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Research Article

EFFECT OF CALCIUM NITRATE ON CHLOROPHYLL-BASED BIOTRANSDUCER CHARACTERIZATION OF *Arthrospira platensis* Gomont

Mulia Safrida Sari [1]*, Rachmad Almi Putra[2], Yonadiah Dwitya[1], M. Ari Fahril[2]

[1]Department of Biology, Faculty of Science and Technology, Universitas Samudra, Langsa 24416, Indonesia;

[2]Department of Geophysic, Faculty of Science and Technology, Universitas Samudra, Langsa 24416, Indonesia.

ARTICLE HIGLIGHTS

- Calcium nitrate is utilized to enhance the growth and chlorophyll quality of *Arthrospira platensis* Gomont, aiming to maximize its potential as a biotransducer molecule.
- Biomass productivity was monitored every three days during a 15-day cultivation period, with specific attention to biomass accumulation and specific growth rates during the stationary phase.
- Chlorophyll concentrations (chlorophyll a, chlorophyll b, and total chlorophyll) were measured using a UV-Vis spectrophotometer at wavelengths of 648 nm and 664 nm.
- Fourier-transform infrared spectroscopy (FTIR) was performed on chlorophyll extracts to assess molecular binding capacity, reinforcing *Arthrospira platensis* Gomont's potential as a biotransducer.
- A concentration of 4.5 g/L of calcium nitrate, in combination with 35 ppt salinity, was found to be optimal for enhancing chlorophyll production during cultivation.

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***Corresponding author, e-mail:**
muliasari03@unsam.ac.id

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ABSTRACT

This study aimed to investigate the potential of calcium nitrate as a specific nutrient capable of enhancing chlorophyll content and optimizing biotransducer characterization in *Arthrospira platensis* Gomont. A two-factor Completely Randomized Design (CRD) was employed, consisting of 12 treatments with 3 replications. Each experimental group was subjected to varying salinity levels: 15 ppt (S15), 25 ppt (S25), and 35 ppt (S35). In the treatment groups, calcium nitrate was applied at concentrations of 2.5 g/L (P1), 3.5 g/L (P2), and 4.5 g/L (P3). Biomass accumulation and specific growth rate were monitored, and data were collected throughout the experimental period. At the conclusion of the treatment period, chlorophyll was extracted, and its concentration was measured using UV-Vis spectrophotometry and FTIR analysis. The addition of calcium nitrate at 4.5 g/L combined with 35 ppt salinity increased the average biomass productivity over 15 days to 5.1 g/L, with a specific growth rate in the stationary phase of 0.12 per day. Supplementation with 4.5 g/L calcium nitrate in 35 ppt salinity increased total chlorophyll concentration to 70.15 µg/mL, supporting its potential as a supplementary nutrient for enhancing biotransducer properties through five key functional groups associated with the stability and binding affinity of analyte molecules in SPR applications.

Keywords: *Arthrospira platensis* Gomont, biotransducer, calcium nitrate, chlorophyll, FTIR

INTRODUCTION

Surface Plasmon Resonance (SPR)-based biosensors have been widely applied in biomedical fields, particularly for the real-time detection of biological analytes. SPR is an optical technique in which changes in the intensity of reflected light at a specific resonance angle on a metal-modified surface are measured. This surface functions as a receptor, with variations in the refractive index near the interface occurring as a result of analyte binding. The analytes may include biomarkers such as DNA, proteins, enzymes, and other biological macromolecules.

In recent years, SPR biosensors have been developed as efficient, cost-effective, and user-friendly alternatives for biomarker detection (Écija-Arenas et al. 2021; D'Agata et al. 2024). However, conventional SPR systems have continued to face limitations, including suboptimal sensitivity and unstable performance, primarily due to inadequacies in the biointerface sensing layer. These limitations highlight the need for incorporating additional external supporting layers beyond the traditional gold (Au) film and prism to enhance signal transduction and reduce energy loss across the optical interface (Syed Nor *et al.* 2022). Consequently, improvement of the biointerface layer has been identified as a critical priority to advance the performance and reliability of SPR biosensors

In efforts to enhance the performance of SPR biosensors, the potential of chlorophyll as a biotransducer has been identified as an attractive alternative. Chlorophyll, present in large quantities in microalgae such as spirulina (*Arthrospira platensis* Gomont), possesses notable optical properties and molecular interaction capabilities, along with high tolerance and stability under varying environmental conditions, which can improve the sensitivity and specificity of detection systems (Mandal & Dutta 2020). When incorporated as the primary component of the biointerface sensing layer, chlorophyll is expected to enhance the performance of SPR biosensors in detecting relevant parameters.

