DJS Vol. 51(2) (2025) pp. 15-27 - ISSN: 1012-5965

Delta Journal of Science

Available online at

https://djs.journals.ekb.eg/

Research Article

MICROBIOLOGY

Influence of nutrient availability in Bold's Basal Medium on growth performance and biochemical productivity of *Chlamydomonas reinhardtii*

Khalil M. Doman*[a], Mohamed M. Gharieb[a], Ahmed M. Abd El-Monem[b] and Hanaa H. Morsi[a]

[a]Department of Botany and Microbiology, Faculty of Science, Menoufia University, Menoufia, Egypt;
[b]Department of Fresh Water and Lakes, National Institute of Oceanography & Fisheries, Cairo, Egypt.
***Corresponding author:** Khalil M. Doman **e-mail**: domankhalil@gmail.com

Received: 12 /10 /2025 **Accepted:** 11 /11/2025

KEY WORDS

Chlamydomonas reinhardtii, Bold's Basal Medium, macronutrients, algal growth, biochemical composition.

ABSTRACT

The unicellular green alga *Chlamydomonas reinhardtii* serves as a model organism for studying physiological and biochemical adaptations of microalgae to nutrient variation. This study investigated the effect of macronutrient modulation nitrogen, phosphorus, and sulfur on the growth, pigment synthesis, and biochemical composition of *C. reinhardtii* cultivated in Bold's Basal Medium under sterile and axenic conditions. Cultures were maintained for 30 days, and biomass accumulation, photosynthetic pigments (chlorophyll *a*, and carotenoids), as well as biochemical constituents (proteins, carbohydrates, phenols, and flavonoids) were quantified. The results demonstrated that *C. reinhardtii* achieved maximal growth and pigment accumulation at the basal nitrogen (0.25 g/L $NaNO_3$) and phosphorus (0.175 g/L KH_2PO_4) concentrations, producing a dry weight of 0.061 g/15 mL, chlorophyll *a* of 15.3 µg/mL, and carotenoids of 6.42 µg/mL. Increasing nitrogen or phosphorus beyond these levels did not enhance productivity. Conversely, moderate sulfur enrichment (0.112 g/L $MgSO_4$) markedly improved metabolic activity, yielding elevated protein (461 mg/g), carbohydrate (300 mg/g), carotenoid (8.48 µg/mL), and total phenolic (9.17 mg/g) contents compared with the basal sulfur level. Collectively, these findings indicate that *C. reinhardtii* exhibits nutrient-specific responses: maintaining basal nitrogen and phosphorus levels ensures optimal growth, while sulfur enrichment enhances pigment and secondary metabolite production. This study provides a refined understanding of macronutrient regulation in microalgal metabolism and a framework for optimizing nutrient composition to boost algal growth and biochemical productivity.

Introduction

Microalgae are photosynthetic organisms that play a central role in aquatic ecosystems as primary producers, while acting as promising resources for biotechnology. They are able to fix carbon dioxide, produce oxygen and synthesize valuable components such as pigments, proteins, carbohydrates and bioactive compounds **(Norena-Caro et al., 2021)**. The diversity and adaptation of microalgae make them ideal candidates for applications in biofuels, nutraceuticals, wastewater treatment and sustainable agriculture **(Balasubramaniam et al., 2021)**.

Among microalgae, Chlamydomonas *reinhardtii* is one of the most used models. It is a unicellular green alga related to Chlorophyceae, which are characterized by simple cultivation in defined media and its small life cycle (**Merchant et al., 2007**). The availability of its well-annotated genome and several mutants have made it an indispensable system to study photosynthesis, nutritional metabolism and stress physiology (**Kong et al., 2019**). Consequently, *Chlamydomonas* is an attractive platform for both basic research and applied biotechnology.

The increase in *Chlamydomonas* and metabolic activity is greatly affected by the availability of macronutrients, especially nitrogen (N), phosphorus (P) and sulfur (S). Nitrogen is an important element for amino acids, nucleic acids and photosynthetic pigments, which directly affects cell division and biomass productivity. Phosphorus is an essential component of ATP, nucleotides and phospholipids, and plays an important role in energy transfer and metabolic regulation. Sulfur contributes to sulfur -containing amino acids (cysteine, methionine) and biosynthesis of cofactors, and contains cellular redox homeostasis (**Zheng et al., 2024**). Environmental stressors, such as nitrogen or salt limitation, often cause distinct physiological and biochemical responses in microalgae. In *Chlamydomonas reinhardtii*, for instance, nitrogen deprivation has been reported to slow down cell growth, while markedly altering the biochemical composition of the cells particularly affecting protein content and pigment accumulation (**Msanne et al., 2012**).

On the other hand, a lack of sulfur prevents photosynthetic performance and modifies carbohydrates and protein allocation (**Zheng et al., 2024**). The phosphorus boundary is reported to limit the accumulation of biomass, while multiplying cellular metabolism in favor of storing carbohydrates and other compounds (**Oz Yasar et al., 2023**).

Several studies on cyanobacteria such as *Anabaena variabilis* have shown that manipulation of N, P and S concentrations in the cultural medium greatly affects bio -dividends and biochemical composition, including chlorophyll, protein and carbohydrates **(Morsi et al., 2023).** However, similar studies on *Chlamydomonas* cultivated in Bold'**s** Basel medium (BBM) -widely used medium for green algae, remain limited. Given the difference between cyanobacteria (prokaryotic) and green algae (eukaryotic), it is important to find out how *Chlamydomonas*.

The purpose of this study is to determine the effect of different concentrations of nitrogen ($NaNO_3$), phosphorus (KH_2PO_4), and sulfur ($MgSO_4 \cdot 7H_2O$) in Bold's Basal Medium (BBM) on the growth, pigment composition (chlorophyll *a* and carotenoids), and biochemical constituents of *Chlamydomonas reinhardtii*. The novelty of this work lies in its focused evaluation of each macronutrient independently, offering updated insights into nutrient-specific influences on algal metabolism compared to previous studies.

