RESEARCH PAPER

Effect of Environmental Factors on Biomass and Insulin Production in the Cyanobacterium *Arthrospira platensis* SPKY1

[1] K. Yuvarani, [2*]A.K. Kathireshan

[1,2] Department of Microbiology, School of life sciences, Vels University, Pallavaram, Chennai, Tamilnadu, India

*Corresponding author: A.K. Kathireshan, Department of Microbiology, School of life sciences, Vels University, Pallavaram, Chennai, Tamilnadu, India,

E mail: kathirhodmicrobiology@gmail.com

ABSTRACT

Arthrospira platensis (*A. platensis*) is a potential bio-factory for producing therapeutic proteins, with reported presence of insulin-like peptides. This study assessed the impact of environmental parameters on biomass accumulation and insulin production in *A. platensis* SPKY1 under outdoor conditions at Ennore, Chennai, India (September 2024–March 2025). Temperature, light intensity, pH, and biomass were monitored every five days throughout each 20-day growth cycle. Biomass was measured gravimetrically; insulin content was quantified by ELISA and HPLC. Regression-based time-series and mediation analyses identified key relationships, while an ARIMA model forecasted insulin trends for April–August 2025. Biomass increased from 1.6 to 2.9 g L^{-1}, corresponding to favorable irradiance (228–407 µmol m^{-2} s^{-1}), pH (9.4–10.5), and temperature (30.3–38.1 °C). Insulin content rose from 22.6 ± 0.3 to 43.8 ± 0.2 µg g^{-1}, showing a strong correlation with biomass (R^2 = 0.944). Biomass mediated environmental effects on insulin, and forecasting predicted a peak yield of 62.7 ± 0.6 µg g^{-1} in May. Optimizing environmental conditions enhances insulin biosynthesis in *A. platensis* SPKY1, highlighting its potential for scalable outdoor production.

Keywords: *Arthrospira platensis* SPKY1; Diabetes; Insulin; Antidiabetic; Anti-glycemic; Biomass; Outdoor cultivation

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INTRODUCTION

Insulin is a critical peptide hormone responsible for regulating glucose metabolism and plays a central role in the management of diabetes mellitus, a metabolic disorder that has reached epidemic proportions worldwide.[1] The rapidly increasing global prevalence of diabetes has led to a substantial rise in insulin demand. Currently, commercial insulin production primarily relies on genetically engineered microorganisms such as *Escherichia coli* and yeast; however, these systems require expensive fermentation infrastructure and complex downstream purification processes, contributing to high production costs.[2,3]

Microalgae have attracted growing interest due to their diverse applications in the food, pharmaceutical, and biofuel industries.[4] Among these, *Arthrospira platensis* (commonly known as *Spirulina*), a filamentous cyanobacterium recognized for its high nutritional value and protein content, has emerged as a promising candidate for biopharmaceutical applications.[5] Its rich composition of proteins and bioactive compounds has stimulated investigations into its potential role in insulin-related therapeutics.[6]

Several studies have reported that *Spirulina* supplementation exerts beneficial effects on glucose metabolism. Consumption of *Spirulina* has been associated with reduced blood glucose levels,[7,8] increased insulin concentration,[9-11] and improved insulin sensitivity.[12-14] However, the specific bioactive molecules responsible for these effects have not been clearly identified. Most investigations have primarily focused on physiological outcomes, without detailed characterization of the exact compounds or

molecular mechanisms underlying the observed antidiabetic effects.

Previous studies reported that certain peptides derived from *A. platensis* exhibit insulin-mimetic or insulin-enhancing properties.[15] Furthermore, insulin-like antigens and approximately 6 kDa protein bands have been detected in several *Spirulina* species, suggesting the presence of insulin-related bioactive molecules.[16-18] However, most earlier investigations were limited to protein detection and lacked genomic confirmation of insulin-associated sequences.

Recent work identified *A. platensis* SPKY1 as a promising insulin-producing strain. Genomic analysis revealed three hypothetical proteins containing amino acid sequences identical to regions of human insulin, along with eight insulinase-family proteins.[19] Subsequent laboratory-based studies evaluated factors influencing insulin production, including culture media composition, pH, light intensity, water sources, carbon supplementation, and greenhouse conditions.[20]

Despite these advances, successful industrial application requires validation under outdoor cultivation systems, where environmental fluctuations significantly influence microalgal physiology, metabolic activity, and product yield. Environmental parameters such as light intensity, temperature, and pH play crucial roles in regulating biomass accumulation and biochemical productivity in microalgae.[21] Therefore, the present study investigates the effects of these key environmental parameters on biomass growth and insulin production in *A. platensis* SPKY1 under outdoor cultivation conditions at Ennore, Chennai, India.

