The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(3): S.I (3), 836-840, 2025 www.thebioscan.com

INFLUENCE OF pH ON THE GROWTH AND MORPHOLOGY OF *ARTHROSPIRA PLATENSIS*

Tehseen Javed[*1], Nafisa Farheen[2], Anitha, W[3], Geetha N B[4] and Geetha, C[5]

[*1]PERI COLLEGE OF PHYSIOTHERAPY, CHENNAI -48

[2 &3] PERI COLLEGE OF PHARMACY, CHENNAI -48

[4]PERI INSTITUTE OF TECHNOLOGY, CHENNAI - 48

[5] PERI COLLEGE OF NURSING, CHENNAI -48

Corresponding mail id: publications@peri.ac.in

DOI: 10.63001/tbs.2025.v20.i03.S.I(3).pp836-840

KEYWORDS
Arthrospira platensis, pH effect, cyanobacteria, biomass production, haematoxylin staining, alkalophilic growth, cyanobacterial morphology, microalgae cultivation.
Received on:
12-07-2025
Accepted on:
10-08-2025
Published on:
17-09-2025

ABSTRACT

The growth and cellular morphology of *Arthrospira platensis*, a commercially important cyanobacterium, are significantly influenced by environmental pH. This study investigates the effects of varying pH levels (3, 5, 9, and 10) on the organism's biomass accumulation and structural integrity. Cultures maintained at alkaline pH, particularly 9 and 10, exhibited enhanced growth, intact trichome structure, and increased chlorophyll content, indicating optimal metabolic function. In contrast, exposure to acidic conditions, especially at pH 3, resulted in severe cellular damage, including rupture of cell walls and disorganization of internal structures, as confirmed by haematoxylin staining. These findings highlight the alkalophilic nature of *A. platensis* and underscore the importance of pH control in optimizing biomass production for biotechnological applications.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes known for their ecological adaptability and biochemical productivity. Among them, *Arthrospira platensis*—commonly known in commercial contexts as *Spirulina*—is a filamentous, helical, multicellular organism widely recognized for its high protein content, essential amino acids, vitamins, and pigments. It is extensively cultivated for nutritional supplements, pharmaceuticals, and feed applications.Despite taxonomical ambiguity in the past, *Arthrospira* is now distinctly classified from *Spirulina*, with *A. platensis* being the most industrially exploited species. Its growth is known to be strongly influenced by environmental parameters such as temperature, light, salinity, and pH. Among these, pH is a critical factor that directly affects cellular metabolism, nutrient uptake, pigment biosynthesis, and overall biomass yield.

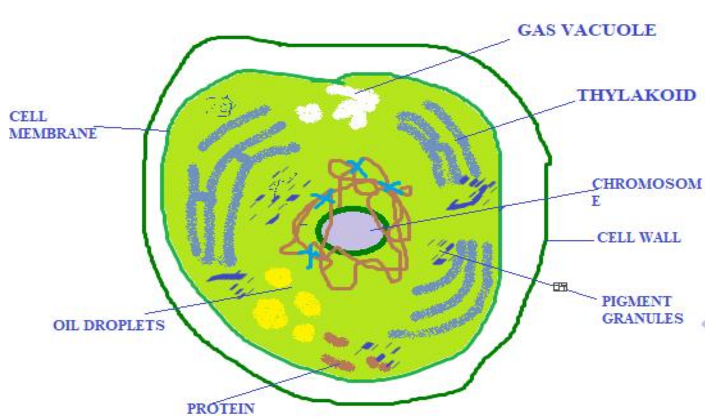

Figure no 1: Cyanobacterium structure

Previous studies have reported that *A. platensis* prefers alkaline conditions, typically thriving in pH ranges between 8.5 and 10.5. Such high pH environments suppress the growth of contaminating organisms, offering a selective advantage for *Arthrospira* cultivation. However, extreme pH levels, particularly on the acidic end, can severely disrupt cellular integrity, causing trichome breakdown, chlorophyll degradation, and reduced photosynthetic activity.In this study, we investigate the influence of different pH levels (3, 5, 9, and 10) on the growth characteristics and cellular morphology of *A. platensis*. The objective is to identify optimal pH conditions for maximizing biomass production and maintaining structural integrity, which are essential for scalable cultivation. Haematoxylin staining and microscopic analyses are employed to observe morphological changes under varying pH environments. ▫ G. Carvalho, J. and Kim,