Material and Method

Experimental Organism and Culture Conditions

The unicellular green alga *Chlamydomonas reinhardtii* was obtained from the Al-Azhar Culture Collection (ACCAZ), Cairo, Egypt. Cultures were maintained in Bold's Basal Medium (BBM), prepared following the formulation of **Bischoff and Bold (1963).**To examine the influence of macronutrient variation on algal growth and biochemical composition, the concentrations of nitrogen ($NaNO_3$), phosphorus (KH_2PO_4), and sulfur ($MgSO_4.7H_2O$) were adjusted while all other constituents were maintained constant. $NaNO_3$ was added at 5, 10 (control), 15, and 20 mL per liter, corresponding to 0.125, 0.25, 0.375, and 0.5 g/L, respectively. KH_2PO_4 was tested at 5, 10 (control), 15, and 20 mL per liter (0.087, 0.175, 0.262, and 0.35 g/L, respectively), while $MgSO_4.7H_2$ O was varied at 5, 10 (control), 15, and 20 mL per liter (0.037, 0.075, 0.112, and 0.15 g/L, respectively). The Bold's Basal Medium (BBM) was sterilized by autoclaving at 121°C for 30 minutes prior to inoculation. Each 500 mL Erlenmeyer flask contained 350 mL of sterile medium and was inoculated with 35 mL of an active *Chlamydomonas reinhardtii* pre culture having an optical density (OD_{750}) of 0.031 to ensure uniform initial cell concentration among all treatments. The cultures were incubated at 31 ± 2°C and pH 7.5 under continuous fluorescent illumination (35 $\mu E\ m^{-2}\ s^{-1}$) for 30 days. The incubation period of 30 days was chosen based on the findings of **Mortensen and Gislerod (2015).**

Growth management

The growth of *Chlamydomonas reinhardtii* was monitored every three days by measuring the optical density at 750 nm (OD_{750}) following **Chioccioli et al. (2014).** The cellular dry weight (CDW) of the algal biomass was determined according to the method described in **APHA (2005).**

Estimation of pigments

Pigments were extracted in methanol 96% (60°C, 30min) and quantified spectrophotometrically (Model UV-1800, Shimadzu, Japan). at 650, 665, and 452 nm. Chlorophyll *a* and carotenoids concentrations were calculated using **Mackinney's e (1941).**

Estimation of total soluble carbohydrates

Total carbohydrates were extracted with 1 N NaOH boiling water bath (HH-4, Jintan, China) 2 h as described by **Payne and Stewart (1988)** and quantified by the phenol–sulphuric acid method **(Kochert, 1978)** using glucose as standard. Absorbance was measured at 490 nm.

Estimation of total soluble proteins

Total soluble proteins were extracted with 1 N NaOH in a boiling water bath for 2 h **(Payne and Stewart, 1988)** and quantified by the Lowry method **(Lowry, 1951)** using bovine serum albumin as standard. Absorbance was measured at 750 nm.

Preparation of microalgae extract

Algal biomass (0.1 g) was extracted twice with 70% methanol (5 mL each) for 24 hours under constant shaking, followed by centrifugation (Model 5810R, Eppendorf, Germany) to separate the supernatant. Preliminary optimization indicated that two extraction cycles were sufficient for efficient recovery of bioactive compounds. The filtrates were combined and concentrated under reduced pressure until dryness using a rotary evaporator rotovap (RE-52A, Henan Lanphan Industry Co., Ltd., China), redissolved in 5 mL of the same solvent, and stored at 4°C until analysis **(Doman et al., 2020**). Total phenolic and flavonoid contents were determined using the same extract.

Estimation of total phenol compounds

The total phenolics was determined using the Folin Ciocalteu method **(Jindal and Singh, 1975)** with pyrogallol as standard. The absorption was measured at 650 nm.

Estimation total flavonoids

The flavonoid content was determined by the aluminum chloride colorimetric method **(Chang et al., 2002)** using quercetin as a standard. The absorbance was measured at 415 nm.

Statistical analysis

The data was expressed and analysis by one -way ANOVA using SPSS v.21. The meaning was considered by testing Tukey on $p \leq 0.05$. Values are mean ± SD (n=3). Different letters in the same column indicate significant differences at $p \leq 0.05$ (a > b > c > d) .

Results

Effect of NaNO$_3$ concentration on growth and biochemical composition of *C. reinhardtii*

Chlamydomonas reinhardtii cultured in various NaNO$_3$ concentrations (0.125, 0.25, 0.375, and 0.5 g/L) in Bolds basal medium. The ideal conditions were used to incubate each experimental flask. The growth parameters and biochemical composition were measured on the twenty-fourth day of incubation. The effect of sodium nitrate concentrations on *C. reinhardtii* growth is depicted in Fig. (1).