MATERIALS AND METHODS

Sample collection and algal isolation

Water samples (2 L) were collected from three locations in the Ennore estuary, Chennai (13.23°N, 80.32°E) at 10 cm depth using sterile containers. Temperature and pH were recorded on-site using a thermometer and digital pH meter. Samples were preserved in 5% neutralized formalin and stored at 4 °C. Algae were isolated by serial dilution and plating on agar containing Zarrouk medium.[22] Colonies were sub-cultured into liquid media, and *Arthrospira* sp. were identified microscopically based on filament color, texture, buoyancy, presence of heterocysts, and overall morphology. To obtain axenic cultures, isolates were treated with cefoxitin (76.9 µg mL^{-1}) for 48 h in the dark, centrifuged at 10,000 rpm for 5 min, washed with distilled water, and transferred to fresh medium.[23]

Production site

Ennore, Chennai, India hosts thermal power plants, petrochemical industries, and port operations contributing to high CO_2 emissions and nutrient-rich saline waters conducive for growing resilient microalgae such as *A. platensis* SPKY1. The tropical coastal climate (25–38 °C) and year-round sunlight support biomass production, although humidity, contamination, and seasonal variations require careful management.

Production module

Outdoor cultivation was performed using Azolla cultivation bags (~25 L capacity), each filled with 20 L of optimized growth medium. About 10% (2 L) of actively growing mother culture was inoculated into each bag to accelerate biomass establishment and minimize contamination risk. The flexible units allowed control of water depth, nutrient replenishment, and exposure to natural conditions. Bags were maintained under shade to prevent excessive light stress.

Batch culture conditions

Environmental parameters were monitored over seven months (September 2024–March 2025). One batch was lost due to heavy rainfall (October 2024); hence, six months of data were included. Since temperature, pH, and light intensity strongly influence biomass productivity, these

were recorded continuously under outdoor conditions.

Temperature

Daily temperature was measured at 12 PM using a centigrade thermometer. Regional temperatures varied from 20–25 °C (March), rising to ~40 °C (May), and stabilizing around 30 °C during monsoon months. Temperatures below 16 °C or above 36 °C are known to inhibit *A. platensis* growth.

Light

Light intensity was measured regularly using a digital lux meter. To prevent photoinhibition during summer, bags were shaded, and cultures were mixed several times a day. During monsoon rains, plastic sheets were used to maintain stable light and water levels.

pH

pH was initially monitored using pH indicator strips during outdoor cultivation and later measured with a calibrated digital pH meter in the laboratory. Optimal growth was maintained between pH 9.0–11.0 to minimize contamination and ensure stable biomass accumulation.

Biomass determination

Biomass was harvested on the 20th day using gravity filtration. Cultures were first passed through cloth to remove debris and then through 380–500 mesh nylon cloth. The biomass was washed with distilled water, dried at room temperature for ~24 h, and ground into fine powder for storage.

Insulin extraction

Five grams of dried biomass were homogenized with 2 mL water, 10 mL 95% ethanol, and 0.72 mL concentrated H_2SO_4. After mixing for 20 min, 13 mL water and 40 mL ethanol were added and pH adjusted to 1.7. The suspension was filtered, and filtrate pH adjusted to 3.0 with ammonium hydroxide. A solvent mixture (150 mL ethanol + 200 mL diethyl ether) was added and stored at 4 °C for 12 h. The pellet was washed with acetone: ether, dissolved in 25% ethanol, pH adjusted to 8.5, and zinc chloride (100 µL, 1 M) added. Incubation at 25 °C for 18 h precipitated insulin.[24]

Insulin quantification (ELISA)

Insulin concentration from all treatments was measured using a Genei ELISA kit with minor modifications.[25] Standards, samples (1:2 dilution), and controls were added to designated wells and incubated at 37 °C for 90 min. After washing, biotin-labeled antibody, HRP–streptavidin, and TMB substrate were sequentially added with respective incubation and wash steps. The reaction was stopped with stop solution, and absorbance was read at 450 nm. A four parameter logistic (4PL) standard curve was used to calculate insulin concentration.

