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Effects of Organic Carbon Sources on Selected Biological Characteristics of the Microalga *Arthrospira platensis* (Gomont, 1892)

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Abstract. *Arthrospira platensis*, a filamentous cyanobacterium rich in nutrients, holds great potential for applications in aquaculture, biotechnology, and functional food products. This study investigates the impact of three organic carbon sources—glucose, sodium acetate, and glycerol—on the growth and biochemical profile of *A. platensis* under mixotrophic conditions. Glucose (1 g/L) and sodium acetate (0.5 g/L) significantly promoted growth rates (0.38 ± 0.04 and 0.37 ± 0.02 day^{-1}, respectively) and biomass production. Conversely, glycerol inhibited growth across all concentrations. While all carbon sources enhanced lipid accumulation—most notably at 2 g/L sodium acetate (6.78 ± 1.16 mg/mL/day)—they simultaneously reduced protein content. These results support the strategic use of carbon sources to optimize *A. platensis* cultivation for high-value biomass production in aquaculture and bioindustry applications.

Keywords: Mixotrophic cultivation, Lipid accumulation, Protein content

1 Introduction

The microalga Arthrospira platensis, commonly known as Spirulina, is a cyanobacterium rich in protein—up to 70%—and contains various valuable bioactive compounds such as fatty acids, vitamins, and pigments [1]. Due to its antibacterial, antiviral, and antioxidant properties, A. platensis has been increasingly applied in medicine, functional foods, and environmental remediation [2, 3]. To improve cultivation efficiency and enhance economic value, it is essential to understand the fluence of environmental factors, particularly carbon sources [1]. Mixotrophic cultivation, which combines light energy with organic carbon sources, has been shown to improve biomass yield and productivity compared to purely autotrophic methods [4]. Among organic carbon sources, glucose, glycerol, and sodium acetate are commonly used in microalgal cultivation, each exerting distinct effects on metabolism and the accumulation of bioactive compounds [5]. However most previous studies has focused on individual carbon sources or limited growth parameters, providing insufficient comparative data on their simultaneous effects on both growth performance and biochemical composition of *A. platensis*. Addressing this gap, the present study aims to evaluate and compare the effects of three organic carbon sources (glucose, glycerol, and sodium acetate) at different concentrations on specific growth rate, protein content, and lipid accumulation under mixotrophic conditions. The novelty of this work lies in integrating growth

kinetics with biochemical profiling to identify optimal carbon supplementation strategies for targeted biomass production or biochemical enrichment. The findings are expected to provide practical guidance for large-scale *A. platensis* cultivation in aquaculture, biotechnology and functional food industries.

2 Materials and Methods

The *Arthrospira platensis* strain was cultured in standard Zarrouk medium under white fluorescent light at an intensity of 40 μmol photons/m²/s, with a photoperiod of 16 hours of light / 8 hours of dark, at a temperature of 25±2°C, and with continuous aeration. The composition of Zarrouk medium was as follows: 0.5 g/L K_2HPO_4; 2.5 g/L $NaNO_3$; 1 g/L K_2SO_4; 1 g/L NaCl; 0.2 g/L $MgSO_4{\cdot}7H_2O$; 0.04 g/L $CaCl_2{\cdot}2H_2O$; 0.01 g/L $FeSO_4{\cdot}7H_2O$; 0.08 g/L EDTA; 16.8 g/L $NaHCO_3$; 2.86 mg/mL H_3BO_3; 2.5 mg/mL $MnSO_4{\cdot}7H_2O$; 0.22 mg/mL $ZnSO_4{\cdot}7H_2O$; 0.079 mg/mL $CuSO_4{\cdot}5H_2O$; 0.0021 mg/mL $Na_2MoO_4{\cdot}2H_2O$. Organic carbon sources (glycerol, sodium acetate, glucose) were added at concentrations of 0.5, 1.5, and 2 g/L. The control treatment consisted of Zarrouk medium with $NaHCO_3$ as the sole carbon source. All experiments were conducted under static conditions (Table 1).