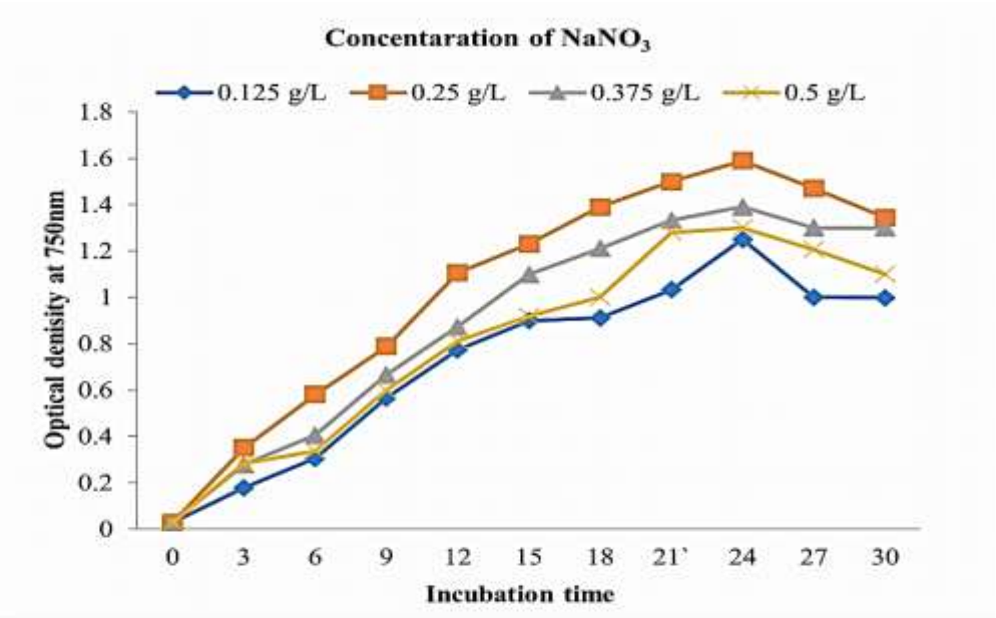

Fig. (1): Effect of NaNO$_3$ concentration on growth of *C. reinhardtii* over 30 days incubation.

As a positive control, a concentration of NaNO$_3$ in the basal medium of 0.25 g/L was found to be optimal for the growth and production of pigmented biomass. Table (1) revealed that at 0.25 g/L NaNO$_3$ the culture of *C. reinhardtii* had a maximum dry weight of 0.061 g/15 ml, carbohydrate content of 261 mg/g, and protein content of 454 mg/g. In the basal medium containing 0.25 g/L NaNO$_3$ *C. reinhardtii* synthesized the highest amounts of Chl. *a* (15.3 μg/ml) and carotenoids (6.42 μg/ml).

Figure (2) shows the total phenolic and flavonoid for various NaNO$_3$ concentrations. At 0.25 g/L of NaNO$_3$, the highest phenolic content was 2.41 mg/g and the highest flavonoid content was 1.23 mg/g.

Table (1): Effect of $NaNO_3$ concentration on growth, proteins, carbohydrates, chorophyll *a* and carotenoids contents of *C. reinhardtii.*

$NaNO_3$ g/L	D. Wt. g/15ml	Proteins mg/g d.wt	Carbohydrates mg/g d.wt	Chlorophyll *a* µg/ml	Carotenoids µg/ml
0.125	$0.023{\pm}0.0004^{c}$	321 ± 6^{c}	$166{\pm}1.7^{d}$	$8.9{\pm}0.031^{c}$	$4.29{\pm}0.011^{c}$
0.25	$0.061{\pm}0.0009^{a}$	$454{\pm}5^{a}$	$261{\pm}0.6^{a}$	$15.3{\pm}0.022^{a}$	$6.42{\pm}0.012^{a}$
0.375	$0.031{\pm}0.0008^{b}$	$421{\pm}3^{b}$	$220{\pm}0.5^{b}$	$12.1{\pm}0.024^{b}$	$5.58{\pm}0.019^{b}$
0.5	$0.024{\pm}0.0002^{c}$	$410{\pm}5^{b}$	$202{\pm}1^{c}$	$9.0{\pm}0.022^{c}$	$4.49{\pm}0.015^{c}$

Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$ (a > b > c> d).

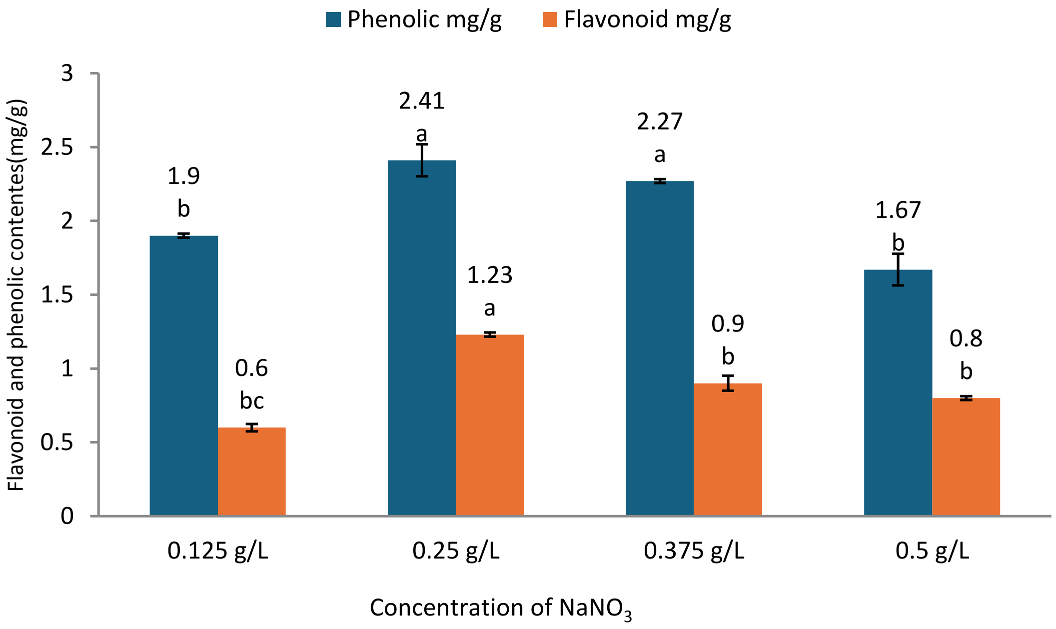

Fig. (2): Effect of $NaNO_3$ concentration on the phenolic and flavonoid contents of *C. reinhardtii.* Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$ (a > b > c).