HPLC analysis

The insulin pellet was reconstituted in 0.1% TFA containing 0.1 M EDTA and loaded on a Phenomenex C18 column (250 × 4.6 mm, 5 µm). Elution was performed with a 30–80% acetonitrile gradient in 0.1% TFA at 1 mL/min. Detection was at 214 nm, and retention time was compared with human insulin standard (50 µg in 500 µL).[25]

Data analysis

Data were cleaned and entered in Excel. Triplicate observations were analyzed using SPSS v25. Mean ± SD were computed for temperature, light intensity, pH, biomass, and insulin yield. Relationships between environmental variables and insulin production were assessed using scatterplots and coefficients of determination (R^2). Seasonal projections of insulin yield were generated using polynomial regression and ARIMA forecasting (April–August 2025). Model accuracy was evaluated using RMSE and MAPE, and paired t-tests compared observed vs. predicted values. Mediation analysis (PROCESS macro-Model 4; Hayes) assessed indirect pathways linking environmental variables (temperature, light, pH) to insulin production via key intermediate factors.

RESULTS

Environmental parameters and biomass production were monitored from September 2024 to March 2025. Temperature ranged from 29.2 °C in December to 38.1 °C in March. Light intensity gradually increased toward late summer, peaking in February–March. Culture pH varied between 9.4 ± 0.6 (December) and 10.5 ± 0.2 (February) (**Table 1**). Biomass concentration increased from 1.6 ± 0.2 g L^{-1} in September to 2.9 ± 0.2 g L^{-1} in March, with a marked rise from December onward, corresponding to improved light exposure and stable alkaline pH (**Figure 1**).

Observed and predicted insulin levels (20th day) from November 2024 to March 2025 showed close agreement (**Table 2**). No significant differences were noted between observed and predicted values ($P > 0.05$). Based on model projections, insulin production is expected to rise further, reaching 62.7 ± 0.6 µg g^{-1} in May 2025, followed by a gradual decline to 50.2 ± 0.5 µg g^{-1} by August 2025, suggesting seasonal enhancement during late summer.

HPLC analysis of the algal extract showed a distinct peak at 6.79 min, matching the retention time of the human insulin standard (**Figure 2**), confirming insulin presence in *A. platensis* SPKY1. Total protein content was ~680 mg g^{-1} dry biomass, with insulin-equivalent content increasing from 15 µg g^{-1} in the mother culture to 43.8 µg g^{-1} in the March batch. The corresponding protein-to-insulin conversion efficiency increased from 0.022% to 0.064%.

Scatterplot analyses (**Figure 3a–3d**) showed positive correlations between insulin production and all assessed environmental factors. Temperature exhibited a moderate association ($R^2 = 0.259$), whereas light intensity showed a stronger effect ($R^2 = 0.536$). pH demonstrated a high positive correlation ($R^2 = 0.843$). Biomass had the strongest linear relationship with insulin production ($R^2 = 0.944$), indicating that insulin synthesis is closely tied to biomass accumulation.

Mediation analysis **(Figure 4)** revealed that biomass acted as a key mediator linking environmental factors to insulin production. Temperature showed both direct ($\beta = 0.213$, $p = 0.014$) and indirect effects via biomass ($p < 0.05$). pH also exerted significant direct ($\beta = 0.343$, $p = 0.001$) and indirect influences ($p < 0.01$). Light intensity had no significant direct effect ($\beta = 0.0002$, $p = 0.182$) but demonstrated a marginal indirect effect fully mediated through biomass ($p = 0.050$). Biomass remained the strongest predictor of insulin output ($\beta = 20.68$, $p = 0.001$). Overall, biomass served as the central regulatory factor connecting environmental variation to insulin biosynthesis in *A. platensis* SPKY1.

Table 1. Monthly environmental parameters recorded during outdoor cultivation of *A. platensis* SPKY1.

Months	Temperature (°C)	Light (µmol photons m^{-2} s^{-1})	Light (lux)	pH 20th day (Mean±SD)
September 2024	32.2-35.6	224-375	12340-20621	9.8±0.2
November 2024	29.8-36.0	241-421	13269-23154	10.2±0.3
December 2024	29.2-36.1	245-418	13500-23000	9.4±0.6
January 2025	29.5-37.5	255-420	14000-23100	9.9±0.4
February 2025	30.3-38.0	291-405	16000-22300	10.5±0.2
March 2025	30.8-38.1	298-400	16400-22000	9.6±0.3

Figure 1. Monthly variation in biomass production of *A. platensis* SPKY1 under outdoor cultivation conditions.

