional parameters. No significant differences were found between any cycles (Kruskal-Wallis statistic=1.12, p=0.49, df=6). These results suggest that MK1B culture medium is suitable for the cultivation of *A. platensis* in semi-industrial volume, as it provides high and stable biomass and protein yields, as well as moderate carbohydrate, lipid, chlorophylls and beta-carotene contents.

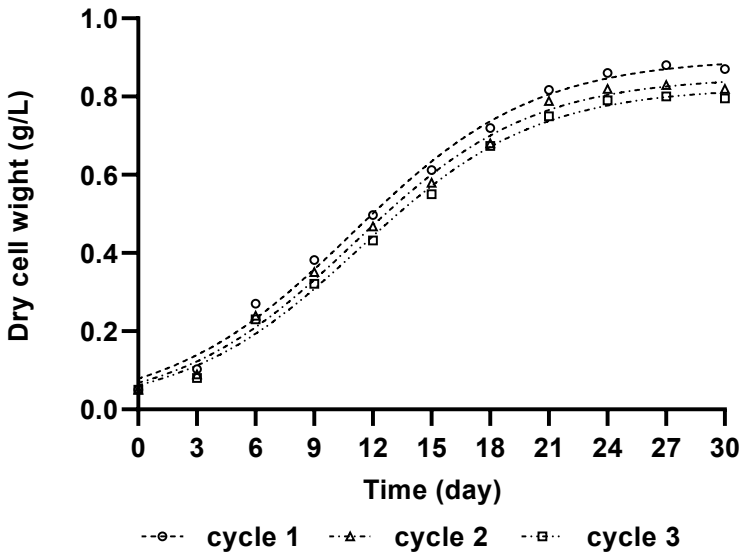

Figure 5: Reproducibility of growth kinetics for *A. platensis* production cycles on a semi-industrial scale in MK1B medium.

Table 8: Growth parameter, Pigment content and Biochemical composition of *A. platensis* grown in MK1B culture medium in three cycles with the same culture conditions.

Production cycles	cycle 1	cycle 2	cycle 3
P_x (mg. L^{-1}. day^{-1})	27.0	26.8	27.4
Specific growth rate μ (day^{-1})	0.1147	0.1134	0.1169
Final biomass concentration (g. L^{-1})	0.860	0.858	0.872
Protein%	57.14	58.07	58.68
Carbohydrate%	27.43	25.21	26.94
Lipid%	8.96	9.83	9.28
chlorophylls (mg. g^{-1})	7.68	8.96	6.83
Beta-carotene (mg. g^{-1})	1.92	2.04	1.75

Discussion

A seawater-based medium (MK1B) for the cultivation of *A. platensis* was developed and optimized in this study. The MK1B medium was formulated by modifying the ionic composition of standard Zarrouk medium and using alternative sources of nitrogen and iron suitable for growth in seawater. The growth performance and productivity of *A. platensis* in the MK1B

medium were comparable to those in the standard Zarrouk medium, which is prepared with freshwater, demonstrating the suitability of MK1B medium for cultivating *A. platensis* using seawater.

The optimization of the MK1B medium involved three stages including adjustment of the ionic composition, optimization of iron concentration, and comparison of iron sources. In the first stage, ions present in sufficient quantities in seawater were excluded from the formulation and different nitrogen and phosphorus sources were tested to prevent precipitation. In the second stage, the effect of different iron concentrations on the growth of A. *platensis* was investigated and 0.003 g. L^{-1} Fe was found to be optimal. Finally, $FeSO_4 \cdot 6H_2O$ and commercial Fe-EDTA chelate fertilizer were applied and compared as iron sources and they were found to have comparable effects on growth.