Moreover, the selection of calcium nitrate as an additional nutrient during the cultivation phase of *Arthrospira platensis* Gomont is considered a crucial factor in enhancing chlorophyll production for use as a biotransducer molecule. Calcium nitrate has been reported to influence the growth and metabolism of *A. platensis*, thereby contributing to increased chlorophyll production (Fakhri *et al.* 2020). However, the effects of nitrate have been shown to vary among species. Rani et al. (2021) reported that increasing nitrate concentrations reduced pigment content in *Chlamydomonas* and *Chlorella* strains.

In contrast, *Chlorella vulgaris* achieved higher biomass at elevated nitrate levels, with improved uptake rates of up to 1,798 mg/L and tolerance exceeding 6,014 mg/L (Jeanfils et al. 1993). Nitrate supplementation has also been observed to improve microalgal growth, with biomass concentrations reaching up to 3,188 mg/L (Vo *et al.* 2022). Therefore, the addition of calcium nitrate during cultivation may serve as an effective strategy to enhance chlorophyll production, which, in turn, could improve the sensitivity of chlorophyll-based biotransducers in SPR biosensors.

Existing research has largely focused on general microalgal growth, with limited attention given to the targeted enhancement of chlorophyll-based biotransducer properties for biosensing applications. In this study, this gap was addressed through an integrated approach combining physiological optimization under controlled environmental conditions with evaluation of biosensor applicability. Using *Arthrospira platensis* Gomont as a model organism, the effects of nutrient and salinity modulation on the performance of chlorophyll-based SPR biosensors for metabolic biomarker detection were investigated.

MATERIALS AND METHODS

Material

The initial *Arthrospira platensis* Gomont inoculum was obtained from a pure culture cultivated by commercial farmers in Tangerang City, Jakarta, Indonesia.

Research Design

A Completely Randomized Design (CRD) with two factors was employed, comprising 12 treatments with 3 replications, resulting in a total of 36 culture groups of *Arthrospira platensis* Gomont. These groups included a negative control and three treatment sets, each subjected to different salinity levels: 15 ppt (S15), 25 ppt (S25), and 35 ppt (S35).

The negative control was maintained without additional nutrients, whereas the treatment groups were supplemented with calcium nitrate at

concentrations of 2.5 g/L (P1), 3.5 g/L (P2), and 4.5 g/L (P3) under the same salinity conditions. Calcium nitrate concentrations were adapted from Fakhri *et al.* (2020) with modifications. The experiment was conducted over a 15-day period, with cultures maintained in aquariums under 4,000 lux lighting (24:0 light cycle) and continuous aeration to ensure uniform nutrient distribution and to prevent sedimentation (Fakhri *et al.* 2020).

Preparation of Culture Media

The culture medium was prepared using sterilized distilled water, treated with 1 mL/L chlorine for 24 hours, and subsequently treated with 1 mL/L sodium thiosulfate for dechlorination. This process was performed to ensure the removal of chlorine residues prior to use in microalgae cultivation (Fakhri *et al.* 2020).

Inoculation, Cultivation, and Treatment Group Assignment

Inoculation Phase

During the inoculation phase, the initial *Arthrospira platensis* Gomont inoculum was expanded to obtain the required quantity for cultivation. A volume of 100 mL of the initial inoculum was added to each liter of culture medium. The inoculum was then incubated for 7 days before initiation of the cultivation phase.

Cultivation Phase and Treatment Group Assignment

Following inoculation, 100 mL of *Arthrospira platensis* Gomont culture was transferred into 2 L of sterile medium, resulting in a 1 : 20 ratio. Cultures were maintained at salinity levels of 15 ppt (S15), 25 ppt (S25), and 35 ppt (S35). Treatment groups were supplemented with calcium nitrate at concentrations of 2.5 g/L (P1), 3.5 g/L (P2), and 4.5 g/L (P3), while the negative control received no nutrient addition. Treatments were applied for 15 days, during which biomass productivity and specific growth rates were monitored and recorded (Fakhri *et al.* 2020).

Biomass Productivity Assessment

Observations were conducted over a 15-day period, from day 0 to day 15, with measurements taken every three days. Biomass productivity was determined by collecting a 25 mL microalgae sample. The sample was subsequently filtered and dried in an oven at 105 °C for 2 minutes before being weighed. The results were calculated using the following formula (Fakhri et al. 2020):

$$Arthrospira\ platensis\ Gomont\ biomass\ \left(g/L\right) = \frac{(B-A)}{Sample\ volume} \times 1000\ L\ of\ media$$

where:

B = Weight of the dried sample and filter paper after being oven-dried at 105 °C for 2 minutes (g);

A = Weight of the filter paper after being oven-dried at 105 °C for 2 minutes (g);

Sample volume = Volume of the sample solution taken from the cultivation medium (mL).