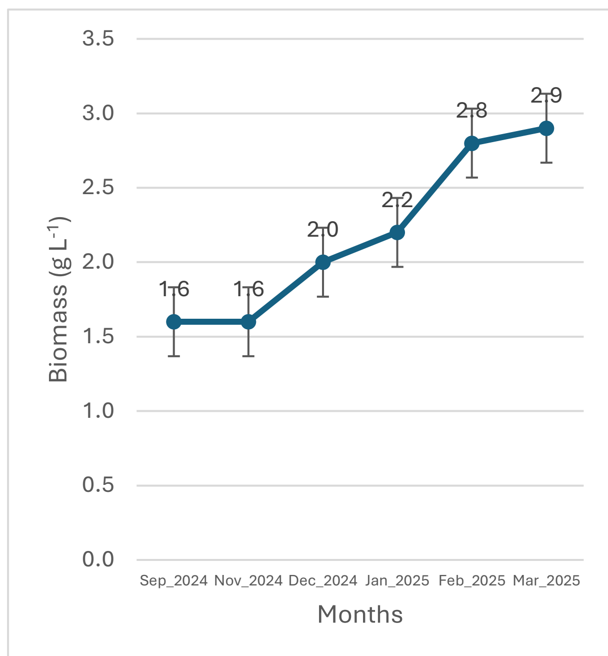


Figure 2. HPLC profiling of standard and *A. platensis* SPKY1 insulin

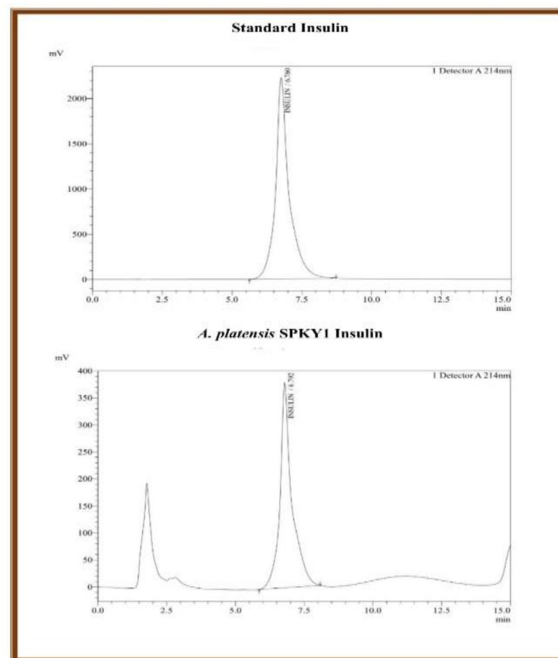
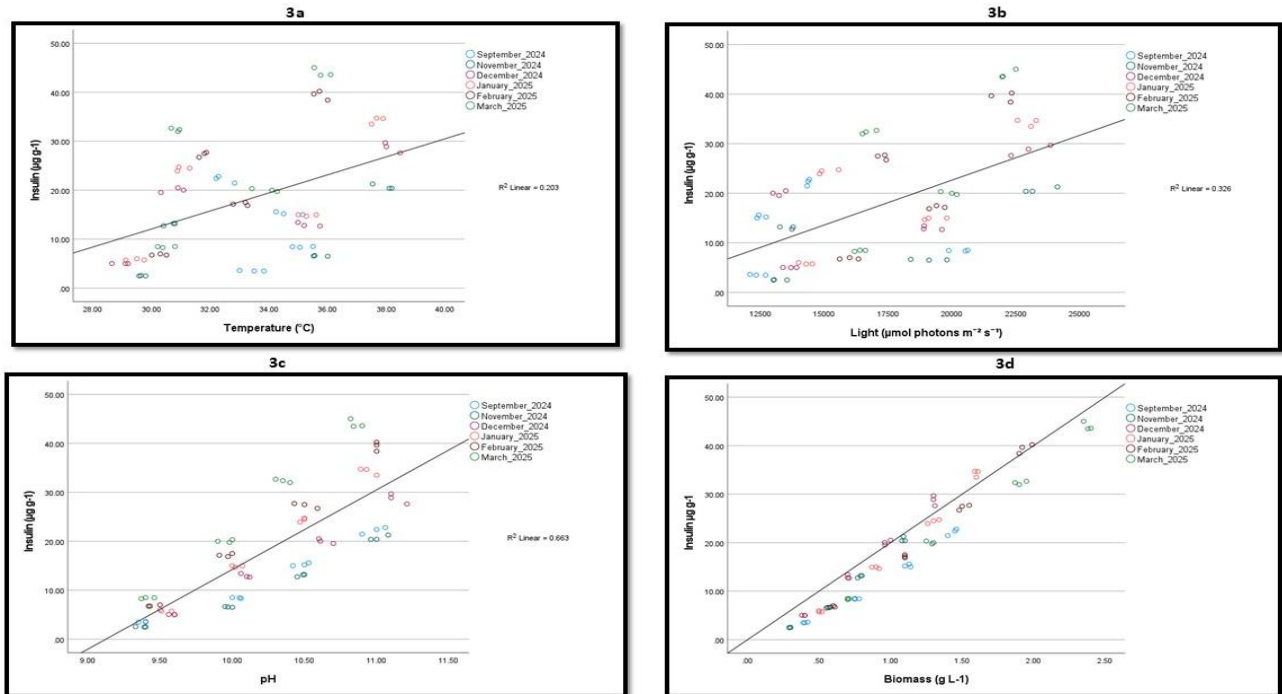