Arthrospira platensis was pre-cultured under standard conditions for 5 days. After this period, culture purity and cell density were verified prior to experimental setup. Each experiment was designed to evaluate the effects of different organic carbon sources (glucose, sodium acetate, glycerol) on *A. platensis*. The culture volume per replicate was 30 mL, maintained for 3 days, with each treatment conducted in triplicate. Each treatment was conducted in triplicate under aseptic conditions, using sterilized glassware and autoclaved culture media to prevent microbial contamination. Growth parameters were monitored daily, while total protein and lipid content were measured at the end of the experiment.

Table 1. Experimental setup for evaluating the effect of glucose, Sodium acetate, and Glycerol on *Arthrospira platensis*

Carbon concentration (g/L)	Glucose volume (mL/L)	Sodium acetate volume (mL/L)	Glycerol volume (mL/L)
0	0	0	0
0.5	1.25	0.08	1.03
1	2.5	0.15	2.06
2	5	0.3	4.12

Growth Rate Assessment

The growth rate of *A. platensis* was determined by measuring optical density (OD) at 680 nm. OD values were converted to dry biomass (g/L) using a pre-established calibration curve (Dry biomass [g/L] = 0.485 × OD_{680} - 0.0198; R^2 = 0.855), generated from parallel gravimetric determinations. The specific growth rate (μ) was calculated using the following equation:

$$\mu = \frac{ln(N_t - N_0)}{\Delta t} \quad (1)$$

where N_0 is the dry biomass on day 0 (g/L), N_t is the dry biomass on day t, and Δt is the cultivation period (days).

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Protein Content Determination

Total protein content was determined using a modified Biuret method [6]. Ten milliliters of algal culture were centrifuged, and the supernatant was discarded. The cell pellet was washed multiple times with distilled water. Cell lysis was achieved by adding 0.5 mL of 1N HCl, followed by incubation at 70°C for 10 minutes (vortexing for 15 seconds every 5 minutes). The lysate was centrifuged, and the pellet was resuspended in 2 mL of 0.9% NaCl. For analysis, 0.5 mL of the sample was mixed with 2 mL of Biuret reagent, shaken well, and left at room temperature for 30 minutes. The absorbance of the resulting blue-colored solution was measured at 540 nm. Protein content (C) was calculated as:

$$C\ (mg/g)\ = \frac{25 \times N \times 100}{a} \quad (2)$$

where N is the protein concentration in the sample (mg/mL) calculated from the OD reading, and a is the sample weight in grams.

Lipid Content Determination

Total lipid content was quantified using the sulpho-phospho-vanillin (SPV) colorimetric method [7], on both the first and last days of the experiment. Five milliliters of algal culture were centrifuged at 4,000 rpm for 5 minutes. The supernatant was discarded, and 2 mL of concentrated sulfuric acid (98%) was added. Samples were heated at 100°C for 10 minutes, then cooled in an ice bath for 5 minutes. Subsequently, 5 mL of phospho-vanillin reagent was added, and the mixture was incubated at 37°C for 15 minutes on a shaking incubator at 200 rpm. Absorbance was measured at 530 nm to determine lipid concentration.

Data Analysis

Descriptive statistics were used to summarize the experimental data. Differences among treatment groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify statistically significant differences. All statistical analyses and visualizations were performed using Python in Google Colab, employing libraries such as *pandas* for data handling, *matplotlib* and *seaborn* for plotting, and *scipy.stats* for statistical testing.

3 Results

3.1 Effects of Organic Carbon Sources on the Growth of *Arthrospira platensis*

Effect of Glucose Concentration

The results clearly indicated that the growth rate of A. platensis increased significantly with the addition of glucose (Fig. 1). The highest specific growth rate was observed at 1 g/L (0.38 ± 0.04 day^{-1}), representing a 73% increase compared to the control (0.26 ± 0.02 day^{-1}), with a statistically significant difference ($p < 0.01$). This suggests that moderate glucose supplementation enhances metabolic efficiency and cellular proliferation. Glucose serves as a rapid energy source under mixotrophic conditions, reducing reliance on photosynthesis and promoting biomass production [8, 5].