Biomass Productivity Assessment and Specific Growth Rate during the Stationary Phase

Upon reaching the stationary phase, which is characterized by consistent and stable biomass growth as well as constant medium turbidity, typically occurring between days 10 to 15, biomass productivity and specific growth rate were measured using the following formula (Fakhri *et al.* 2020):

$$\mu = \frac{(ln(X2) - (ln(X1))}{(t2-t1)}$$

where:

μ = Specific growth rate per unit of time (per day);

X_2 = Biomass measured at the final time point (g/L);

X_1 = Biomass measured at the initial time point (g/L)'

t_2 = Final time (days);

t_1 = Initial time (days).

Chlorophyll Extraction

On day 15, 250 mL of microalgae samples were collected from each group and filtered using Whatman No. 1 filter paper with a pore size of 11 µm. The biomass was air-dried, weighed (~1 g), and dissolved in 10 mL of acetone. The solution was transferred to 10 mL bottles, wrapped in aluminum foil to prevent light exposure, and centrifuged at 3,000 rpm for 20 minutes. The supernatant was collected, placed in cuvettes, and its absorbance was measured at 648 nm and 664 nm using a UV-Vis spectrophotometer (Aryono et al. 2022). The concentration of pigments in the extracts was determined using the following equations (Lichtenthaler & Wellburn 1983):

Chlorophyll a concentration (µg/mL): Ca = (13.36 × A664) - (5.19 × A648)

Chlorophyll b concentration (µg/mL): Cb = (27.43×A648) - (8.12×A664)

Total Chlorophyll (µg/mL) = Ca + Cb

FTIR (Fourier Transform Infrared Spectroscopy) Analysis

FTIR analysis was conducted using a Shimadzu IR Prestige 21/FTIR 8400 instrument, which was warmed up for at least 30 minutes to stabilize performance. After powering on the detector, the IR Solution software was launched for data acquisition. Approximately 0.5 mL of the liquid sample, free of water or interfering solvents, was applied onto the ATR crystal plate and evenly distributed across its surface. The ATR assembly was then placed into the sample holder. A background spectrum was first obtained to eliminate external interference and ensure accurate sample measurement. Subsequently, the spectrum of the liquid sample was recorded, capturing absorption peaks characteristic of the sample's molecular structure. The resulting spectrum was analyzed using the IR Solution software to identify key absorption bands.

Data Analysis

Biomass increase, specific growth rate, and chlorophyll concentration were statistically analyzed using two-factor ANOVA in SPSS 16 software, followed by Duncan's multiple range test at a significance level of 5% (P = 0.05).

RESULTS AND DISCUSSION

Biomass Productivity of *Arthrospira platensis* Gomont during 15-day of the Study

Biomass productivity of *Arthrospira platensis* Gomont was monitored every three days over a 15-day period under various nutritional and salinity treatments (Figs. 1a & 1b). At the onset (Day 0), all experimental groups exhibited similarly low productivity, indicating uniform initial conditions. Treatments supplemented with medium to high concentrations of calcium nitrate (P2 and P3) demonstrated a progressive increase in biomass, reaching a peak on Day 15. This trend suggests that elevated nitrate availability supports cellular growth, consistent with previous findings linking nitrogen availability to increased biomass in Chlorella sp. (Vo *et al.* 2022).

Unexpectedly, considerable biomass accumulation was also observed in the control group (KN), which received no additional nutrients, particularly on Days 12 and 15 of the cultivation period. This phenomenon may be attributed to adaptive metabolic strategies, such as efficient utilization of residual nutrients or internal nitrogen recycling mechanisms in nutrient-limited microalgal cultures (Bezerra et al. 2020; Villaró et al. 2023).