This study demonstrated that increasing iron concentration in the MK medium resulted in a reduction of the specific growth rate (μ), Px, and PV. Specifically, the highest growth rate (0.1606 day^{-1}), Px (0.0884 g. L^{-1} day^{-1}), and PV (0.1371 g. L^{-1} day^{-1}) were observed in the MK1 medium containing 0.003 g. L^{-1} Fe. As the iron concentration increased to 0.007 g. L^{-1} in the MK3 medium, the specific growth rate decreased to 0.1474 day^{-1}, Px to 0.0670 g. L^{-1} day^{-1}, and PV to 0.1003 g.L^{-1}. These findings align with previous research indicating that while iron is essential for the growth and metabolic activities of *A. platensis*, excessive iron concentrations can have inhibitory effects. Kougia *et al.* (2023) reported that iron concentrations up to 244 mg Fe.L^{-1} do not inhibit growth, but our results suggest that even lower concentrations can negatively impact growth parameters if not optimized. Delrue *et al.* (2017) found that replacing iron sulfate with iron EDTA increased iron content in biomass, but the effectiveness of Fe-EDTA can be influenced by pH and light conditions (Kean *et al*., 2015). This study highlights the importance of optimizing iron concentrations to balance the benefits of iron supplementation with the potential inhibitory effects of excessive iron. The results indicate that 0.003 g.L^{-1}·day^{-1} Fe is the optimal concentration for maximizing growth and productivity in the MK medium. This is consistent with findings from El-sheekh *et al.* (2024), who reported that a $FeSO_4$ concentration of 0.1 g. L^{-1} resulted in the highest growth rate and pigment content, suggesting that careful optimization of iron levels is crucial for achieving optimal growth. This study underscores the need for precise control of iron concentrations in *A. platensis* cultivation. The observed reduction in growth parameters with increased iron concentration highlights the delicate balance required to optimize nutrient supplementation for enhanced biomass production and biochemical composition.

The optimization of nitrogen sources for *A. platensis* cultivation has been a subject of extensive research, with varying results depending on the specific conditions and desired outcomes. Our study indicates that NH_4^+ is a more suitable source of nitrogen, as evidenced by its higher productivity compared to other nitrogen sources. Previous studies have shown mixed results regarding the optimal nitrogen source for *A.*

platensis cultivation. El-sheekh *et al.* (2021) reported potassium nitrate as the best nitrogen source. In contrast, Cruz-Martínez *et al.* (2015) demonstrated that ammonium nitrate was an effective alternative, providing both readily assimilable nitrogen and a reserve source, achieving high cell concentration and productivity. Mirhosseini *et al.* (2021) investigated ammonium sulfate and sodium nitrate, finding that nitrogen starvation surprisingly resulted in the highest biomass and phycobiliprotein production. However, increasing ammonium sulfate concentrations decreased both biomass and phycobiliprotein content. These conflicting results suggest that the optimal nitrogen source may vary depending on specific cultivation conditions and desired outcomes.

Our findings align with the results of Cruz-Martínez *et al.*(2015), demonstrating that ammonium-based nitrogen sources can be highly effective for *A. platensis* cultivation. The higher productivity observed with NH4+ in our study suggests that it provides a readily available nitrogen source that supports robust growth and biomass accumulation. The variation in results across different studies highlights the complexity of optimizing nitrogen sources for *A. platensis* cultivation. Factors such as the specific strain of *A. platensis*, the composition of the culture medium, and environmental conditions can all influence the effectiveness of different nitrogen sources. Therefore, further research is needed to explore the interactions between these factors and to identify the most suitable nitrogen source for various cultivation scenarios. Nutritional, physical, or chemical stresses can cause abnormalities in the typical spiral trichome structure of *A. platensis*. Continuous morphological monitoring during cultivation is necessary to avoid suboptimal conditions. Increased salinity has been shown to cause the trichome spiral to become looser in *Arthrospira* spp. (Wu *et al*., 2005). This study demonstrated that *A. platensis* cells cultured in Zarrouk medium exhibited a tighter coil morphology compared to those in the MK1B medium with seawater or coastal well water. Trichomes in the seawater-based MK1B medium were observed to be slightly less coiled compared to those in coastal well water. Despite these differences, the cell size, number of coils, and spiral structure remained consistent across all media. These findings align with previous research indicating that morphological changes can occur in different media. Toyoshima *et al.* (2015) observed that cells in seawater-based media showed more loosely coiled trichomes, similar to our observations in the MK1B medium. Additionally, Chen (2011) reported that media shifts could induce fragmentation and straightening of filaments, which may explain the slight differences in trichome coiling observed in our study. The consistency in cell size, number of coils, and spiral structure across all media suggests that while the medium composition can influence trichome morphology, it does not significantly affect the overall structural integrity of *A. platensis* cells. This is an important consideration for large-scale cultivation, as maintaining consistent cell morphology is crucial for the quality and functionality of the biomass produced. Trichomes in the