Effect of KH_2PO_4 concentration on growth and biochemical composition of *C. reinhardtii*

Chlamydomonas reinhardtii cultured in various KH_2PO_4 concentrations (0.087, 0.175, 0.262, and 0.35 g/L) in Bolds basal medium. The ideal conditions were used to incubate each experimental flask. The growth parameters and biochemical composition were measured on the twenty-fourth day of incubation. The effect of KH_2PO_4 concentration on *C. reinhardtii* growth is depicted in Fig. (3). Concentration of KH_2PO_4 present in the basal medium 0.175 g/L, as a positive control, was proven to be the optimal for the growth and production of pigmented biomass. Table (2) revealed that the culture of *C. reinhardtii* recorded the maximum dry weight 0.052 g/15 ml, carbohydrate content 300 mg/g and protein 494 mg/g at 0.175 g/L of KH_2PO_4. From Table (2) *C. reinhardtii* synthesized maximum amounts of Chl. *a* 15.3 μg/ml and carotenoids 7.42 μg/ml in the basal medium containing 0.175 g/L of KH_2PO_4.

Total flavonoid and phenolic of the different concentration of KH_2PO_4 are presented in Fig. (4). The highest flavonoid content 2.5 mg/g and phenolic 6.77 mg/g at 0.175 g/L of KH_2PO_4.

Effect of $MgSO_4$ concentration on growth and biochemical composition of *C. reinhardtii*

Chlamydomonas reinhardtii grown in Bolds basal medium containing different concentrations of $MgSO_4$ (0.037, 0.075, 0.112 and 0.15 g/L). All the experimental flasks were incubated under the optimal conditions. On day 24 of incubation, the growth parameters and biochemical composition were quantified Fig. (5): show the effect of $MgSO_4$ concentration on growth of *C. reinhardtii.*

Optimum concentration of $MgSO_4$ for production of most pigmented biomass was found to be 0.112 g/L, as shown in Table (3), of cultured *C. reinhardtii* recorded maximum growth of 0.052 g/15 ml, carbohydrate content of 300 mg/g and protein of 461 mg/g dry weights.

In presence of the mentioned level of sulfur source, pigmentation of *C. reinhardtii* was increased; carotenoids 8.48 μg/ ml and Chl.*a* content 12 μg/ml, are shown in Table (3).

The effects of different concentrations of sulfur on total flavonoid and phenolic of *C. reinhardtii* is shown in Fig. (6). The highest flavonoid content 2.0 mg/g and phenolic was obtained 9.17 mg/g at 0.112 g/L, concentrations of $MgSO_4$.

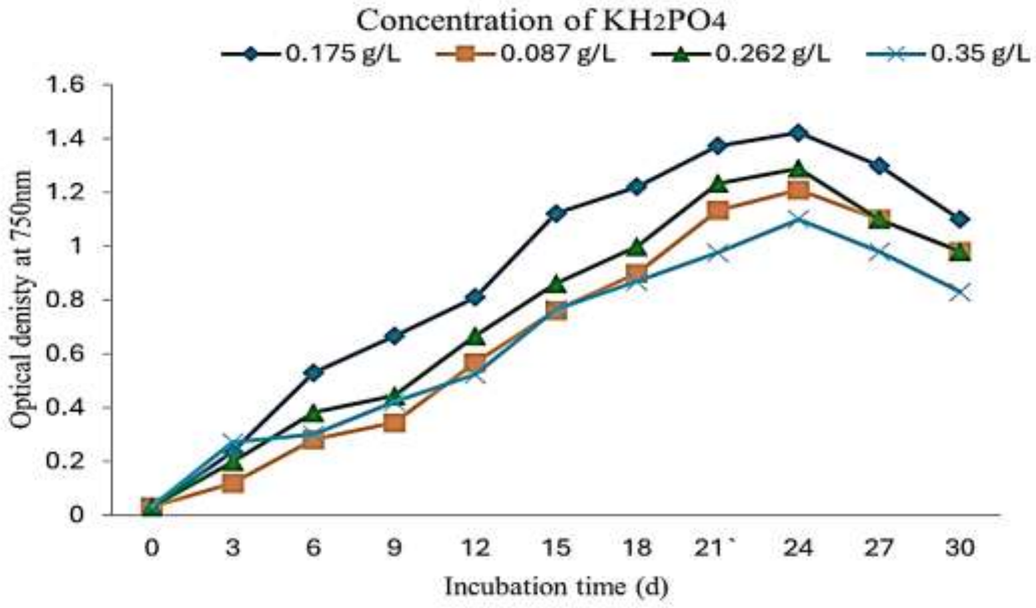

Fig. (3): Effect of KH_2PO_4 concentration on growth of *C. reinhardtii* over 30 days incubation

Table (2): Effect of KH_2PO_4 concentration on growth, proteins, carbohydrates, chlorophyll *a* and carotenoids contents of *C. reinhardtii*.

KH_2PO_4 g/L	D. Wt. g/15ml	Proteins mg/g d.wt	Carbohydrates mg/g d.wt	Chlorophyll *a* µg/ml	Carotenoids µg/ml
0.087	0.027 ± 0.0008^{c}	391 ± 6^{d}	190 ± 0.6^{d}	10.9 ± 0.127^{bc}	4.29 ± 0.011^{bc}
0.175	0.052 ± 0.0004^{a}	494 ± 7^{a}	300 ± 1.8^{a}	15.3 ± 0.331^{a}	7.42 ± 0.021^{a}
0.262	0.042 ± 0.0009^{b}	451 ± 2^{b}	250 ± 0.4^{b}	11.1 ± 0.034^{b}	5.88 ± 0.019^{b}
0.350	0.033 ± 0.0005^{c}	430 ± 5^{c}	220 ± 1^{c}	9.5 ± 0.042^{c}	4.76 ± 0.025^{b}

Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$, (a > b > c > d)