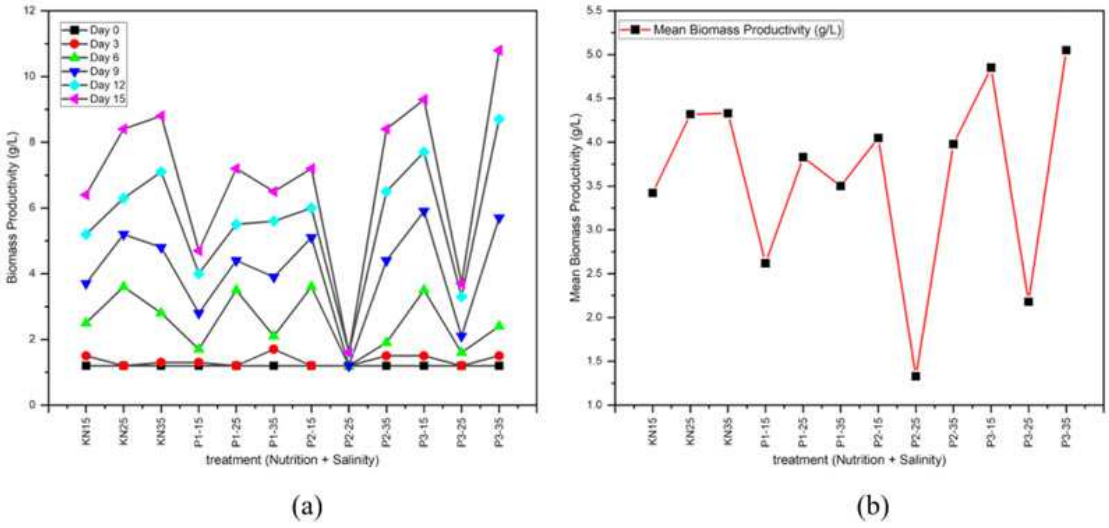


Figure 1 Biomass productivity of *Arthrospira platensis* Gomont during the 15-day study

Notes: a) Biomass productivity was measured five times over 15 days of study; b) Mean biomass productivity.

Biomass productivity was enhanced under higher salinity conditions (35 ppt) compared to lower salinity treatments (15 and 25 ppt), particularly during the final observation period. The combination of 35 ppt salinity and 4.5 g/L calcium nitrate (P3) yielded the highest mean biomass productivity at 5.1 g/L. These findings are consistent with previous studies demonstrating that moderate salinity stress stimulates osmoregulatory mechanisms in microalgae, promoting the synthesis of compatible solutes and enhancing ionic regulation, which collectively support increased biomass production (Bezerra *et al.* 2020; Villaró *et al.* 2023). Furthermore, calcium ions introduced through calcium nitrate are believed to contribute to membrane stabilization and activation of signaling pathways involved in stress tolerance, thereby further improving growth under high-salinity conditions (White & Broadley 2003; Haider et al. 2024).

Despite the observed trends, two-factor ANOVA indicated that the effects of calcium nitrate supplementation ($F = 0.611$; $P = 0.611$), salinity ($F = 1.579$; $P = 0.215$), and their interaction ($F = 1.191$; $P = 0.324$) on biomass productivity were not statistically significant at the 0.05 level (Table 1). The adjusted R^2 value (0.016) further suggested that variability in biomass productivity was only marginally explained by the tested variables. These findings may have been influenced by intrinsic biological variation or the relatively short duration of the experimental period. It is important to emphasize that the absence of statistical significance does not necessarily negate the biological relevance of the observed trends, particularly in microalgal systems where subtle physiological responses may not be captured by conventional significance thresholds (Quinn & Keough 2002).

Although calcium nitrate supplementation and elevated salinity did not produce statistically significant differences in biomass productivity, the consistent increase observed in treatments P2 and P3, particularly under high salinity, suggests a potential synergistic effect of nitrate availability and osmotic conditioning in enhancing the growth performance of *Arthrospira platensis* Gomont. Further investigations involving extended cultivation periods, increased replication, and refined physiological measurements are warranted to elucidate the underlying mechanisms and validate these observed trends.

Biomass Productivity of *Arthrospira platensis* Gomont during the Stationary Phase

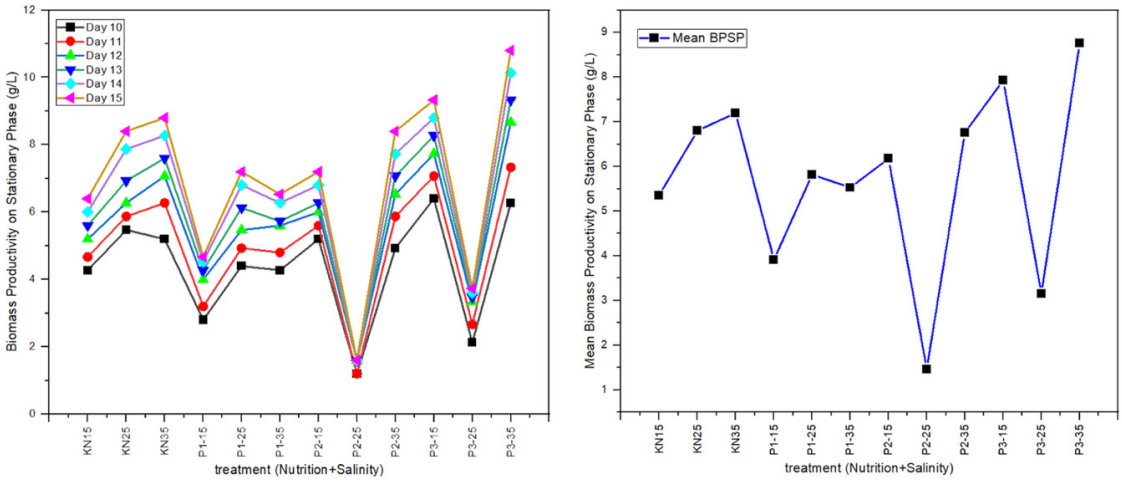
The stationary phase typically reflects a condition in which cellular growth slows due to nutrient limitation, waste accumulation, or other environmental constraints (Richmond 2004). Despite these limitations, sustained or even enhanced biomass productivity was observed in *Arthrospira platensis* Gomont during the stationary phase (days 10 - 15), particularly under specific combinations of calcium nitrate supplementation and salinity levels (Figs. 2a & 2b).