seawater-based MK1B medium were observed to be slightly less coiled compared to those in coastal well water. Despite these differences, the cell size, number of coils, and spiral structure remained consistent across all media. These findings suggest that MK1B medium can support the standard growth and morphology of *A. platensis*, even when saline water sources are used. The preservation of morphological characteristics across different media underscores the potential of MK1B medium for the large-scale cultivation of *A. platensis* in varied saline conditions.

The MK1B medium not only supported the acceptable growth of *A. platensis* in seawater, but also enhanced some of its biochemical and pigment characteristics. The carbohydrate content, beta-carotene content, and beta-carotene/chlorophylls ratio of *A. platensis* were increased in the MK1B medium compared to the control medium (Zarrouk). The control group, which was cultivated in conventional media, exhibited the highest chlorophyll content, with a concentration of 15.31 mg. g^{-1}, and the highest beta-carotene concentration, measured at 3.25 mg. g^{-1}. This suggests that the standard cultivation media provide an optimal environment for the synthesis of these pigments. However, it is noteworthy that the experimental groups G1 and G2, which were cultivated in seawater-based media, displayed significantly different beta-carotene/chlorophyll ratios compared to the control. This indicates a differential allocation of metabolic resources towards beta-carotene synthesis in the presence of seawater constituents. The altered ratio could be attributed to the ionic composition of seawater (Leema *et al*., 2010). This alteration in pigment production is further influenced by the presence of Mg^{2+} and Cu^{2+} ions, which can affect the levels of phycocyanin, allophycocyanin, and chlorophyll a (Urek and Kerimoglu, 2019). Additionally, the presence of trace elements in seawater might act as cofactors, enhancing the production of beta-carotene. The observed variations in pigment concentration and their ratios underline the potential of seawater-based media to modulate the metabolic profile of *A. platensis*, which could be leveraged for targeted bioproduct synthesis (Sandeep *et al*., 2015).

Leema *et al.* (2010) found that *A. platensis* grown in seawater media had increased carbohydrate content, beta-carotene content, and beta-carotene/chlorophylls ratio compared to the control medium. Satchasataporn *et al.* (2022) further demonstrated that the combined supplementation of glycerol and phosphorus can lead to enhanced production of carotenoids in *A. platensis*. Similarly, Forján *et al* (2007) showed that the limitation of essential nutrients such as phosphate can also increase carotenoid production in marine microalgae. Lastly, Wu *et al.* (2021) reported that the use of a nitrogen-free and seawater-supplemented medium can lead to a significant increase in carbohydrate content in *A. platensis*. These findings collectively suggest that the MK1B medium, which is seawater-based, can indeed enhance the biochemical and pigment characteristics of *A. platensis*, making it a more valuable food supplement and source of natural pigments.

The cultivation of *A. platensis* in media with increased salinity levels, as observed in groups G1 and G2, resulted in a decrease in protein content and a concomitant increase in carbohydrate content (Panyakampol *et al*., 2016; Ismaiel *et al*., 2018). This shift in metabolic allocation is a common stress response in many microorganisms, including cyanobacteria (Joset *et al*., 1996). The response of *A. platensis* to the combined stress of nitrogen depletion and high temperature is different from its response to individual stress, with a reduction in protein content and an increase in carbohydrate content (Panyakampol *et al*., 2016). Similarly, the cyanobacterium *Synechococcus elongatus* showed an increase in protein content under salt stress (Rezayian *et al*., 2016). These findings suggest that the decrease in protein content and increase in carbohydrate content in *A. platensis* under high salinity levels may be a result of the osmotic stress induced by the salt, leading to a reduction in nitrogen uptake and assimilation, and a shift in metabolic energy towards the synthesis of osmoprotectants.

