Optimization of Biomass and Protein Content of Microalga *Arthrospira* (*Spirulina*) *platensis* Using Different Nitrogen Sources

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Abstract

The increasing interest in Arthrospira (Spirulina) can be attributed to its high protein content, ease of digestion, and significant levels of vitamins, minerals, amino acids, and pigments. Nitrogen is known to exert a strong influence on the metabolism of lipids and proteins in various microalgae. In the present study, the production of *Arthrospira platensis* was optimized in terms of biomass and protein by utilizing different nitrogen sources: KNO_3, NH_4NO_3, and urea. *A. platensis* was grown in Zarrouk's medium within a 3000 ml Erlenmeyer flask, where KNO_3, NH_4NO_3, and urea replaced $NaNO_3$ at concentrations of 0.010 M, 0.025 M, and 0.050 M. The results clearly indicated that *A. platensis* can be successfully cultivated using different nitrogen regimes; although maximum biomass production occurred in medium containing NH_4NO_3, there were no significant differences between treatments ($p > 0.05$). The highest protein content was obtained from cultures containing NH_4NO_3 followed by KNO_3; treatments had no significant differences ($p > 0.05$). Furthermore, for all *A. platensis* cultures examined, increases in nitrogen concentrations led to corresponding increases in both maximum biomass and protein content. Chlorophyll *a* content increased with rising nitrogen concentrations across all treatments; relatively high values (9.18 $\mu g.ml^{-1}$) occurred when KNO_3 was used as the nitrogen source on day fourteen of the culturing period. Overall, the results from this study suggest that using NH_4NO_3 can be considered as a promising nitrogen source for cultivating *A. platensis* aimed at achieving optimal biomass and protein production.

Keywords: Nitrogen, Protein, Biomass, Chlorophyll-*a*, Carotenoid, *Arthrospira platensis*

Introduction

Microalgae are organisms capable of producing valuable compounds such as pigments, proteins, minerals, amino acids, fatty acids, and vitamins, which can be used as food additives or for medicinal and health purposes. At present, three microalgae species of considerable economic importance are *Chlorella, Arthrospira*, and *Dunaliella.* Of these, *Arthrospira*, often

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referred to as *Spirulina*, is considered the most advantageous choice in the field of microalgae biotechnology, owing to its straightforward cultivation methods and uncomplicated harvesting and drying techniques (Jiménez et al., 2003). The microalga *Arthrospira* is thermophilic and thrives at an optimum growth temperature of 35-37 ºC; consequently, commercial cultivation of this species is primarily limited to tropical and subtropical regions.

Arthrospira is cultivated globally as a supplement in the preparation of valuable food products. This microalga contains a high protein content (60-70% dry weight), vitamins (especially vitamin B_{12}), minerals, along with many essential amino acids and fatty acids. Since the cell wall of this species does not contain cellulose, 85 to 95% can be utilized by organisms. Therefore, it is used in aquaculture as feed for fish, crustaceans, mollusks, and bivalves (Oliveira et al., 1999). Studies have shown that more than 85% of the protein in Arthrospira can be digested and absorbed within 18 hours (Sasson, 1997). Microalgae—known as single-celled microorganisms—produce biomass that can be used either for human consumption or in aquaculture as a nutritious food source for fish and shrimp (Spolaore et al., 2006). The large-scale production of lipids and metabolites from blue-green algae such as Arthrospira depends on various factors including temperature (Richmond et al., 1992), light intensity (Pandey and Tiwari, 2010), carbon dioxide availability (Zilli & Converti; Ravelonandro et al., 2011), nitrogen sources (Uslu et al., 2011), with ammonium being one such source (Yuan et al., 2011).

Researchers have indicated that growth rates, along with biomass accumulation and Chlorophyll production in blue-green algae like *Arthrospira* vary under different light intensities and temperatures when using various nitrogen sources. For instance, cultivation with urea resulted in better growth compared to potassium nitrate treatments (Danesi et al., 2011); moreover, increasing potassium nitrate levels did not significantly impact either growth rates or protein content within this algae species (Colla et al., 2007). Adequate nitrogen presence in culture media supports protein production within algal cells while inhibiting carbohydrate synthesis; conversely, a significant reduction in nitrogen levels promotes carbohydrate synthesis instead (Fernandez-Reiriz et al.,1989).