Arthrospira platensis Gomont exhibited a substantial increase in biomass productivity, reaching up to 10.8 g/L with an average productivity of 8.8 g/L, particularly in cultures treated with 4.5 g/L calcium nitrate under 35 ppt salinity. This suggests that specific physiological mechanisms supported sustained metabolic activity despite potentially growth-limiting conditions. The enhanced growth is likely attributed to the dual role of calcium nitrate: nitrate (NO_3^-) continues to support nitrogen

Table 1 Two-factorial ANOVA analysis on mean biomass productivity of *Arthrospira platensis* Gomont during the 15-day study

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	79.618[a]	11	7.238	1.103	0.375
Intercept	944.676	1	944.676	143.974	0.000
Treatment	12.021	3	10.362	0.611	0.611
Salinity	20.724	2	7.812	1.579	0.215
Treatment*Salinity	46.872	6	6.561	1.191	0.324
Error	393.687	60			
Total	1,417.980	72			
Corrected total	473.304	71			

Note: a: R2 = .168 (Adjusted R2 = .016).



(a) (b)

Figure 2 Biomass productivity of *Arthrospira platensis* Gomont during the stationary phase

Notes: a) Biomass productivity; b) Mean biomass productivity.

metabolism and chlorophyll synthesis, while calcium (Ca^{2+}) contributes to stabilization of thylakoid membranes, regulation of enzymatic activity, and facilitation of photosynthetic protection mechanisms under stress (Cui *et al.* 2024; The *et al.* 2021; Hachiya & Sakakibara, 2017; Hochmal *et al.* 2015; White & Broadley 2003; Haider *et al.* 2024).

However, this stimulatory effect was not consistent across all treatment groups. Interestingly, the KN group, which received no additional nutrients, achieved comparably high productivity at 35 ppt salinity, highlighting the remarkable adaptive capacity of *Arthrospira platensis* Gomont. Elevated salinity may act as a mild stressor, triggering metabolic compensation mechanisms that enable continued biomass accumulation despite nutrient limitations (Bezerra *et al.* 2020; Villaró *et al.* 2023).

These findings underscore the interactive effects of nutrient concentration and salinity, where physiological outcomes are nonlinear and dependent on threshold levels and organismal adaptive responses. While high calcium nitrate concentrations combined with elevated salinity promoted growth, intermediate nutrient levels or suboptimal salinity conditions did not elicit the same effect.

In some cases, the absence of supplementation under high salinity conditions paradoxically stimulated biomass accumulation through internal regulatory mechanisms.

Specific Growth Rate of *Arthrospira platensis* Gomont during the Stationary Phase

The specific growth rate for each treatment group was measured after *Arthrospira platensis* Gomont entered the stationary phase, as indicated by stable turbidity between Days 10 and 11. This measurement was conducted to evaluate the culture's capacity for biomass accumulation following the onset of nutritional stress, which results from intensified intra-population competition and depletion of essential nutrients (Richmond 2004). The specific growth rate results for each group are presented in Figure 3.