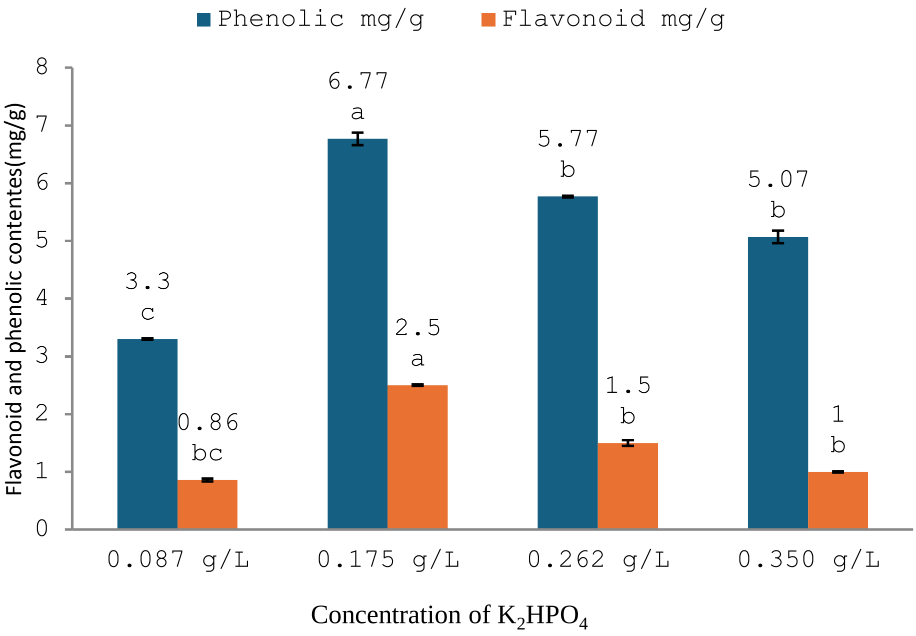

Fig. (4): Effect of KH_2PO_4 concentration on the phenolic and flavonoid contents of *C. reinhardtii*. Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$ (a > b > c).

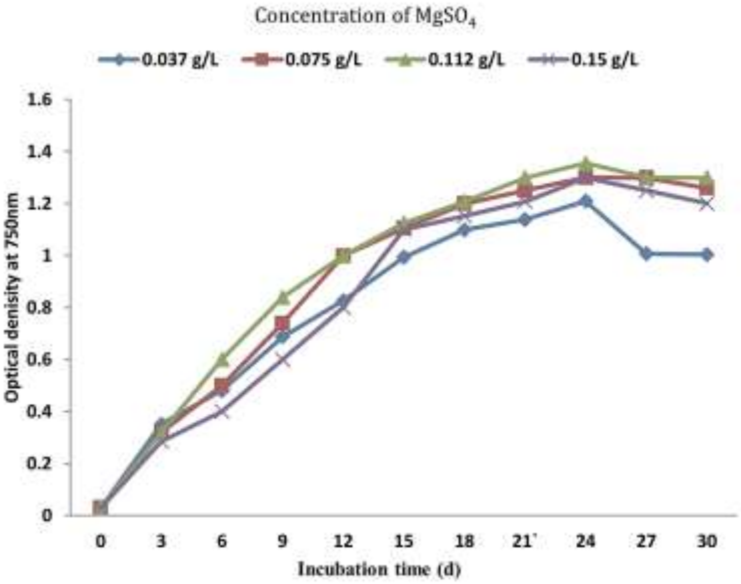

Fig. (5): Effect of $MgSO_4$ concentration on growth of *C. reinhardtii* over 30 days incubation

Table (3): Effect of $MgSO_4$ concentration on growth, proteins, carbohydrates, chlorophyll *a* and carotenoids contents of *C. reinhardtii*.

$MgSO_4$ g/L	D. Wt. g/15ml	Proteins mg/g d.wt	Carbohydrates mg/g d.wt	Chlorophyll *a* µg/ml	Carotenoids µg/ml
0.037	0.037 ± 0.0006^{c}	311 ± 4^{c}	190 ± 5^{c}	7.0 ± 0.037^{cb}	4.79 ± 0.023^{c}
0.075	0.047 ± 0.0003^{b}	344 ± 7^{c}	200 ± 7^{bc}	8.1 ± 0.031^{b}	6.42 ± 0.031^{b}
0.112	0.052 ± 0.0009^{a}	461 ± 6^{a}	300 ± 0.5^{a}	12 ± 0.239^{a}	8.48 ± 0.220^{a}
0.15	0.045 ± 0.0027^{b}	410 ± 5^{b}	210 ± 6^{b}	9.1 ± 0.182^{b}	5.66 ± 0.175^{bc}

Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$ (a > b > c).