Table 2: Forecasting analysis of insulin production by *A. platensis* SPKY1

Months	Observed Insulin (µg g⁻¹) (Mean±SD)	Predicted Insulin (µg g⁻¹) (Mean±SD)	P*
Nov_2024	22.6±0.3	22.4±0.4	0.667
Dec_ 2024	28.5±0.3	28.4±0.3	0.578
Jan_ 2025	33.6±0.2	33.3±0.1	0.128
Feb_ 2025	38.7±0.4	38.2±0.4	0.119
Mar_ 2025	43.8±0.2	43.6±0.5	0.578
Apr_ 2025	–	48.4±0.3	–
May_ 2025	–	62.7±0.6	–
Jun_ 2025	–	58.4±0.4	–
Jul_ 2025	–	53.1±0.2	–
Aug_ 2025	–	50.2±0.5	–

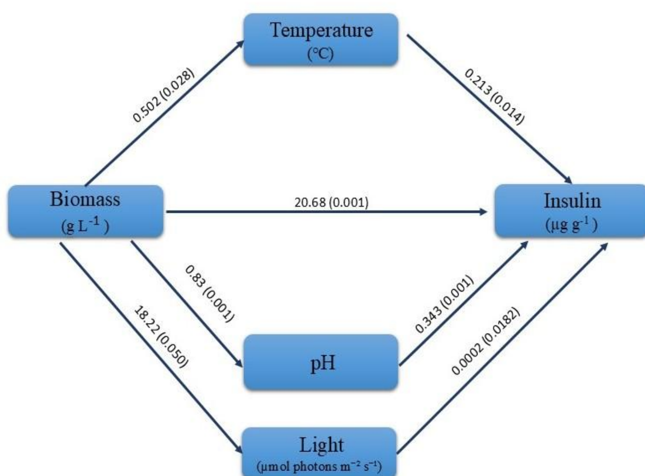
P-values represent comparison between observed and predicted values

Figure 3: Association between environmental factors and insulin



Footnote: Scatter plots (3a–3d) shows the relationship between insulin production and environmental variables across different months. Each point represents a single observation, color-coded by month. Linear regression lines and R^2 values indicate the strength of association.

Figure 4: Mediation model of environmental factors influencing insulin production



Footnote: Values on arrows indicate standardized regression coefficients (β), with p-values in parentheses. Biomass (g L^{-1}) serves as a mediator between environmental variables (temperature, light, pH) and insulin production (µg g^{-1}). Significant direct and indirect pathways highlight the mediating role of biomass in linking environmental conditions to insulin yield

DISCUSSION

The present study provides critical insights into the environmental regulation of biomass accumulation and insulin production in *A. platensis* SPKY1 cultivated under outdoor conditions from September 2024 to March 2025, with projections extended to August 2025. A combination of monthly monitoring, regression analysis, and mediation modeling was used to understand how temperature, light intensity, and pH influence growth and insulin biosynthesis.