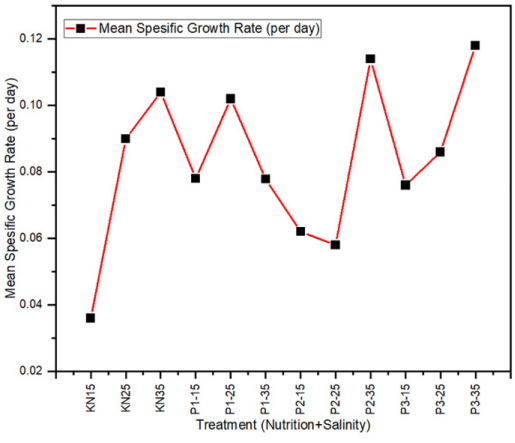


Figure 3 Mean specific growth rate of *Arthrospira platensis* Gomont during the stationary phase

In this study, cultures maintained at 35 ppt salinity demonstrated an average specific growth rate of 0.104 per day. When calcium nitrate was

supplemented at concentrations of 3.5 g/L and 4.5 g/L, the specific growth rate increased to 0.11 and 0.12 per day, respectively. The highest growth rate was recorded in the P3-35 group, followed by the P2-35 group. These findings suggest that under elevated salinity, appropriate nitrate supplementation may enhance nitrogen assimilation pathways, support chlorophyll synthesis, and maintain cell division even during the later stages of cultivation (Cui et al. 2024; Vo *et al.* 2022).

However, this effect was not consistent across all treatment combinations. Groups receiving calcium nitrate at 2.5 g/L (P1), or those cultivated at lower salinities (15 ppt and 25 ppt), exhibited lower specific growth rates. This inconsistency indicates that the physiological response of *Arthrospira platensis* Gomont to nutrient addition is modulated by salinity, and not all nutrient–salinity combinations yield synergistic effects. Interestingly, the KN-35 group exhibited a growth rate comparable to those of the P2-35 and P3-35 groups. This suggests that *Arthrospira platensis* Gomont is capable of activating osmoregulatory mechanisms and utilizing internal nutrient reserves to maintain essential metabolic functions despite nutrient constraints, which may contribute to sustaining growth rates under high-salinity stress (Bezerra *et al.* 2020; Villaró *et al.* 2023).

Two-factor ANOVA analysis (Table 2) revealed that calcium nitrate supplementation ($F = 0.184$; $P = 0.906$), salinity ($F = 1.690$; $P = 0.195$), and their interaction ($F = 0.416$; $P = 0.865$) did not produce statistically significant differences in specific growth rate ($P > 0.05$). Although these statistical results do not confirm significant treatment effects, observable patterns suggest that increasing salinity levels (from 15 ppt to 35 ppt) across all treatments tend to correlate with higher biomass productivity and specific growth rates, particularly in the KN and P3 groups. The P2-25 treatment exhibited a significant drop in productivity, which may be attributed to unfavorable salinity-nutrient interactions under this condition. The P3-35 treatment appeared to be the most optimal condition for biomass production, showing the highest values across all metrics, productivity and growth rate, indicating that this specific nutrient-salinity combination may create physiological conditions conducive to enhanced microalgal growth and biomass accumulation.

The data presented in Table 3 suggest that higher salinity levels (35 ppt) generally support enhanced biomass productivity and specific growth rates in *Arthrospira platensis* Gomont, particularly under the P3 treatment, which consistently outperformed other conditions. However, the interaction between salinity and treatment varied, with the P2-25 condition identified as unfavorable, indicating the necessity for optimization based on specific growth or production targets.

Table 2 Two-factorial ANOVA analysis on specific growth rate of *Arthrospira platensis* Gomont during the stationary phase

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	0.032[a]	11	0.003	0.585	0.832
Intercept	0. 420	1	0.420	84.423	0.000
Treatment	0. 003	3	0.001	0.184	0.906
Salinity	0. 017	2	0.008	1.690	0.195
Treatment*Salinity	0. 012	6	0.002	0.416	0.865
Error	0.239	48	0.005		
Total	0.691	60			
Corrected total	0.271	59			

Note: a: R2 = .118 (Adjusted R2 = .084).

Table 3 Measurement results of mean biomass productivity, mean stationary phase biomass productivity and mean specific growth rate

Treatment	Mean biomass productivity (g/L)	Mean biomass productivity on stationary phase (g/L)	Mean specific growth rate (per day)
KN15	3.4^{ab}	5.4^{ab}	0.04^{a}
KN25	4.3^{ab}	6.8^{ab}	0.09^{ab}
KN35	4.3^{ab}	7.2^{ab}	0.10^{b}
P1-15	2.6^{ab}	3.9^{ab}	0.08^{ab}
P1-25	3.8^{ab}	5.8^{ab}	0.10^{b}
P1-35	3.5^{ab}	5.5^{ab}	0.08^{ab}
P2-15	4.1^{ab}	6.2^{ab}	0.06^{ab}
P2-25	1.3^{a}	1.5^{a}	0.06^{ab}
P2-35	4.0^{ab}	6.8^{ab}	0.11^{b}
P3-15	4.9^{b}	7.9^{b}	0.08^{ab}
P3-25	2.2^{ab}	3.2^{ab}	0.09^{ab}
P3-35	5.1^{b}	8.8^{b}	0.12^{b}