However, at 2 g/L, the growth rate slightly declined to 0.33 ± 0.02 day^{-1}. This decrease may be attributed to feedback inhibition or osmotic stress induced by high glucose concentration. Excess glucose can lead to the accumulation of intermediates such as pyruvate or NADH, disrupting intracellular redox balance and reducing energy conversion efficiency [9, 10]. Additionally, surplus glucose may redirect carbon flux toward lipid synthesis rather than biomass accumulation, especially under nitrogen-limited or unbalanced nutritional conditions [5]. Nevertheless, under sufficient nutrient availability, cells still prioritize growth, which explains why the growth rate remains relatively high at 2 g/L, although it is lower than the optimal

rate of 1 g/L. In summary, glucose is an effective organic carbon source for enhancing the growth of A. platensis under mixotrophic conditions, with a concentration of 1 g/L being optimal for this purpose.

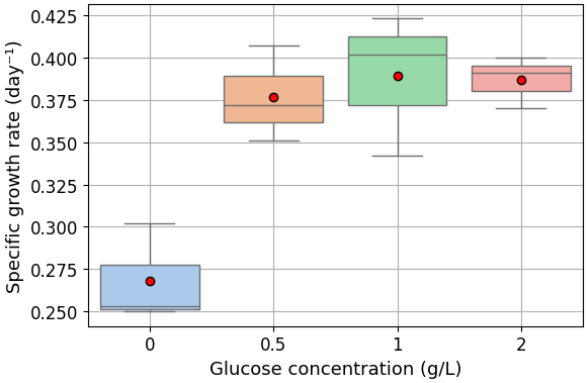

Fig. 1. Growth rate of *A. platensis* under different glucose concentrations

Effect of Sodium Acetate Concentration

Sodium acetate also significantly enhanced the growth of *A. platensis* under mixotrophic conditions, particularly at appropriate concentrations (Fig. 2). The highest growth rate was recorded at 0.5 g/L (0.37 ± 0.02 day^{-1}), approximately 68% higher than the control (0.22 ± 0.02 day^{-1}). This demonstrates the alga's ability to effectively absorb and metabolize acetate as an auxiliary energy source.

Nevertheless, growth rates slightly declined at 1 g/L (0.33 ± 0.03 day^{-1}) and 2 g/L (0.30 ± 0.01 day^{-1}). The reduction may result from acetate-induced pH shifts or osmotic stress, which can impair enzyme activity and cellular homeostasis [3]. At high concentrations, excess acetyl-CoA may be diverted to lipid biosynthesis—a process demanding substantial energy and precursors—thereby reducing resources available for cell division [11]. Thus, 0.5 g/L sodium acetate is optimal for promoting A. platensis growth; exceeding this level may shift metabolism toward lipid storage and stress response.

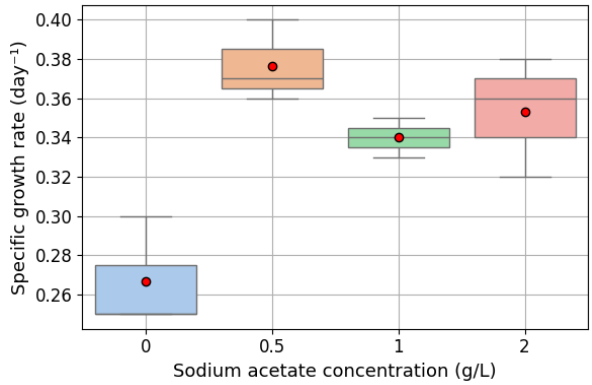

Fig. 2. Growth rate of *A. platensis* under different sodium acetate concentrations

Effect of Glycerol Concentration

Contrary to glucose and acetate, glycerol did not enhance growth; instead, it inhibited *the growth of A. platensis* at all tested concentrations (Fig. 3). Growth rates at 0.5, 1, and 2 g/L were 0.19 ± 0.02, 0.17 ± 0.01, and 0.13 ± 0.02 day^{-1}, respectively, all lower than the control (0.22 ± 0.02 day^{-1}). This indicates inefficient utilization of glycerol as a carbon source under the tested mixotrophic conditions.