In contrast, the group cultivated in Zarrouk media, which is specifically formulated for the growth of *Arthrospira* species and is characterized by its optimal nutrient balance and lower salinity, exhibited a significantly higher protein content. This underscores the importance of a well-balanced growth medium for protein synthesis in *A. platensis*. The Zarrouk media's composition supports efficient nutrient absorption and utilization, leading to enhanced protein production (Diaconu *et al*., 2020).

These observations highlight the intricate relationship between medium composition, particularly salinity levels, and the metabolic prioritization in *A. platensis*. Understanding this relationship is essential for tailoring cultivation conditions to maximize the desired bioproduct yield, whether it be protein or carbohydrate-based compounds.

This study has some novel aspects and unexpected outcomes that distinguish it from previous studies. First, coastal well water was used as an alternative water source for preparing the MK1B medium, which had a lower salinity than seawater. The growth of *A. platensis* in coastal well water-based MK1B medium was comparable to that in seawater-based MK1B medium, suggesting that coastal well water can be a viable option for *A. platensis* cultivation in areas where seawater is not readily available. Second, the performance and quality of the MK1B medium were tested in semi-industrial cultivation using a 200 L plastic tub. The MK1B medium provided high and stable biomass and protein yields, as well as moderate carbohydrate, lipid, chlorophylls and beta-carotene contents, across three production cycles. This indicates that the MK1B medium is suitable for large-scale cultivation of *A. platensis* using seawater or coastal well water.

The primary aim of this study was to develop a seawater-based culture medium. The results demonstrate that the novel MK1B medium rivals the performance of traditional spirulina cultivation media, as evidenced by comparable levels of chlorophyll, protein content, and dry biomass yield. Unlike the Zarrouk medium, which necessitates distilled or fresh water

for effective cultivation, the MK1B medium is specifically designed for and has been assessed in seawater-based cultivation systems. Furthermore, a cost analysis revealed that producing 1000 liters of Zarrouk medium incurs an expense of 7,504,549.47 Iranian Rials, whereas the MK1B medium can be prepared at a significantly lower cost of 2,613,000.00 Iranian Rials, representing a cost reduction of approximately 65.2%, based on the pricing provided by local chemical suppliers. The advantages of the MK1B medium are thus twofold: it serves as an economical alternative and a potent cultivation agent. This makes it an attractive option for coastal communities engaged in the large-scale production of spirulina-enriched biomass, offering both financial and productivity benefits.

Conclusion

This research contributes to the field of *A. platensis* cultivation by developing and optimizing a seawater-based medium (MK1B) that supports the growth and quality of *A. platensis* using seawater or coastal well water. This research has the potential to impact the development of a sustainable and cost-effective approach to producing this valuable microalga, which can be used as a food supplement and a source of natural pigments. This research also provides a basis for further improvement and application of the MK1B medium for large-scale *A. platensis* production and other microalgae cultivation. Future studies could further elucidate the role of specific nutrients in seawater characteristics in optimizing biomass production for commercial *A. platensis* cultivation.

Acknowledgements

We are immensely grateful to *Zist Darya Parvaran Anahita* and *Jolbak Sabz Caspian* companies for their generous support and for providing the capacities of their esteemed organizations, which were crucial to the success of our project. Their commitment and resources have been invaluable in our pursuit of excellence. The authors gratefully acknowledge financial support from UCSI University through its research grant scheme REIG-FAS-2025/004.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Abushaban, A., Salinas-Rodriguez, S.G., Pastorelli, D., Schippers, J.C., Mondal, S., Goueli, S. and Kennedy, M.D., 2021. Assessing pretreatment effectiveness for particulate, organic and biological fouling in a full-scale SWRO desalination plant. *Membranes,* 11(**3**), 167. DOI:10.3390/membranes11030167

Akbarnezhad, M., Shamsaie Mehrgan, M., Kamali, A. and Javaheri Baboli, M., 2020. Effects of microelements (Fe, Cu, Zn) on growth and pigment contents of *Arthrospira (Spirulina) platensis*. *Iranian Journal of Fisheries Sciences*, 19(**2**), 653-668. DOI:10.22092/ijfs.2019.120614

Bamba, B., Yu, X., Lozano, P., Ouattara, A., Abert Vian, M. and Lozano, Y.,

2014. Photobioreactor-based procedures for reproducible small-scale production of microalgal biomasses. *Journal of Algal Biomass Utlilization,* 5(**1**), 1–14.