Notes: Different superscript letters indicate statistically significant differences at $P < 0.05$ (Duncan's multiple range test); identical or shared letters (e.g., ab) indicate no significant differences.Results of Chlorophyll Content Measurement

Chlorophyll a content was consistently higher than chlorophyll b across all treatment groups (Fig. 4). Based on measurements of chlorophyll a and total chlorophyll concentrations, the KN-35, P3-35, P3-15, and P2-35 groups exhibited higher levels compared to other groups. These results indicate that a salinity of 35 ppt combined with calcium nitrate supplementation at concentrations of 2.5 g/L and 3.5 g/L can enhance chlorophyll content.

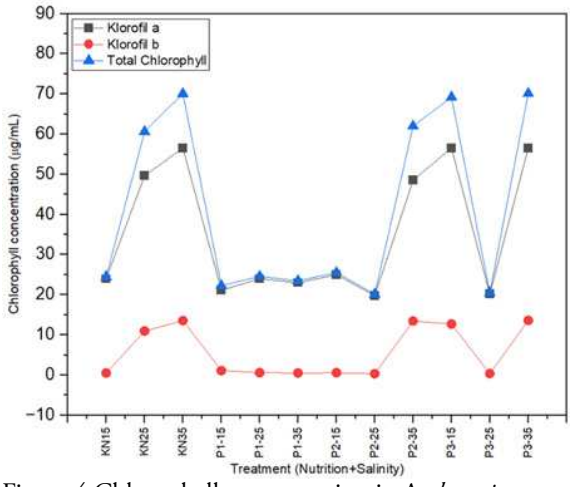


Figure 4 Chlorophyll concentration in A*rthrospira platensis* Gomont

When cultivated at 35 ppt salinity without nutrient supplementation, *Arthrospira platensis* Gomont exhibited elevated concentrations of chlorophyll a, chlorophyll b, and total chlorophyll, measured at 56.55, 13.57, and 70.12 µg/mL, respectively. Similarly, under 35 ppt salinity with 4.5 g/L calcium nitrate supplementation, chlorophyll a, b, and total chlorophyll levels were recorded at 56.54, 13.61, and 70.15 µg/mL, respectively. Moreover, in several treatment groups, calcium nitrate supplementation did not result in a significant enhancement of chlorophyll content in *Arthrospira platensis* Gomont.

Under 15 ppt salinity without nutrient supplementation (KN-15), chlorophyll content in *Arthrospira platensis* Gomont was relatively low, indicating that suboptimal salinity may not enhance chlorophyll production. Treatments at 25 ppt salinity (KN-25, P1-25, P3-25) showed slight improvements in total chlorophyll compared to KN-15; however, these increases remained lower than those observed in treatments at 35 ppt.

At 35 ppt salinity (KN-35, P2-35, P3-35), increases in chlorophyll content were observed in *Arthrospira platensis* Gomont. The highest enhancement occurred in the P3-35 treatment, which achieved a total chlorophyll concentration of 70.15 µg/mL. These results suggested that this nutrient-salinity combination provides optimal conditions for biomass growth and chlorophyll accumulation in *Arthrospira platensis* Gomont.

The results indicated that nutrient supplementation combined with appropriate salinity levels plays a significant role in enhancing chlorophyll content in *Arthrospira platensis* Gomont. Nitrate, absorbed through NRT1 and NRT2 transporters, supports amino acid and

nucleotide biosynthesis essential for cell growth and biomass accumulation. Moreover, nitrate serves as a nitrogen donor for porphyrin ring formation, the core structure of chlorophyll molecules (Cui *et al.* 2024), and modulates gene expression under saline stress (The *et al.* 2021; Hachiya & Sakakibara 2017). In calcium nitrate, both nitrate and calcium components contribute to chlorophyll biosynthesis, with calcium stabilizing chloroplast membranes and supporting photosynthetic activity through multiple physiological roles. As a component of the oxygen-evolving complex (Mn_4CaO_5) in photosystem II (PSII), calcium facilitates water splitting and oxygen evolution, while also regulating Calvin cycle enzymes such as fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase). Under salinity stress, calcium maintains thylakoid membrane integrity and activates protective mechanisms such as cyclic electron flow (CEF) and non-photochemical quenching (NPQ), thereby preserving pigment biosynthesis (Hochmal *et al.* 2015). These multifaceted roles likely explain the elevated chlorophyll content observed in high-calcium nitrate treatments (notably P3-35), underscoring its potential to enhance photosynthetic efficiency and pigment accumulation under environmental stress