Glycerol metabolism requires phosphorylation by glycerol kinase to form glycerol-3-phosphate, which is then converted to dihydroxyacetone phosphate (DHAP) before entering glycolysis. This longer, enzyme-dependent pathway is less efficient than glucose or acetate metabolism [12]. Moreover, high glycerol concentrations may induce osmotic stress, disrupting ion balance, enzyme activity, and membrane integrity, further impairing growth [7]. Glycerol may also promote lipid accumulation over cell proliferation under suboptimal conditions [4], aligning with the present findings.

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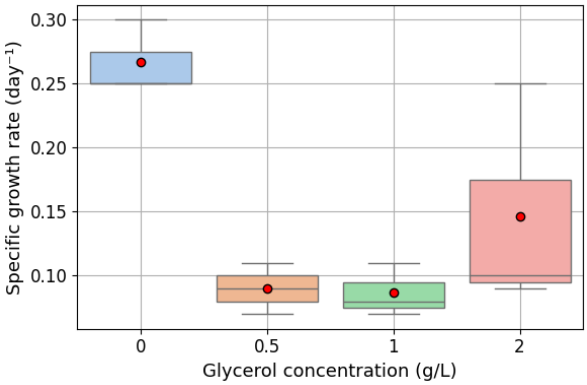

Fig. 3. Growth rate of *A. platensis* under different glycerol concentrations

3.2 Effects of Organic Carbon Sources on the Biochemical Composition of *Arthrospira platensis*

Effect of Glucose Concentration on Biochemical Composition

Glucose supplementation significantly affected the protein content of A. platensis, whereas changes in lipid content were marginal (Fig. 4). Lipid content slightly increased from 4.27 ± 1.08 mg/mL/day (0 g/L) to 5.11 ± 0.63 mg/mL/day (0.5 g/L), then decreased at 2 g/L (4.07 ± 0.37 mg/mL/day), with no statistically significant differences ($p = 0.35$). Conversely, protein content markedly declined with increasing glucose levels ($p = 0.0007$), from 56.38 ± 9.75 mg/mL/day (control) to 27.44 ± 3.41 mg/mL/day at 2 g/L. These findings suggest high glucose concentrations may divert carbon flux toward lipid synthesis at the expense of protein production, consistent with previous studies [13].

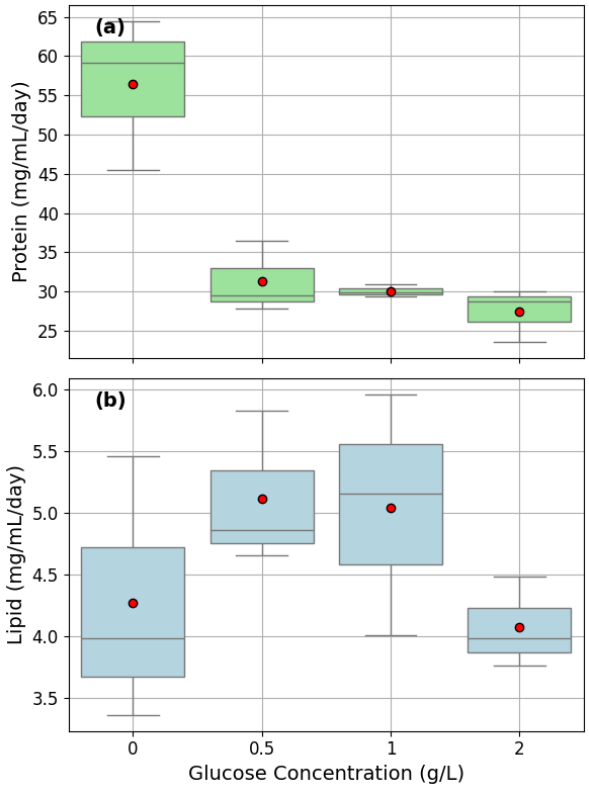

Fig. 4. Protein and lipid content of *A. platensis* under different glucose concentrations

Effect of Sodium Acetate Concentration on Biochemical Composition

Sodium acetate supplementation significantly increased lipid content ($p = 0.034$), from 4.27 ± 1.08 mg/mL/day to 6.78 ± 1.16 mg/mL/day at a concentration of 2 g/L (Fig. 5). In contrast, protein content declined sharply at 0.5 g/L (33.66 ± 7.93 mg/mL/day), followed by a slight increase at 1 and 2 g/L, but still remained below the control level (56.38 ± 9.75 mg/mL/day). This suggests that sodium acetate may initially shift metabolism toward lipid accumulation rather than protein synthesis. These results align with previous findings that acetate enhances biomass production at optimal levels, but higher concentrations may negatively affect pH and metabolic balance [3].