Barati, B., Zeng, K., Baeyens, J., Wang, S., Addy, M., Gan, S.Y. and El-Fatah Abomohra, A., 2021. Recent progress in genetically modified microalgae for enhanced carbon dioxide sequestration. *Biomass and Bioenergy*, 145. DOI:10.1016/j.biombioe.2020.105927

Barati, B., Zafar, F.F., Qian, L., Wang, S. and El-Fatah Abomohra, A., 2022. Bioenergy characteristics of microalgae under elevated carbon dioxide. *Fuel,* 321. DOI:10.1016/j.fuel.2022.123958

Berden Zrimec, M., Sforza, E., Pattaro, L., Carecci, D., Ficara, E., Idà, A., Ferrer-Ledo, N., Canziani, S., Mangini, S., Lazar, B., Papadaki, S., Markou, G., Tzovenis, I. and Reinhardt, R., 2024. Advances in spirulina cultivation: Techniques, challenges, and applications. In: Severo, I.A., Martinez-Burgos, W.J. and Ordonez, J. (eds.) Insights into algae-fundamentals, culture techniques and biotechnological uses of microalgae and cyanobacteria. *IntechOpen,* pp 1-30. DOI:10.5772/intechopen.1005474

Chen, Y.C., 2011. The effect of shifts in medium types on the growth and morphology of *Spiruuna platensis* (*Arthrospira platensis*). *Journal of Marine Science and Technology*, 19, 565–570. DOI:10.51400/2709-6998.2171

Colla, L.M., Oliveira Reinehr, C., Reichert, C. and Costa, J.A.V., 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresource Technology*, 98, 1489–1493. DOI:10.1016/j.biortech.2005.09.030

Cruz-Martínez, L.C., Jesus, C.K.C., Matsudo, M.C., Danesi, E.D.G., Sato, S. and Carvalho, J.C.M., 2015. Growth and composition of a*rthrospira (spirulina) platensis* in a tubular photobioreactor using ammonium nitrate as the nitrogen source in a fed-batch process. *Brazilian Journal of Chemical Engineering*, 347–356. DOI:10.1590/0104-6632.20150322s00003062

Danesi, E.D.G., Rangel-Yagui, C.O., Sato, S. and de Carvalho, J.C.M., 2011. Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. *Brazilian Journal of Microbiology*, 42, 362–373. DOI:10.1590/S1517-83822011000100046

Dehghani, J., Adibkia, K., Movafeghi, A., Barzegari, A., Pourseif, M.M., Maleki Kakelar, H., Golchin, A. and Omidi, Y., 2018. Stable transformation of *Spirulina* (*Arthrospira*) *platensis*: a promising microalga for production of edible vaccines. *Applied Microbiology and Biotechnology*,102, 9267–9278. DOI:10.1007/s00253-018-9296-7

Delrue, F., Alaux, E., Moudjaoui, L., Gaignard, C., Fleury, G., Perilhou, A., Richaud, P., Petitjean, M. and Sassi, J.F., 2017. Optimization of *Arthrospira platensis* (*Spirulina*) growth: From laboratory scale to pilot scale. *Fermentation*, 3. DOI:10.3390/fermentation3040059

Diaconu, M., Volf, I., Cretescu, I. and Soreanu, G., 2020. Study of different liquid media influence on *Arthrospira platensis* microalgae cultivation for environmental applications.

Environmental Engineering And Management Journal, 19, 353–358. DOI:10.30638/eemj.2020.033

Dineshkumar, R., Narendran, R. and Sampathkumar, P., 2016. Cultivation of *Spirulina platensis* in different selective media. *Indian Journal of Geo-Marine Sciences,* 45, 1749–1754. DOI:10.9790/264X-0701011014

Dortch, Q., Clayton, J.R., Thoresen, S.S. and Ahmed, S.I., 1984. Species differences in accumulation of nitrogen pools in phytoplankton. *Marine Biology*, 81, 237–250.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry Journal* 28, 350–356.