Few studies have focused on utilizing different nitrogen sources specifically for cultivating Arthrospira alga (Rodrigues et al., 2011; Madkouret et al., 2012). Given that nutrient prices significantly influence overall Arthrospira production costs after labor expenses, reducing costs through economical cultivation media plays a key role in advancing Arthrospira production. Although nitrate is commonly used as a conventional nitrogen source in preparing culture media for this alga, alternative inexpensive nitrogen sources such as urea can provide the necessary nitrogen for its growth (Danesi et al., 2011). Therefore, the purpose of this research is to identify optimal nitrogen sources aimed at enhancing both growth rate and yield of Arthrospira with respect to overall protein content.

Material and methods

Alga cultivation

The location of this research is the shrimp breeding workshop in Sandorof, located 5 km east of Jask. The blue-green alga *A. platensis* was obtained from the Aquaculture Department of the Persian Gulf and Sea of Oman Ecology Research Institute, and placed in 3-liter jars containing 2.5 liters of sterilized seawater with Zarrouk's medium (1966). This medium includes different sources of nitrogen: urea (NH_2CONH_2), ammonium nitrate (NH_4NO_3), and potassium nitrate (KNO_3) at concentrations of 0.010, 0.025, and 0.050 M. The alga was cultivated under identical conditions with a temperature range of 22-25 °C, salinity at 30 ppt, alkalinity pH 8, and a photoperiod of 12:12 hours (dark: light) (Guillard and Ryther, 1962).

Determination of dry biomass

To determine the amount of biomass, we took a sample of 250 ml from *A. platensis* cultivated in different media during the logarithmic growth phase and filtered it through a plankton net with a mesh size of 25 µm. The collected algae on weighed filter paper were then placed in an incubator at a temperature of 50 °C for 12 hours to achieve constant dry weight (AOAC, 1995).

Determining protein content

The total protein content in the algal samples was determined using the Lowry assay (Lowry et al., 1951) employing bovine serum albumin standards and measuring absorbance at 750 nm as described by Waterborg (2002) with light modification.

Determination of Chlorophyll a and carotenoids

To assess Chlorophyll *a* and carotenoid content, we took a specific volume (5-10 ml) from algal samples at the end of the experiment which was then filtered through a membrane filter with pore size 0.45 µm; Chlorophyll *a* was extracted using 90% acetone before its concentration was measured via spectrophotometry using equations provided by Strickland and Parsons (1989).

The total carotenoid content—comprising both xanthophylls and carotenes—was determined via spectrophotometry following Wellburn's method (1994).

Data analysis

$$C_a\ (\mu g/ml) = 11.24\ A_{661.6} - 2.04\ A_{644.8}$$

$$C_b\ (\mu g/ml) = 20.13\ A_{644.8} - 4.19\ A_{661.6}$$

$$C_{(x+c)}\ (\mu g/ml) = 1000\ A_{470} - 1.90\ C_a - 63.14\ C_b$$

Statistical analysis was performed using SPSS software with parametric tests including one-way analysis of variance and Duncan's multiple range test to compare data sets; $p<0.05$ was considered statistically significant.

Results

Table 1 shows the biomass of *A. platensis* alga cultivated with different culture media. According to Table 1, although the maximum algal biomass ($g.L^{-1}$) was obtained in the culture medium containing NH_4NO_3, it did not show a significant difference compared to other nitrogen sources ($p> 0.05$). Additionally, as nitrogen concentration increased, algal biomass also rose, with a maximum observed in the culture medium at a molarity of 0.05 M,

which demonstrated a significant difference from other concentrations studied ($p< 0.05$). Table 2 presents the protein content in *A. platensis* alga cultivated with various culture media. As shown in Table 2, the highest protein content was observed in culture media containing NH_4NO_3 and KNO_3; however, there was no significant difference compared to cultures containing urea ($p> 0.05$). Furthermore, an increase in nitrogen concentration led to an increase in protein levels across all cultures.

The amount of Chlorophyll *a* (Chl *a*) in *A. platensis* alga during a 16-day cultivation period with different nitrogen sources at concentrations of 0.010, 0.025, and 0.050 M is illustrated in Figure 1. From this figure, it can be seen that the maximum amount of Chl *a* was recorded on day 14 across all three treatments; however, its concentration decreased by day 16. On day 14, Chl *a* levels reached less than 7 $\mu g.ml^{-1}$ at a treatment concentration of 0.025 M and less than 10 $\mu g.ml^{-1}$ at a concentration of NH_4NO_3.