In the screening study by Anwer *et al.*[18] insulin production was detected in 16 out of 23 *Arthrospira* and *Spirulina* sp., with concentrations ranging from 2.3 to 33.9 µg g^{-1}. Among these, *S. platensis* (CFTRI, Mysore) exhibited the highest insulin yield (33.9 µg g^{-1}),

followed by *S. platensis* S5 (31.4 µg g^{-1}), *Spirulina* NCCU-482 (25.1 µg g^{-1}), and *Spirulina* NCCU-483 (23.2 µg g^{-1}), all isolated from freshwater habitats. Moderate insulin levels were observed in *A. maxima* (SAE-25780; 17.5 µg g^{-1}) and *Spirulina* NCCU-481 (17.8 µg g^{-1}), whereas *A. indica* (SAE-85, Lonar-CSV, PCC7940) produced between 8.8 and 9.6 µg g^{-1}. The marine strain *S. subsalsa* displayed the lowest insulin content (2.3 µg g^{-1}), suggesting that freshwater isolates may be more efficient producers. In the later screening by Khursheed *et al.*[17] *Spirulina* NCCU-482, *Spirulina* NCCU-483, and *S. platensis* S5 were found to be positive for insulin-like activity, demonstrating the inherent potential of *Spirulina* sp. to synthesize insulin-like peptides.

In the present study, the laboratory-maintained mother culture of *A. platensis* SPKY1 produced 15.9 µg g^{-1} of insulin, which is lower than the yields reported by *S. platensis* (CFTRI, Mysore; 33.9 µg g^{-1}) and *S. platensis* S5 (31.4 µg g^{-1}) strains. However, when cultivated under outdoor conditions, *A. platensis* SPKY1 demonstrated a substantial increase in insulin production, reaching 43.8 ± 0.2 µg g^{-1} in March 2025. This indicates that environmental factors, particularly natural light intensity and temperature, may play a crucial role in enhancing insulin biosynthesis in *A. platensis*. Insulin levels rose in parallel with biomass, increasing from 22.6 ± 0.3 µg g^{-1} in November 2024 to 43.8 ± 0.2 µg g^{-1} in March 2025, and biomass explained 94% of insulin variability ($R^2 = 0.944$).

Mediation analysis showed that temperature and pH each had significant direct effects on insulin as well as indirect effects through biomass. Light had no significant direct effect ($\beta = 0.0002$, $p = 0.182$) but a marginal indirect effect via biomass, indicating that light enhances insulin production primarily by increasing cell density rather than directly upregulating insulin synthesis. All outdoor culture batches produced higher insulin concentrations compared to the mother culture (15.9 µg g^{-1}), indicating enhanced productivity of *A. platensis* SPKY1 under optimized environmental conditions. In addition, HPLC analysis of insulin extracted from *A. platensis* SPKY1 revealed a retention time of 6.79 minutes, showing a chromatographic profile comparable to that of standard human insulin. Previous studies have reported insulin retention times of 6.95 minutes in *B. variegate,*[26] 17.2 minutes in *Arabidopsis thaliana* seeds,[27] and 31.0 minutes in *Vigna unguiculata.*[28]

Temperature emerged as a key determinant of both biomass and insulin production. The cultivation period experienced a progressive rise in temperature from 29.2 °C in December to 38.1 °C in March, closely mirrored by a steady increase in both biomass concentration and insulin yield. This positive trend is supported by prior literature, which defines the optimal temperature range for *Spirulina* growth between 24 °C and 38 °C.[29] Richmond,[30] reported 18 °C as the minimum viable temperature for outdoor cultivation, while Barsanti and Gualtieri.[31] noted that temperatures above 35 °C can be lethal for certain strains, though SPKY1 appeared to tolerate high temperatures well, particularly in March.

Light plays a crucial role in *Spirulina* photosynthesis and biomass accumulation. In the present study, irradiance increased from approximately 220 µmol photons m^{-2} s^{-1} in September 2024 to 415 µmol photons m^{-2} s^{-1} in November, and subsequently ranged between 285 and 400 µmol photons m^{-2} s^{-1} through March 2025. During the initial months, when light intensity was relatively low, the biomass yield on day 20 remained around 1.6 g L^{-1}. As irradiance levels rose from December onward, biomass gradually increased, reaching 2.9 g L^{-1} by March, indicating that light availability directly influenced growth.