El-Daim, A., El Asely, A., Kandiel, M., Abd El-Gawad, E., Elabd, H., Shaheen, A. and Abbass, A. A. A., 2021. Effect of *Spirulina platensis* and *Azolla nilotica* as feed additives on growth performance, antioxidant enzymes and fecundity of *Oreochromis niloticus*. *Iranian Journal of Fisheries Sciences*, 20(3), 846-862. DOI:10.22092/ijfs.2021.124136

El-Sheekh, M.M., Hassan, L.H.S. and Morsi, H.H., 2021. Growth enhancement of spirulina platensis through optimization of media and nitrogen sources. *Egyptian Journal of Botany,* 61–69. DOI:10.21608/ejbo.2020.27927.1487

El-Sheekh, M.M., Salman, J.M., Grmasha, R.A., Abdul-Adel, E., Saleh, M.M. and Al-sareji, O.J., 2024. Influence of Fe^{+2} on the biomass, pigments, and essential fatty acids of *Arthrospira platensis*. *Biomass Conversion Biorefinery*, 14, 621–629. DOI:10.1007/s13399-022-02470-9

Forján E., Garbayo I., Casal C. and Vílchez C., 2007. Enhancement of carotenoid production in *Nannochloropsis* by phosphate and sulphur limitation. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Microbiology Book Series,* 1, 356-364.

Hoseini, S.M., Khosravi-Darani, K. and Mozafari, M.R., 2013. Nutritional and medical applications of Spirulina microalgae. *Mini-Reviews in Medicinal Chemistry*, 13, 1231–1237.

Hussian, A.E.M., 2018. The role of microalgae in renewable energy production: challenges and opportunities. *Marine Ecology - Biotic and Abiotic Interactions* DOI:10.5772/intechopen.73573

Iijima, H., Nakaya, Y., Kuwahara, A., Hirai, M.Y. and Osanai, T., 2015. Seawater cultivation of freshwater cyanobacterium *Synechocystis* sp. PCC 6803 drastically alters amino acid composition and glycogen metabolism. *Front Microbiology*, 6: 326. DOI:10.3389/fmicb.2015.00326

Ismaiel, M.M.S., Piercey-Normore, M.D. and Rampitsch, C., 2018. Proteomic analyses of the cyanobacterium *Arthrospira (Spirulina) platensis* under iron and salinity stress. *Environmental and Experimental Botany,* 147, 63-74. DOI:10.1016/j.envexpbot.2017.11.013

Johnson, P.D., Girinathannair, P., Ohlinger, K.N., Ritchie, S., Teuber, L. and Kirby, J., 2008. Enhanced Removal of Heavy Metals in Primary Treatment Using Coagulation and Flocculation. *Water Environment Research,* 80(**5**), 472-479. DOI:10.2175/106143007x221490

Joset, F., Jeanjean, R. and Hagemann, M., 1996. Dynamics of the response of

cyanobacteria to salt stress: Deciphering the molecular events. *Physiologia Plantarum*, 96(**4**), 738-744. DOI:10.1111/j.1399-3054.1996.tb00251.x

Joventino, I.P., Alves, H.G.R., Neves, L.C., Pinheiro-Joventino, F., Leal, L.K.A.M., Neves, S.A., Ferreira, F.V., Brito, G.A.C. and Viana, G.B., 2012. The microalga *Spirulina platensis* presents anti-inflammatory action as well as hypoglycemic and hypolipidemic properties in diabetic rats. *Journal of Complementary and Integrative Medicine*, 9(**1**). DOI:10.1515/1553-3840.1534

Kean, M.A., Brons Delgado, E., Mensink, B.P., Bugter, M.H.J., 2015. Iron chelating agents and their effects on the growth of algae Iron chelating agents and their effects on the growth of *Pseudokirchneriella subcapitata*, *Chlorella vulgaris, Phaeodactylum tricornutum* and *Spirulina platensis* in comparison to Fe-EDTA. J*ournal of Algal Biomass Utilization*, 6(**1**), 56-73.DOI:10.1000/xyz999