Table 3 displays Chl *a* amounts on the fourteenth day of cultivation for *A. platensis* alga grown with various media types. The Chl *a* content increased significantly across all treatments as concentrations rose ($p< 0.05$). Moreover, the highest level of Chl *a* was observed in the medium containing KNO_3, with significant differences noted compared to other treatments ($p< 0.05$).

Table 4 displays the Carotenoid content on the fourteenth day of cultivation for *A. platensis* alga grown with various media types. The Carotenoid content increased significantly across all treatments as concentrations rose ($p< 0.05$). Moreover, the highest level of Carotenoid was observed

Table 1. The biomass of *A. platensis* alga cultivated with different nitrogen sources on the 14th day

Culture media	Biomass ($g.L^{-1}$)		
Molarity	0.010	0.025	0.05
KNO_3	1.08±0.05	1.35±0.18	1.50±0.10
NH_4NO_3	1.16±0.22	1.40±0.07	1.72±0.18
NH_2CONH_2	0.95±0.30	1.12±0.04	1.47±0.35

Table 2. Protein Content of *A. platensis* alga cultivated in different nitrogen sources on the 14th day

Culture media	Protein ($mg.g^{-1}$)		
Molarity	0.01	0.02	0.05
KNO_3	563±60	573±20	586±30
NH_4NO_3	572±73	584±80	607±45
NH_2CONH_2	563±25	559±15	570±30

Table 3. Chl *a* content of *A. platensis* cultivated with different nitrogen sources on the 14th day

Culture media		Chl a (μg.mg^{-1})		
	Molarity	0.010	0.025	0.050
KNO_3		4.84±0.26	6.97±0.14	9.18±0.23
NH_4NO_3		5.02±0.30	6.95±0.42	7.89±0.17
NH_2CONH_2		3.95±0.18	5.13±0.39	7.44±0.40

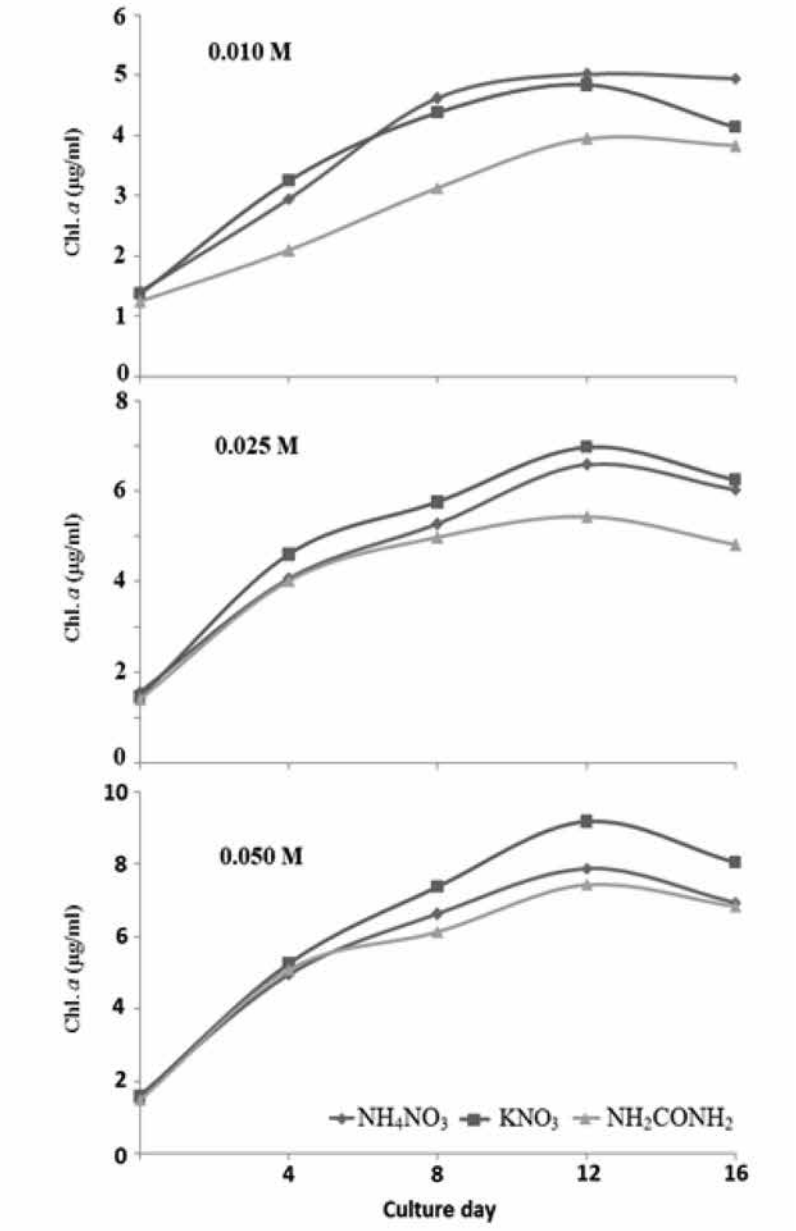

Fig. 1. The concentration of Chl *a* in *A. platensis* during the cultivation period with various nitrogen sources