Analysis of Functional Groups in Chlorophyll of *Arthrospira platensis* Gomont

FTIR analysis revealed that key functional groups essential for biosensor applications were present in chlorophyll extracted from *Arthrospira platensis* Gomont cultivated with 4.5 g/L calcium nitrate at 35 ppt salinity (Fig. 5). A strong absorption at 3,389/cm indicated the presence of hydroxyl (–OH) groups, which are known to contribute to hydrogen bonding and enhance molecular interaction stability (Nandiyanto et al. 2023). The carbonyl (C=O) group was detected at 1,704/cm, which is typically found in ester or ketone structures of chlorophyll and is recognized for its role in increasing binding affinity through electrostatic interactions with target molecules (Mansour *et al.* 2022).

An absorption band at 1,371/cm was identified as alkane (CH) groups that participate in hydrophobic interactions and van der Waals forces, though these interactions are weaker than hydrogen bonds (Wu & Prausnitz 2008). An absorption at 1,222/cm was attributed to C–O

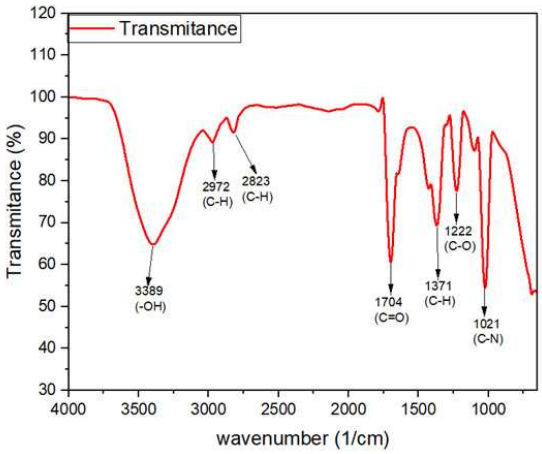


Figure 5 FTIR analysis of chlorophyll from P3-35 group

bonds, characteristic of esters or carboxylic acids, which have been found to enhance chlorophyll's ability to interact with target molecules through polar interactions (Chemistry LibreTexts 2024). Meanwhile, the band at 1,021/cm was associated with C–N bonds, which are commonly found in amine or amide groups and the porphyrin structure of chlorophyll. These groups are known to enable electrostatic interactions with charged molecules, thereby strengthening the binding affinity between chlorophyll and target analytes in sensor applications (Mansour *et al.* 2022).

Overall, these FTIR results indicate that stable chemical interactions with target molecules can be formed by chlorophyll due to the presence of hydroxyl, carbonyl, alkane, C–O, and C–N groups. The role of these functional groups in biosensing applications has been supported by their contribution to enhancing molecular binding affinity. Furthermore, a positive enhancement in chlorophyll production under optimal conditions reinforcing the potential of chlorophyll as a biotransducer for molecular detection (Mansour *et al.* 2022).

This study highlights the potential of optimized nutrient conditions to enhance the biotransducer properties of *Arthrospira platensis* Gomont. Although some treatments did not yield statistically significant results, the increased presence of specific functional groups, as revealed by FTIR analysis, supports their role in strengthening binding affinity in SPR biosensors. Cultivation under appropriate calcium nitrate concentrations and salinity levels was shown to improve chlorophyll content and promote the expression of functional groups favorable for analyte interaction. These

findings underscore the potential of *Arthrospira platensis* Gomont as an effective biotransducer for SPR-based detection systems. Future research should investigate the scalability and real-world applicability of this approach in diagnostic settings. The FTIR-detected functional groups further validate the chemical complexity of chlorophyll and its suitability as a molecular recognition element, particularly due to the presence of groups capable of polar and electrostatic interactions.

CONCLUSION

The addition of calcium nitrate was found to positively impact the increase in biomass productivity of *Arthrospira platensis* Gomont, correlating with a rise in total chlorophyll concentration. Calcium nitrate at a concentration of 4.5 g/L under 35 ppt salinity (P3-35) demonstrated greater potential in enhancing biomass production compared to lower calcium nitrate concentrations or salinity levels. The presence of hydroxyl, carbonyl, alkane, C–O, and C–N functional groups confirmed that chlorophyll extracted from the optimized cultivation condition (P3-35) possesses enhanced stability and interactive properties as a biotransducer. These findings can inform the formulation of biointerface layers in SPR biosensors. Future studies are recommended to validate these outcomes in real-world sensor platforms to confirm binding efficiency and diagnostic relevance.

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