Usharani et al.[32] reported that moderate light intensity favored optimal *Spirulina* growth, yielding 0.85 g/500 mL culture with high protein (64.3%) and chlorophyll *a* (9.8 mg/g), while excessive light could lead to

photodamage. Danesi et al.[33] observed that approximately 60 µmol photons $m^{-2} s^{-1}$ sufficed for maximal growth, beyond which shading effects limited productivity. Similarly, Delrue et al.[34] noted no photoinhibition even at high irradiances (85–430 µmol photons $m^{-2} s^{-1}$) and reported a specific growth rate of 0.12 day in a 2 L reactor. However, continuous illumination is not advisable, as dark intervals are essential for protein synthesis and respiration.[35]

Spirulina thrives in alkaline conditions (pH 8.5–10.5), which limit competing microbes and optimize photosynthesis. In December 2024, the culture's mean pH was lowest (9.4 ± 0.6) with biomass of 2.0 g L^{-1}, whereas February 2025 saw peak pH (10.5 ± 0.2) and biomass of 2.8 g L^{-1}, suggesting that a pH near or just above 10 supports growth when light and temperature are favorable. Although September and November 2024 had pH values of 9.8 ± 0.2 and 10.2 ± 0.3, biomass remained at 1.6 g L^{-1}, indicating light and temperature were more limiting then.

By March 2025, pH dipped slightly to 9.6 ± 0.3, yet biomass peaked at 2.9 g L^{-1}, showing that small pH shifts (9.5–10.0) do not hinder growth under optimal irradiance and temperature. *Spirulina* sensitivity to pH arises from bicarbonate uptake: below 8.5, carbon fixation is limited, while above 10.5, carbon exists mainly as unusable carbonate, leading to stress.[36,37] Previous studies similarly report optimal growth at pH 9–10.[38-40]

Validation of our predictive model for November 2024–March 2025 (predicted vs. observed $p > 0.05$) establishes confidence in its ability to forecast insulin levels through August 2025 indicating the reliability and robustness of the model. The model forecasts a peak insulin concentration of 62.7µg g^{-1} in May 2025, followed by a steady decline to 50.2 µg g^{-1} by August. This seasonal trend likely reflects the interplay of declining natural irradiance and moderating temperatures as summer progresses into monsoon and post-monsoon periods in the India subcontinent. Moreover, as day length shortens after June, cumulative daily photon flux diminishes even if midday irradiance remains high potentially limiting overall carbon assimilation and insulin synthesis.

Such seasonally shaped profiles have been reported in outdoor photobioreactor studies: biomass and metabolite yield often crest in late spring/early summer and taper off during periods of lower insolation or high humidity.[41] Therefore, the forecasting model can inform operational decisions: harvests should be scheduled around late April–early May to capture peak insulin content, whereas continuous cultivation between June and August may produce lower-value biomass better suited for non-therapeutic applications (e.g., nutraceutical *Spirulina* powder).

Insulin yields peak around May and decline thereafter, so a semi-continuous or daily-harvest strategy in April–May is optimal. By harvesting when cell densities reach approximately 2.8–3.0 g L^{-1} just before the projected biomass plateau cultures remain in late-exponential phase, sustaining specific growth rates near 0.12 day. This minimizes downtime and avoids repeated restarts while maximizing both biomass and insulin output over extended production cycles.

It is well established that nitrogen sources (e.g., nitrate or urea) and macronutrient ratios (N:P:Fe) play a critical role in directing carbon partitioning between native protein synthesis and recombinant product formation.[42-44] In insulin-expressing *A. platensis* SPKY1, modest increases in nitrogen availability have the potential to enhance total soluble protein levels, thereby boosting insulin accumulation at the cellular level. Consistent with this, our media optimization studies demonstrated that elevated concentrations of key medium constituents including $NaHCO_3$, $NaNO_3$, NaCl, K_2SO_4, and K_2HPO_4 positively correlate with insulin yield. Among these, K_2SO_4 at a concentration of 1.4 g L^{-1} yielded the highest insulin production, highlighting its pivotal role in optimizing native insulin-like protein expression in *A. platensis*.[20]

High biomass densities also simplify downstream processing by concentrating the target product. At an expression level of 0.5% insulin by dry weight, a culture with a biomass concentration of 2.9 g L^{-1} yields approximately 14.5 mg of insulin/ liter. When scaled to a 10,000-liter raceway system, this corresponds to over 145 grams of insulin/batch. Such yields are competitive with conventional microbial expression systems, provided that purification steps are efficient and cost-effective.[45,46]