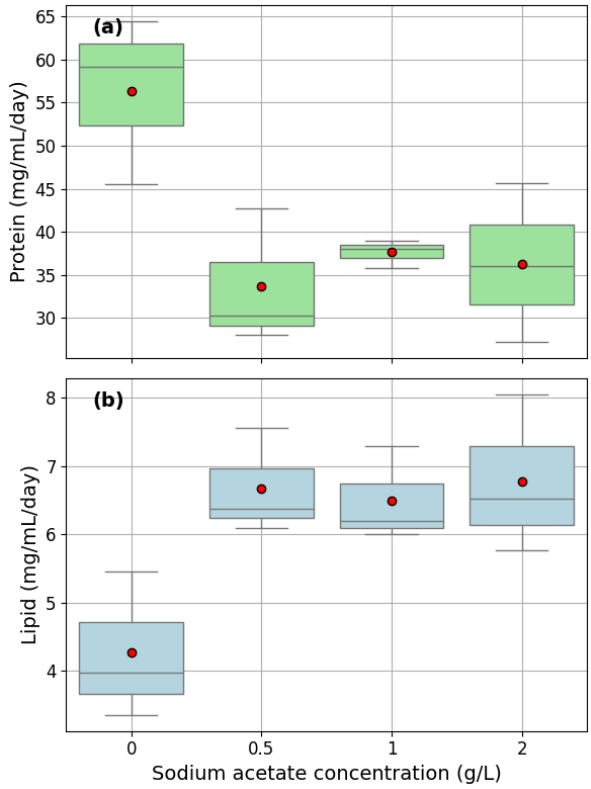

Fig. 5. Protein and lipid content of *A. platensis* under different sodium acetate concentrations

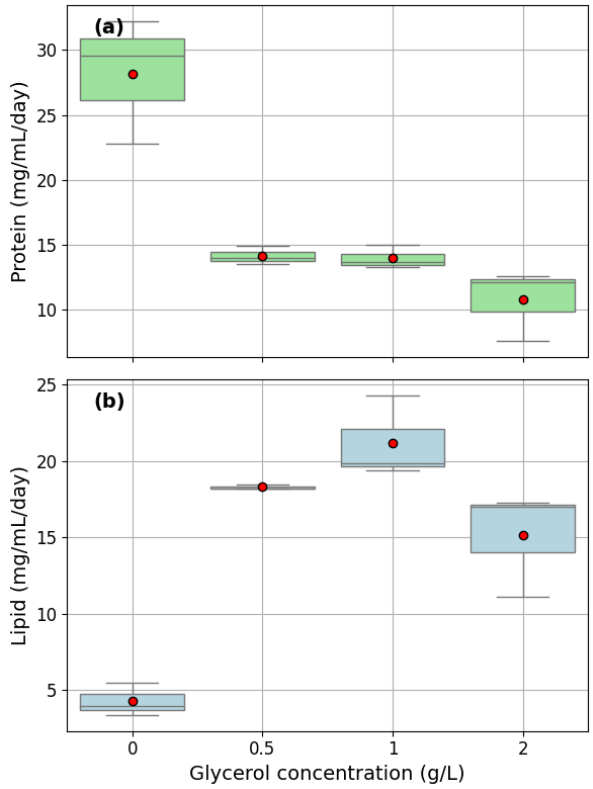

Fig. 6. Protein and lipid content of *A. platensis* under different glycerol concentrations