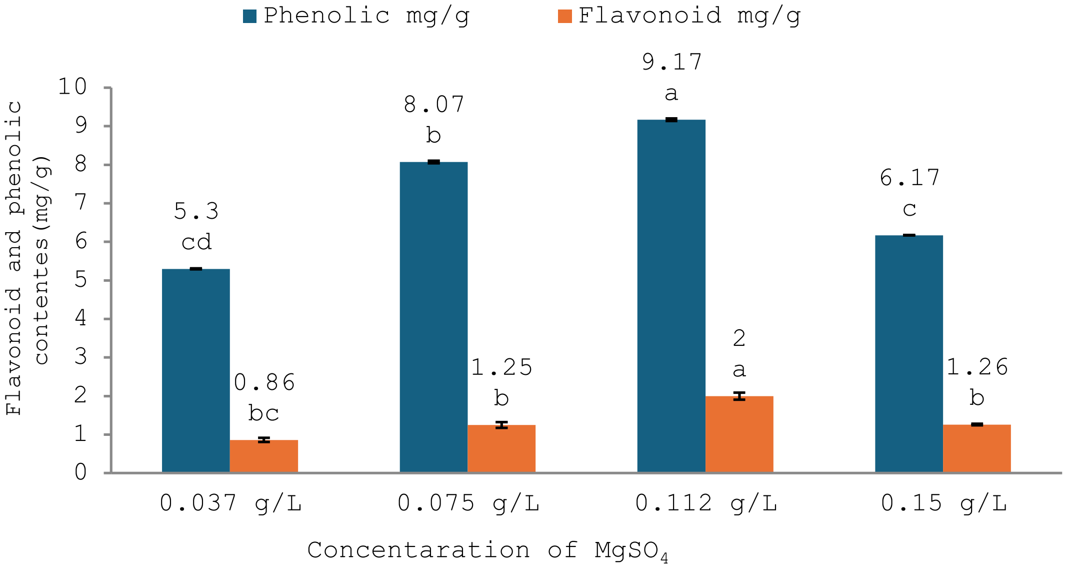

Fig. (6): Effect of $MgSO_4$ concentration on the phenolic and flavonoid contents of *C. rein hardtii*. Values are presented as mean ± SD (n = 3). Different letters within the same column indicate significant differences at $p \leq 0.05$ (a > b > c).

Discussion

In the current study, the growth of the *Chlamydomonas reinhardtii* was monitored under different macronutrient conditions, focusing on the availability of nitrogen (N), phosphorus (P) and sulfur (S). After 24 days of incubation, growth began to subside, which is in line with **El-Monem et al. (2021)**, which reported a decline in increase in batch cultures after 21 days, while **Norena-Caro et al. (2021)**, who found that optimal development had reached after about 14 days. These results indicate that while *C. reinhardtii* can maintain long -term exponential growth compared to many other microalgae, the nutritional limit eventually leads to a decline in biomass accumulation.

Nitrogen emerged as the most critical macronutrient for growth. In this study, maximum dry weight, chlorophyll a, and protein accumulation were obtained at the basal concentration of sodium nitrate (0.25 g/L) in Bold's Basal Medium (BBM). This is consistent with **Msanne et al. (2012),** who demonstrated that nitrogen is indispensable for sustaining growth and pigment accumulation in *C. reinhardtii*. Similarly, basal medium for sodium nitrate and bold was also reported to promote optimal growth and pigment accumulation in other algae treasures such as *Synchronous pevalekii* and *Tetradesmus obliquus*, which confirmed the widespread effect of this medium and nitrogen source **(El-Sheekh et al., 2025).** Nitrogen deprivation is known to induce major physiological changes, including reduced photosynthetic activity, chlorosis, and redirection of carbon fluxes toward neutral lipid accumulation. **López García de Lomana et al. (2015)** showed that nitrogen starvation rapidly triggers transcriptional and metabolic reprogramming, leading to triacylglycerol (TAG) storage as an energy reserve. These stress responses underline nitrogen's dual role: while sufficient supply promotes protein and pigment synthesis, limitation induces survival mechanisms that may be exploited for biofuel production. Since nitrogen constitutes 7–10% of algal dry weight **(El-Sheekh et al., 2021)** its availability is a key determinant of both primary productivity and secondary metabolic pathways.

Phosphorus also played an important role, where the maximum increase and pigment accumulation were seen in basal phosphate concentration (0.175g/L KH_2PO_4). This discovery corresponds to **Oz Yasar et al. (2023)** who demonstrated that phosphorus supplementation around 5 mg/L optimized growth and nutrient recovery efficiency in *C. reinhardtii*. According to reports, *A. variabilis* biochemical composition, especially its proteins and carbohydrates, and pigment production both significantly increased when phosphorus was added at a concentration of 0.06 g/L **(Morsi et al, 2023).**

At the molecular level, phosphorus is central to energy metabolism as an important component of ATP, nucleic acid and phospholipids. **Zheng et al. (2024)** reported that phosphor homeostasis is necessary to maintain metabolic balance, while the transcription factor PSR1's aversion inspires luxury uptake and storage of phosphorus. Both the defect and the oversight were harmful, indicating that phosphorus balance is important. Reduced causes impaired nucleic acid synthesis and photosynthetic efficiency decreases, while ove dose can trigger metabolic imbalance and stress reactions.

Sulfur performed a separate pattern compared to nitrogen and phosphorus. In this study, development and pigment accumulation were not increased in basal concentration, but during the revised sulfur supply. In the present study, the optimal concentration of $MgSO_4$ for producing the most pigmented biomass and favorable biochemical composition was found to be 0.112 g/L. This observation corresponds to **Zheng et al. (2024),** which stated that sulfur deficiency weakened photosynthetic capacity and changed carbon metabolism in *C. reinhardi*. **Gonzalez-Balester et al. (2010)** in the same way, showed that the availability of sulfur controls photosynthetic performance and claims stress.

Sulfur plays multiple roles in algal physiology. It is an essential component of amino acids (cysteine and methionine), coenzymes, and redox-active proteins, and contributes to electron transport and antioxidant defense. **Pollock et al. (2005)** demonstrated that sulfur deprivation in *Chlamydomonas reinhardtii* triggers the activation of the sulfate assimilation system, which is stringently regulated at the transcriptional level. This regulatory response enables the alga to adapt its metabolism under sulfur-limited conditions. In addition, sulfur Hunger has been linked to an increase in alternative oxidations (AOX1) activity, which reflects changes in energy metabolism and redox balance **(Zalutskaya et al., 2018**). Unlike nitrogen and phosphorus, which supports an increase in basal concentrations, sulfur requires accurately modulation based on the target of agriculture: induction of metals related to high biomass versus stress.