2. LITERATURE REVIEW

1. Influence of pH on Growth Performance of *Arthrospira platensis*

Alkaline pH (especially 9–10) is widely reported to enhance the growth and metabolic activity of *A. platensis*. It improves chlorophyll content, protein synthesis, and biomass accumulation, making it ideal for large-scale cultivation.pH 9–10 significantly improves biomass productivity and maintains cellular integrity [1], [15], [19].Acidic pH leads to reduced photosynthetic efficiency and cellular damage [2], [13].pH control is essential to optimize nutrient uptake, particularly nitrogen and phosphorus [15], [19].Ammonium feeding under pH- regulated environments enhances nitrogen uptake [26].

2. Morphological Changes Under pH Stress

The morphology of *A. platensis* is strongly influenced by the culture pH. Alkaline conditions maintain the filamentous helical structure, whereas acidic pH causes rupture of cell walls and loss of trichomes.Morphological studies using microscopy confirm cell integrity at pH 9–10 [5], [12], [22].pH 3–5 causes trichome fragmentation and deformation of cell structure [2], [30].Changes in filament morphology affect harvesting efficiency and cellular viability [22].

3. Impact of pH on Biochemical Composition

Optimal pH not only affects growth but also influences pigment (phycocyanin, chlorophyll-a), antioxidant content, and protein composition.Alkaline pH increases chlorophyll-a and phycocyanin content significantly [13], [25], [30].Organic acids combined with alkaline pH enhance phycocyanin stability [30].Media rich in bicarbonate at alkaline pH boost productivity of valuable biocompounds [3], [25].

4. Cultivation Strategies and Media Composition

Adjusting the medium composition, including nitrogen sources and bicarbonates, under optimal pH conditions improves growth and sustainability.Cultivation in bicarbonate-rich or ammonium-supplemented media is efficient under pH 9–10 [3], [15], [26].Cultivation in different nutrient sources and wastewaters shows comparable growth performance under alkaline pH [6], [10].Alternative carbon sources like palm oil mill effluent were effectively utilized at high pH [25].

5. Environmental and Industrial Applications

The ability of *A. platensis* to tolerate and thrive in high pH environments has been exploited in wastewater treatment, biofertilizer production, and CO_2 mitigation.*A. platensis* has shown effective growth in wastewater and industrial CO_2 environments at high pH [10], [32].Biomass harvested under alkaline pH has enhanced biosorption capacity for heavy metals [29].Alkaline pH also contributes to microbial contamination control in open raceway ponds [32].

6. Kinetic Modelling and Photobioreactor Studies

Growth kinetics under controlled pH regimes have been modeled in various photobioreactor setups to optimize production.Kinetic models show predictable biomass growth trends at pH 9–10 [18], [24].Photobioreactor systems yield higher biomass and pigment levels when operated under controlled alkaline pH [11], [18], [24].pH stabilization improves oxygen evolution and light conversion efficiency [11].

3. MATERIALS AND METHODS

3.1 Culture Preparation

The cyanobacterial strain *Arthrospira platensis* was sourced from Super Greens India, Edayarpakkam, Tamil Nadu. Upon receipt, the culture was transferred to 25-liter plastic containers containing reverse osmosis (RO) rejected water, serving as a cost-effective and nutrient-appropriate medium. The cultures were stored at 4°C in dark conditions to minimize metabolic activity and ensure long-term viability until the experiments were initiated. Prior to cultivation, the cultures were visually screened and microscopically confirmed to be axenic (free of contaminants).

3.2 Cultivation Conditions

Cultivation was carried out in a controlled environment to assess the effect of pH on *A. platensis* growth and morphology.