Effect of Glycerol Concentration on Biochemical Composition

Glycerol significantly increased lipid accumulation, particularly at 1 g/L (21.21 ± 2.69 mg/mL/day), which was nearly five times higher than the control (4.27 ± 1.08 mg/mL/day) (Fig. 6). This suggests that glycerol strongly induces lipid biosynthesis via the acetyl-CoA pathway. Conversely, protein content declined significantly with increasing glycerol concentration, from 28.19 ± 4.87 mg/mL/day (control) to 14.13 ± 0.71 mg/mL/day at 0.5 g/L and further at higher concentrations. These results suggest that glycerol may inhibit protein synthesis by diverting carbon flux or inducing metabolic imbalance. While consistent with previous lipid findings [11], the protein trend may vary depending on culture conditions and algal strains. Overall, glycerol is suitable for lipid enrichment but not for maintaining high protein levels.

4 Discussions

The results of this study demonstrate that different organic carbon sources have a significant impact on the growth and biochemical composition of Arthrospira platensis under mixotrophic conditions. Glucose was found to be the most effective carbon source in promoting growth when applied at an appropriate concentration. The highest specific growth rate (0.38 ± 0.04 day^{-1}) was recorded at 1 g/L, representing an approximately 72% increase compared to the control. This can be primarily attributed to the efficient uptake and rapid metabolism of glucose via glycolysis, which supplies ATP and carbon skeletons for biomass and amino acid synthesis [7].

However, at higher concentrations (2 g/L), the growth rate slightly declined, possibly due to energy overflow regulation or intracellular stress

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caused by increased osmotic pressure, which can impair cellular metabolic activity [13]. From a biochemical perspective, glucose slightly enhanced lipid accumulation but significantly reduced protein content. This shift in carbon flux toward lipid synthesis at high glucose levels is a common mechanism observed in microalgae under conditions of energy excess [7].

Sodium acetate also proved to be an effective carbon source, with the highest growth rate observed at 0.5 g/L (0.37 ± 0.02 day^{-1}). Acetate can be directly converted to acetyl-CoA, which enters the TCA cycle to produce energy efficiently [14]. However, at concentrations of 1–2 g/L, the growth rate declined gradually, suggesting a saturation threshold or negative effects from acetate accumulation on intracellular homeostasis [3]. Although acetate contributed to an increase in lipid content, protein levels decreased significantly at 0.5 g/L and remained lower than the control at higher concentrations. This reflects a metabolic preference for fatty acid synthesis over protein synthesis under certain environmental conditions.

In contrast, glycerol did not enhance growth and showed inhibitory effects at all tested concentrations. Glycerol requires phosphorylation by glycerol kinase to form glycerol-3-phosphate, which is then converted to DHAP before entering glycolysis—a more complex and energy-demanding pathway compared to glucose or acetate [12, 5]. Additionally, high glycerol concentrations may cause osmotic stress or create suboptimal conditions for enzyme activity, thereby slowing down cell division. However, glycerol strongly promoted lipid accumulation, particularly at 1 g/L (21.21 ± 2.69 mg/mL/day), the highest among all carbon sources tested. This response reflects a typical metabolic adaptation in microalgae, redirecting excess carbon toward lipid storage rather than cell growth or protein production [4].

In conclusion, A. platensis exhibited distinct physiological responses to different organic carbon sources. While glucose and sodium acetate at optimal concentrations effectively enhanced growth, glycerol favored lipid accumulation but was less effective for promoting growth. These findings suggest that the choice of carbon supplementation strategy should align with specific cultivation objectives—whether biomass production or lipid enrichment.

5 Conclusion

This study confirms that organic carbon sources have a significant influence on the growth and biochemical composition of Arthrospira platensis under mixotrophic cultivation. Glucose (1 g/L) and sodium acetate (0.5 g/L) markedly enhanced growth, while glycerol inhibited it at all tested concentrations. All three carbon sources increased lipid accumulation but reduced protein content, especially at higher concentrations. Glycerol and acetate at 1–2 g/L yielded the highest lipid levels, though protein levels declined, particularly with glycerol. Overall, supplementing glucose at 1 g/L or sodium acetate at 0.5 g/L is recommended to optimize both growth and biochemical profiles. These findings offer valuable insights for optimizing A. platensis cultivation for biomass production, functional foods, or lipid-rich bioresources.

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