From a biotechnological perspective, the results highlight the importance of optimizing nutrient availability to maximize both biomass yield and biochemical productivity. Nitrogen and phosphorus play crucial roles in maintaining the effective growth of *Chlamydomonas reinhardtii* under basal BBM concentrations, whereas sulfur requires more precise regulation depending on the desired outcome whether it is maximizing biomass accumulation or enhancing stress-induced metabolite production. Such understanding is fundamental for developing large-scale cultivation strategies aimed at producing high-value algal compounds such as pigments, proteins, and other bioactive metabolites **(Balasubramaniam et al., 2021).**

Conclusion

The results of this study show that nitrogen (N) and phosphorus (P) in their basal concentrations in Bold's basal medium are optimal for promoting algae growth and biochemical productivity, while sulfur (S) required admission beyond standard levels to increase performance. These findings highlight the important role of the availability of balanced nutrients in regulating algae physiology, corresponding to previous studies on both green algae and cyanobacteria. Macronutrient concentration adaptation, especially sulfur, can therefore serve as an effective strategy to improve biomass yield and pigment composition in *Chlamydomonas reinhardtii* and other algae species, with promising implications for biotechnological applications.

References

APHA, A.-A. P. H. A. et al. (2005). Standard method for examination of water and wastewater, 21st edit. AWWA, WPCF, Washington DC. ISBN:9780875530475.

Balasubramaniam, V., Gunasegavan, R. D.-N., Mustar, S., Lee, J. C., and Mohd Noh, M. F. (2021). Isolation of industrially important bioactive compounds from microalgae.

Molecules, *26*(4), 943. DOI.org/10.3390/molecules26040943.

Bischoff, H.W and Bold, H.C. (1963). Phycological Studies IV. Some Soil Algae from Enchanted Rock and Related Algal Species, pp. 1-95. Univ. Texas Publ, 6318p.

Chang, C.-C., Yang, M.-H., Wen, H.-M., and Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*, 10(3). DOI:10.38212/2224-6614.2748.

Chioccioli, M., Hankamer, B., and Ross, I. L. (2014). Flow cytometry pulse width data enables rapid and sensitive estimation of biomass dry weight in the microalgae *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. PloS one, 9(5): e97269.
DOI: 10.1371/journal.pone.0097269.

Doman, K. M., El-Monem, A., Ahmed, M., and Gharieb, M. M. (2020). Effect of ultraviolet-B radiation on the biochemical composition and antibacterial activity of *Spirulina platensis*. *J. Basic Environ. Sci*, 7(1), 72-78.
DOI: 10.21608/JBES.2020.371149.

El-Monem, A., Ahmed, M., Gharieb, M. M., and Doman, K. M. (2021). Chemical constituents of zarrouk's medium affect growth, pigments and metabolites productions of *Spirulina platensis*. *Egypt. J. Bot*, 61(3):681–691. DOI:10.21608/ejbo.2019.6052.1245.

El-Sheekh, M. M., Morsi, H., and Hassan, L. (2021). Growth enhancement of *Spirulina platensis* through optimization of media and nitrogen sources. *Egypt. J. Bot*, 61(1):61–69. DOI:10.21608/ejbo.2020.27927.1487.

El-Sheekh, M. M., Shaaban, M. T., Ali, A. G., Saber, A. A. and Morsi, H. H.(2025). Evaluating the growth performance of *Synechocystis pevalekii* and *Tetradesmus obliquus* under different culture conditions. *Egypt.J. Phycol*, 26(1),12-20. DOI:10.21608/egyjs.2024.325808.1035.

Gonzalez-Ballester, D., Casero, D., Cokus, S., Pellegrini, M., Merchant, S. S., and Grossman, A. R. (2010). RNA-Seq analysis of sulfur-deprived Chlamydomonas cells reveals aspects of acclimation critical for cell survival. *Plant Cell,* 22(6), 2058–2084. DOI: doi.org/10.1105/tpc.109.071167.

Jindal, K. K. and Singh, R. N. (1975). Phenolic content in male and female *Carica papaya*: a possible physiological marker for sex identification of vegetative seedlings. *Physiol. Plant*, 33(1):104–107. ID:86754108.

Kochert, G. (1978). Carbohydrate determination by the phenol-sulfuric acid method. *Handb. Phycol. Methods.*, pages 95–97. ID:99274484.

Kong, F., Yamaoka, Y., Ohama, T., Lee, Y., and Li-Beisson, Y. (2019). Molecular genetic tools and emerging synthetic biology strategies to increase cellular oil content in *Chlamydomonas reinhardtii*. *Plant Cell Physiol*, *60*(6), 1184-1196.
DOI.org/10.1093/pcp/pcz022.

López García de Lomana, A., Schäuble, S., Valenzuela, J., Imam, S., Carter, W., Bilgin, D. D., ... and Baliga, N. S. (2015). Transcriptional program for nitrogen starvation-induced lipid accumulation in *Chlamydomonas reinhardtii*. *Biotechnol. Biofuels*, *8*(1),207.
DOI.org/10.1186/s13068-015-0391-z.

Lowry, O., Rosebrough, N., Farr, A. L., and Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem*, 193(1):265–275.

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem*,140(2):315–322. DOI:10.1016/S0021-9258(18)51320-X.

Merchant, S. S., Prochnik, S. E., Vallon, O., Harris, E. H., Karpowicz, S. J., Witman, G. B., … and Grossman, A. R. (2007). The *Chlamydomonas genome* reveals the evolution of key animal and plant functions. Science, 318(5848), 245–250.DOI. 10.1126/science.1143609.

Morsi, H. H., Gharieb, M. M., Abd El-Monem, A. M., and Khalil, D. M. (2023). The influence of nutrient manipulation on growth and cultivation constituents of *Anabaena variabilis*. *Egypt. J. Phycol*, 24, 55–77. DOI :10.21608/egyjs.2023.218813.1020.

Mortensen, L. M., and Gislerød, H. R. (2015). The growth of *Chlamydomonas reinhardtii* as influenced by high CO2 and low O2 in flue gas from a silicomanganese smelter. *J. Appl. Phycol*, 27(2), 633-638.