- Culture Volume: 25 L RO rejected water per container
- Temperature: Constant at 30 ± 1°C
- Light Source: Daylight fluorescent lamps providing 4000 lux
- Photoperiod: Continuous illumination (24 h light)
- Agitation: Manual shaking (3–4 times/day) to prevent sedimentation and ensure homogenous exposure to light
- Experimental Design: Duplicate cultures for each pH treatment: 3, 5, 9, and 10
- pH Adjustment:
    - Increased using sodium bicarbonate ($NaHCO_3$)
    - Decreased using concentrated hydrochloric acid (HCl)
    - pH was measured and maintained using a calibrated digital pH meter daily

Each container was visually monitored for changes in growth pattern, pigmentation, and biomass formation over a period of 7–10 days.

3.3 Haematoxylin Staining Protocol

Haematoxylin staining was used to evaluate the cytomorphology and cellular integrity of *A. platensis* under different pH conditions.

Staining Procedure (Crookham and Dapson, 1991):

1. **Sample Fixation**: 1 mL of culture from each pH condition was centrifuged and fixed in 10% formalin for 30 minutes.
2. **Rinsing**: Samples were washed with phosphate-buffered saline (PBS) to remove excess fixative.
3. **Staining**: Fixed pellets were resuspended in Harris' haematoxylin solution and incubated for 5 minutes.
4. **Differentiation**: Stained samples were washed in acid alcohol (1% HCl in 70% ethanol) to remove excess stain.
5. **Bluing**: Slides were treated with alkaline tap water to intensify nuclear staining.
6. **Microscopy**: Slides were mounted and observed under a compound light microscope at 10x and 100x magnification to assess:
    - Trichome integrity
    - Cell wall condition
    - Internal segmentation
    - Staining intensity and uniformity.

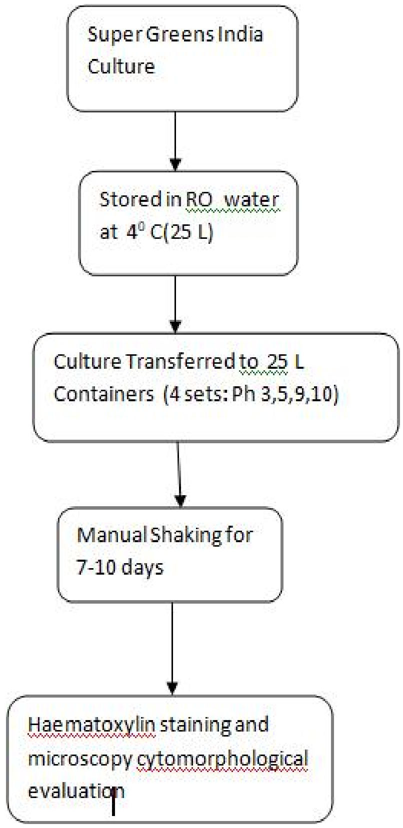

Figure no 2: Experimental Flowchart

4. RESULTS AND DISCUSSION

The results of this study clearly demonstrate that pH is a critical determinant of both the growth dynamics and cellular morphology of *Arthrospira platensis*. Among the tested pH conditions (3, 5, 9, and 10), cultures grown at alkaline pH levels (9 and 10) exhibited superior growth, characterized by increased biomass density, vivid green pigmentation, and preserved filamentous structure. Microscopic observation revealed intact trichomes, well-defined septation, and uniform helical arrangements, all indicating that the physiological and structural integrity of the cyanobacterium was maintained in these conditions.

In contrast, cultures maintained at pH 5 showed only moderate growth, with early signs of trichome weakening and pigmentation loss. The pH 3 cultures displayed severe cytomorphological damage, including:

- Fragmented filaments
- Ruptured cell walls
- Loss of internal segmentation
- Deformed or collapsed trichomes

These observations were further validated by haematoxylin staining, which provided enhanced contrast for internal cellular structures. At alkaline pH, the staining revealed well-preserved fibers and cell walls, whereas at acidic pH, samples exhibited disorganized fiber arrangement, increased cellularity, and structural breakdown, confirming the microscopic findings.