Table 4. Carotenoid content of *A. platensis* cultivated with different nitrogen sources on the 14th day

Culture media	Carotenoid (μg.mg^{-1})		
Molarity	0.010	0.025	0.050
KNO_3	24.07±1.05	31.49±1.93	39.52±2.50
NH_4NO_3	24.68±1.34	31.40±2.01	36.10±2.38
NH_2CONH_2	19.48±1.68	25.22±1.74	34.03±1.95

in the medium containing KNO_3, with significant differences noted compared to other treatments ($p< 0.05$).

Discussion

The lack of nutrients in the culture medium, especially nitrogen, affects the growth and biochemical composition of microalgae in various ways. For example, sufficient nitrogen (in higher concentrations) in the culture medium of Arthrospira microalga supports both the amount of biomass and protein content within these cells. Conversely, carbohydrate synthesis increases when nitrogen levels in the culture medium are limited (Fernandez-Reiriz et al., 1989). The results of this study clearly demonstrated that increasing nitrogen concentrations across all culture conditions enhanced protein levels in *A. platensis*, with the maximum protein content observed in the culture medium containing NH_4NO_3; however, this did not show a significant difference from other culture conditions ($p> 0.05$).

It appears that nitrogen depletion occurs more rapidly in cultures with higher biomass compared to those with lower biomass since insufficient nitrogen (at lower concentrations) reduces algal cell efficiency (Göksan et al., 2007). Overall, this study also indicated that lower concentrations of nitrogen across all three culture environments led to a significant decrease in lipid and protein content. Therefore, it seems that adjusting or limiting nitrogen levels in Arthrospira microalgae's culture medium can increase or decrease both the type and amount of desired biochemical compounds. Danesi et al. (2011), through research on different nitrogen sources for cultivating Arthrospira microalga, found that urea resulted in better growth compared to potassium nitrate. On the other hand, Colla et al. (2007) showed that increasing potassium nitrate concentration had no significant effect on growth rate or protein content for this microalga.

In contrast, recent studies have clearly indicated successful cultivation of Arthrospira microalga using Zarrouk's culture medium containing different nitrogen sources without significant differences noted among lipid and protein contents across treatments. This suggests that varying results from different studies may be attributed to intrinsic characteristics of *A. platensis* regarding its response to various nitrogen sources.

Fluctuations in nitrogen concentration

within the culture medium not only affect Arthrospira biomass and protein levels but can also alter Chlorophyll-*a* and carotenoid pigment amounts, resulting in observable color changes within the media (Cohen, 1997). In this study, an increase was noted for Chlorophyll-*a* and carotenoid pigments as nitrogen content rose; however, no significant differences were observed between treatments using different nitrogen sources.

Sarada et al. (1998) reported a change from green-blue to green color within Arthrospira cultures after day 13 due to declining available nitrogen following consumption by algal cells—a characteristic typical among cyanophytes. They also noted that when nitrogen in the culture medium is depleted, phycocyanin pigment—responsible for the blue-green color of this microalga—is utilized as a nitrogen source, causing a shift in color from blue-green to green. Furthermore, a consistent Chlorophyll color indicates sufficient nitrogen presence in the cultivation environment.

In conclusion, the production of microalgae in aquaculture facilities accounts for approximately 30% of the total production costs (Coutteau and Sorgeloos, 1992). Therefore, one important factor in microalgae production is reducing costs by using inexpensive culture media. Nitrates are commonly used as one of the traditional nitrogen sources in preparing *A. platensis* culture media. The results obtained from this study clearly showed that Arthrospira grew well with the applied nitrogen sources, and there were no significant differences in the amounts of lipids, proteins, and pigments across the experimental treatments. Therefore, it can be considered a cheaper nitrogen source compared to KNO_3 and NH_4NO_3, urea can be used in the production of this microalga.

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