Although the *A. platensis* SPKY1 strain has been optimized for insulin expression, it may still fall short of its maximum protein production potential. Strain improvement can be pursued through both random and targeted strategies. Physical mutagenesis, such as UV irradiation.[47,48] and chemical mutagenesis, using agents like Ethyl Methane Sulfonate (EMS) or N-Methyl-N′-Nitro-N-Nitrosoguanidine (MNNG)[49-51] can induce genetic diversity and lead to mutants with enhanced expression, though these methods require extensive screening. In parallel, advanced genetic engineering approaches such as codon optimization, strong endogenous or synthetic promoters, and subcellular targeting to the thylakoid lumen offer more precise control over gene expression and protein localization.[52-53]

While maximizing biomass productivity and insulin titers is a key scientific objective, the commercial feasibility of *Spirulina*-based insulin production ultimately hinges on a robust techno-economic analysis. This evaluation must compare the cost-effectiveness of *A. platensis* SPKY1 to conventional microbial platforms such as *E. coli* [54,55], or yeast[56,57], which already dominate recombinant insulin production.

Capital expenditures (CAPEX) include infrastructure investments such as closed photobioreactor systems or open raceway ponds, each with trade-offs in sterility, light penetration, land use, and temperature control. Operational expenditures (OPEX) encompass energy inputs for artificial lighting, CO_2 delivery, pH maintenance, and thermal regulation especially significant in regions with variable climates.[58,34] While microalgae cultivation may have lower nutrient requirements and reduced need for sterile conditions compared to *E. coli*, these savings can be offset by the energy-intensive nature of maintaining optimal growth conditions at scale. Downstream processing represents another major cost driver. Although *Spirulina* produces low endotoxin levels a significant advantage over gram-negative bacteria like *E. coli* the efficient extraction and purification of intracellular insulin from cyanobacterial biomass remain technically challenging and cost-intensive.[59,60] Preliminary observations indicate that *A. platensis* SPKY1 culture maintenance and nutrient costs are considerably lower than standard fermentation-based systems.

LIMITATIONS AND FUTURE DIRECTIONS

Despite the promising findings, this study has certain limitations. The present work primarily optimized environmental parameters influencing insulin production in *A. platensis* SPKY1; however, the therapeutic efficacy of the extracted insulin, including its receptor-binding activity and structural stability was not assessed. Although a strong correlation was observed between biomass accumulation and insulin yield, the molecular pathways regulating insulin gene expression under varying environmental conditions remain unexamined. Finally, while the forecasting model showed robust short-term accuracy, it may require recalibration for extended cultivation periods or alternate environmental regimes. Future research should focus on evaluating the biosafety, immunogenicity, and regulatory feasibility of *A. platensis* derived insulin. Further studies are also needed to assess production cost-effectiveness, validate therapeutic potential through in vivo experiments, and elucidate the molecular mechanisms governing insulin gene regulation under diverse culture conditions.

CONCLUSION

This study establishes *A. platensis* SPKY1 as a novel and promising biological platform for sustainable insulin production. The findings demonstrate that biomass accumulation serves as the principal mediator linking environmental factors including temperature, light intensity, and pH to insulin yield. Through mediation and predictive modeling, the study identifies optimal culture conditions (30–38 °C, ≥ 16,000 lux, and pH 9.5–10.5) and predicts peak insulin yield in May, providing a data-driven framework for large-scale cultivation and harvest planning.

The novelty of this work lies in integrating environmental optimization with statistical and forecasting approaches to model insulin production dynamics in *A. platensis* SPKY1 under real-world outdoor conditions. Actionable next steps include validating these findings across different *Arthrospira* species, exploring molecular pathways regulating insulin gene expression, assessing insulin bioactivity and biosafety, and incorporating bioreactor or AI-driven cultivation systems to improve yield precision and scalability.

DECLARATIONS

Ethical Approval: Not applicable.

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Conflicts of Interest: The authors declare no conflicts of interest.

Authors' Contributions: K. Yuvarani conceptualized the study and conducted literature review. A.K. Kathireshan analysed, interpreted, and validated the data. K. Yuvarani wrote the manuscript. A.K. Kathireshan reviewed and authorized the final version for publication.

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