Msanne, J., Xu, D., Konda, A. R., Casas-Mollano, J. A., Awada, T., Cahoon, E. B., and Cerutti, H. (2012). Metabolic and gene expression changes triggered by nitrogen deprivation in the green alga *Chlamydomonas reinhardtii*. *Plant Cell*, *24*(2), 524–545. DOI.org/10.1016/j.phytochem.2011.12.007.

Norena-Caro, D. A., Malone, T. M., and Benton, M. G. (2021). Nitrogen sources and iron availability affect pigment biosynthesis and nutrient consumption in *Anabaena* sp. UTEX 2576. *Microorganisms*, 9(2):431. DOI:10.3390/microorganisms9020431.

Oz Yasar, C., Fletcher, L., & Camargo-Valero, M. A. (2023). Effect of macronutrients (carbon, nitrogen, and phosphorus) on the growth of *Chlamydomonas reinhardtii* and nutrient recovery under different trophic conditions. *Environ. Sci. Pollut. Res*, *30*(51),111369-111381. DOI.org/10.1007/s11356-023-30231-2.

Payne, J. K. and Stewart, J. R. (1988). The chemical composition of the thallus wall of *Characiosipho rivularis* (Characiosiphonaceae, Chlorophyta). *Phycologia*, 27(1):43–49. DOi:10.2216/i0031-8884-27-1-43.1.

Pollock, S. V., Pootakham, W., Shibagaki, N., Moseley, J. L., and Grossman, A. R. (2005). *Insights into the acclimation of Chlamydomonas reinhardtii to sulfur deprivation*. *Photosynth. Res*, 86, 223–236. DOI: org/10.1007/s11120-005-4048-9.

Zalutskaya, Z., Filina, V., Ostroukhova, M., and Ermilova, E. (2018). Regulation of alternative oxidase 1 in *Chlamydomonas reinhardtii* during sulfur starvation. *Eur. J. Protistol*, 63, 26-33. DOI.org/10.1016/j.ejop.2018.01.001.

Zheng, S., Sun, S., Zou, S., Song, J., Hua, L., Chen, H., and Wang, Q. (2024). Effects of culture temperature and light regimes on biomass and lipid accumulation of *Chlamydomonas reinhardtii* under carbon-rich and nitrogen-limited conditions. Bioresour. Technol, 399, 130613. DOI .org/10.1016/j.biortech.2024.130613.

تأثير توفر العناصر الغذائية في وسط Bold’s Basal على أداء النمو والإنتاجية الكيميائية الحيوية للطحلب *Chlamydomonas reinhardtii*

د/ خليل محمد دومان[1]، أ.د/ محمد مدحت غريب[1]، أ. د/ أحمد محمد عبد المنعم [2]، أ.د/ هناء حسنين مرسي[1]

[1]قسم النبات وعلم الأحياء الدقيقة، كلية العلوم، جامعة المنوفية، المنوفية، مصر
[2]قسم المياه العذبة والبحيرات، المعهد القومي لعلوم البحار والمصايد، القاهرة، مصر

يُعد الطحلب الأخضر وحيد الخلية *Chlamydomonas reinhardtii* نموذجًا بحثيًا لدراسة التكيفات الفسيولوجية والكيميائية الحيوية للطحالب الدقيقة تجاه تغير توافر العناصر الغذائية. تهدف هذه الدراسة إلى تحليل تأثير تعديل المغذيات الكبرى النيتروجين، الفوسفور، والكبريت على النمو، وتخليق الصبغات، والتركيب الكيميائي الحيوي للطحلب *C. reinhardtii* المزروع في وسط Bold’s Basal تحت ظروف معقمة ونقية. تمت متابعة نمو الطحلب لمدة ٣٠ يومًا، وتم تحديد تراكم الكتلة الحيوية، والصبغات (الكلوروفيل a والكاروتينات)، بالإضافة إلى المكونات الكيميائية الحيوية (البروتينات، الكربوهيدرات، الفينولات، والفلافونويدات). أظهرت النتائج أن الطحلب *C. reinhardtii* حقق أعلى معدل نمو وتراكم للأصباغ عند التركيزات الأساسية من النيتروجين 0.25 جرام/لتر $NaNO_3$ والفوسفور 0.175 جرام/لتر $KH_2 PO_4$، حيث بلغ الوزن الجاف 0.061جرام/١٥ مل، وتركيز الكلوروفيل a بمقدار ١٥,٣ ميكروجرام/مل، والكاروتينات بمقدار ٦,٤٢ ميكروجرام/مل.لم يُؤدِّ رفع تركيز النيتروجين أو الفوسفور عن هذه المستويات إلى زيادة في الإنتاجية. وعلى النقيض، فإن الزيادة المعتدل بالكبريت 0.112 جرام/لتر $MgSO_4$ حسّن النشاط الأيضي بشكل ملحوظ، مما أدى إلى زيادة محتوى البروتين (٤٦١ ملي جرام/جرام)، والكربوهيدرات (٣٠٠ ملي جرام/جرام)، والكاروتينات (٨,٤٨ ميكروجرام/مل)، والفينولات الكلية (٩,١٧ ملي جرام/جرام) مقارنة بمستوى تركيز الكبريت الأساسي. بشكل عام، تشير هذه النتائج إلى أن *C. reinhardtii* يُظهر استجابات نوعية تجاه المغذيات؛ إذ إن الحفاظ على التركيزات الأساسية من النيتروجين والفوسفور يضمن النمو الأمثل، في حين أن زيادة بالكبريت يعزز بشكل فعّال من تخليق الأصباغ والمركبات الثانوية. وتُسهم هذه الدراسة في تقديم فهم أكثر دقة لتنظيم المغذيات الكبرى في أيض الطحالب الدقيقة، وتوفر إطاراً لتصميم تركيبات غذائية مثلى في التطبيقات البيوتكنولوجية للطحالب.