Kougia, E., Ioannou, E., Roussis, V., Tzovenis, I., Chentir, I. and Markou, G., 2023. Iron (Fe) biofortification of *Arthrospira platensis*: Effects on growth, biochemical composition and in vitro iron bioaccessibility. *Algal Research*, 70, 103016. DOI:10.1016/j.algal.2023.103016

Leca, M.A., Michelena, B., Castel, L., Sánchez-Quintero, Á., Sambusiti, C., Monlau, F., Le Guer, Y. and Beigbeder, J.B., 2023. Innovative and sustainable cultivation strategy for the production of *Spirulina platensis* using anaerobic digestates diluted with residual geothermal water. *Journal of Environmental Management,* 344, 118349. DOI:10.1016/j.jenvman.2023.118349

Leema, J. M., Kirubagaran, R., Vinithkumar, N. V., Dheenan, P. S. and Karthikayulu, S., 2010. High value pigment production from *Arthrospira (Spirulina) platensis* cultured in seawater. *Bioresource Technolology,* 101(**23**), 9221-9227. DOI:10.1016/j.biortech.2010.06.120

Lichtenthaler, H.K. and Buschmann, C., 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-Vis spectroscopy. *Current Protocols in Food Analytical Chemistry*, 1(**1**), DOI:10.1002/0471142913.faf0403s01

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275. DOI:10.1016/S0021-9258(19)52451-6

Madkour, F.F., Kamil, A.E.W., Nasr, H.S., 2012. Production and nutritive value of *Spirulina platensis* in reduced cost media. *Egyptian Journal of Aquatic Research,* 38, 51–57. DOI:10.1016/j.ejar.2012.09.003

Mehar, J., Shekh, A., Nethravathy, M.U., Sarada, R., Chauhan, V.S. and Mudliar, S., 2019. Automation of pilot-scale open raceway pond: A case study of CO_2-fed pH control on *Spirulina* biomass, protein and phycocyanin production. *Journal of CO_2 Utilization.* 33, 384–393. DOI:10.22092/ijfs.2021.351071.0

Mirhosseini, N., Davarnejad, R., Hallajisani, A., Cano-Europa, E., Tavakoli, O., Franco-Colín, M. and Blas-Valdivia, V., 2021. Cultivations of *Arthrospira maxima (Spirulina)* using ammonium sulfate and sodium nitrate as

an alternative nitrogen sources. *Iranian Journal of Fisheries Sciences,* 20, 475–489. DOI:10.22092/ijfs.2021.351071.0

Mishra, S.K., Suh, W.I., Farooq, W., Moon, M., Shrivastav, A., Park, M.S. and Yang, J.W., 2014. Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. *Bioresource Technology*, 155, 330–333. DOI:10.1016/j.biortech.2013.12.077

Mohebi Najafabadi, M. and Naeimpoor, F., 2023. Boosting β-carotene and storage materials productivities by two-stage mixed and monochromatic exposure stresses on *Dunaliella salina*. *International Journal of Phytoremediation*, 25, 609–620. DOI:10.1080/15226514.2022.2095976

Narala, R.R., Garg, S., Sharma, K.K., Thomas-Hall, S.R., Deme, M., Li, Y. and Schenk, P.M., 2016. Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system. *Frontiers in Energy Research*, 4. DOI:10.3389/fenrg.2016.00029

Olguín, E.J., Galicia, S., Camacho, R., Mercado, G. and Pérez, T.J., 1997. Production of *Spirulina* sp. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. *Applied Microbiology and Biotechnology.* 48, 242–247. DOI:10.1007/s002530051045

Panyakampol, J., Cheevadhanarak, S., Senachak, J., Dulsawat, S., Siangdung, W., Tanticharoen, M. and Paithoonrangsarid, K., 2016. Different effects of the combined stress of nitrogen depletion and high temperature than an individual stress on the synthesis of biochemical compounds in *Arthrospira platensis* C1 (PCC 9438). *Journal of Applied Phycology* 28, 2177–2186. DOI:10.1007/s10811-015-0765-4

Papalia, T., Sidari, R. and Panuccio, M.R., 2019. Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass. *Molecules,* 24(**15**), 2810. DOI:10.3390/molecules24152810

Rafa, N., Ahmed, S.F., Badruddin, I.A., Mofijur, M. and Kamangar, S., 2021. Strategies to produce cost-effective third-generation biofuel from microalgae. *Frontiers in Energy Research,* 9. DOI:10.3389/fenrg.2021.749968

Raoof, B., Kaushik, B.D. and Prasanna, R., 2006. Formulation of a low-cost medium for mass production of *Spirulina*. *Biomass and Bioenergy,* 30, 537–542. DOI:10.1016/j.biombioe.2005.09.006

Rezayian, M., Niknam, V. and Faramarzi, M.A., 2016. Effect of salinity on some physiological and biochemical traits in grape (*Vitisvinifera*). *International Journal of Advanced Biological and Biomedical Research*, 4, 67–77. DOI:10.26655/ijabbr.2016.6.3

Richmond, A., 2013. Biological principles of mass cultivation of photoautotrophic microalgae. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. Chichester. pp. 171–204. John Wiley & Sons. DOI:10.1002/9781118567166

Sandeep, K.P., Shukla, S.P., Vennila, A., Purushothaman, C.S. and ManjuLekshmi, N., 2015. Cultivation of *Spirulina (Arthrospira) platensis* in low cost seawater based medium for extraction of value added pigments. *Indian Journal of Geo-Marine Sciences,* 44, 3.

Satchasataporn, K., Duangsri, C.,

Charunchaipipat, W., Laloknam, S., Burut-Archanai, S., Powtongsook, S., Akrimajirachoote, N. and Raksajit, W., 2022. Enhanced production of poly-3-hydroxybutyrate and carotenoids by *Arthrospira platensis* under combined glycerol and phosphorus supplementation. *ScienceAsia,* 48, 509–517. DOI:10.2306/scienceasia1513-1874.2022.072

Soni, R.A., Sudhakar, K. and Rana, R.S., 2019. Comparative study on the growth performance of *Spirulina platensis* on modifying culture media. *Energy Reports*, 5. DOI:10.1016/j.egyr.2019.02.009

Syarina, P.N.A., Karthivashan, G., Abas, F., Arulselvan, P. and Fakurazi, S., 2015. Wound healing potential of *Spirulina platensis* extracts on human dermal fibroblast cells. *EXCLI Journal,* 14, 385. DOI:10.17179/excli2014-697

Toyoshima, M., Aikawa, S., Yamagishi, T., Kondo, A. and Kawai, H., 2015. A pilot-scale floating closed culture system for the multicellular cyanobacterium *Arthrospira platensis* NIES-39. *Journal of Applied Phycology*. 27, 2191–2202. DOI:10.1007/s10811-014-0484-2

Urek, R.O. and Kerimoglu, Y., 2019. Evaluation of Effects of $Mg^{2+}$and Cu^{2+}on Pigment-Metabolite Production and Photosystem II Activity of *Arthrospira platensis* Gomont 1892. *Turkish Journal of Fisheries and Aquatic Sciences,* 19, 873–883. DOI:10.4194/1303-2712-v19_10_07

Vaquero, M., Morán, L., Álvarez, C., Cabral, E.M. and Lafarga, T., 2023. Effect of seawater on the biomass composition of *Spirulina* produced at a pilot-scale. *New Biotechnology*, 78, 173–179. DOI:10.1016/j.nbt.2023.11.002

Wu, H., Gao, K., Villafañe, V.E., Watanabe, T. and Helbling, E.W., 2005. Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis. Applied and Environmental Microbiology*, 71, 5004–5013. DOI:10.1128/AEM.71.9.5004-5013.2005

Wu, H., Li, T., Lv, J., Chen, Z., Wu, J., Wang, N., Wu, H. and Xiang, W., 2021. Growth and biochemical composition characteristics of *Arthrospira platensis* induced by simultaneous nitrogen deficiency and seawater-supplemented medium in an outdoor raceway pond in winter. *Foods*, 10(**12**), 2974. DOI:10.3390/foods10122974