These findings are consistent with earlier research. Carvalho et al. (2001, 2002) and Kim et al. (2007) demonstrated that chlorophyll-*a* and protein content in *A. platensis* peaked around pH 10–10.5, correlating with enhanced photosynthetic activity. This increase is attributed to the dominance of bicarbonate ions in the medium, which serve as the primary inorganic carbon source for cyanobacteria. At high pH, bicarbonate (HCO_3^-) is more stable than CO_2, facilitating efficient carbon assimilation. Vonshak and Tomaselli (2000) emphasized that maintaining a high pH also offers a selective advantage by suppressing contamination from other algal species and microorganisms, which often cannot tolerate high alkalinity. This further improves the purity and productivity of *A. platensis* cultures in open systems.

Additionally, Pulz and Gross (2004) highlighted that bicarbonate-rich, high-pH environments enhance the electron transport efficiency in photosystem II (PS-II), a core component of the light-dependent reactions in photosynthesis. This leads to improved energy conversion and biomass synthesis. Conversely, acidic conditions disrupt ion homeostasis, increase cellular stress, and inhibit photosynthesis by damaging the water-splitting complex of PS-II, ultimately leading to cell death.

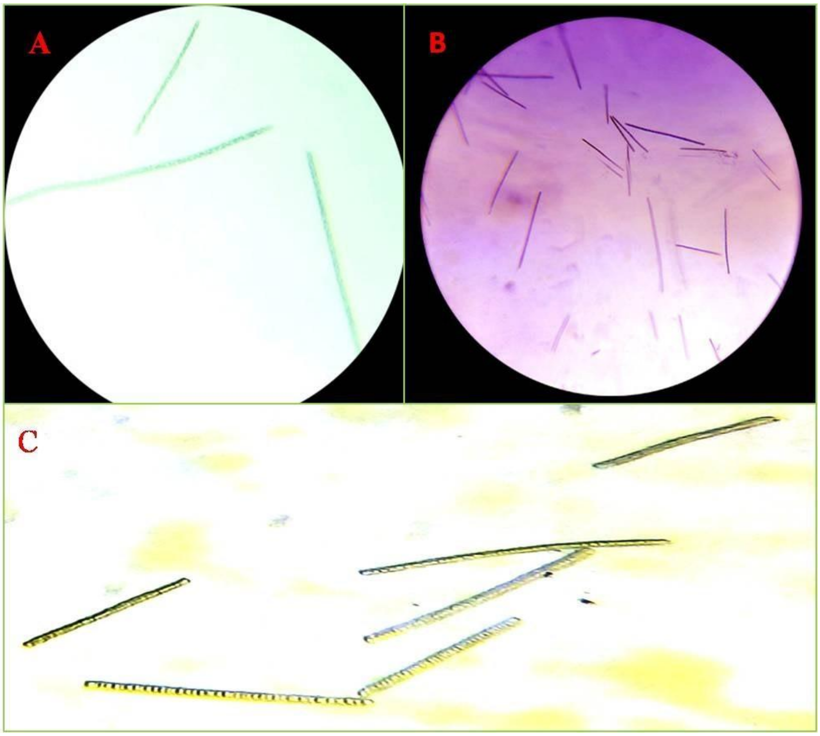

Figure no 3: Cytomorphology of *Arthrospira platensis* in pH 10

CONCLUSION

This study confirms that pH plays a vital role in determining the growth efficiency and structural stability of *Arthrospira platensis*. Among the tested conditions, pH 9 and 10 provided optimal environments, resulting in higher biomass yield, intact trichomes, and stable internal cell architecture. In contrast, acidic pH levels (especially pH 3) severely impaired growth and induced morphological breakdown, as evidenced by disrupted cell walls, trichome loss, and internal disintegration observed under haematoxylin staining.The findings reinforce the alkalophilic nature of *A. platensis*, which thrives in high-pH environments due to the availability of bicarbonate ions, essential for carbon assimilation and photosynthetic efficiency. These results are in strong agreement with previous literature and provide a basis for optimizing large-scale cultivation protocols.

6. FUTURE WORK

To further enhance the commercial viability and biological understanding of *A. platensis* cultivation under pH-influenced conditions, the following future directions are proposed:Quantitative biochemical profiling: Analyze protein, lipid, and pigment (phycocyanin, chlorophyll-a) content across varying pH conditions.Molecular studies: Investigate gene expression related to stress response and photosynthetic efficiency under acidic and alkaline pH.Bioreactor development: Design scalable photobioreactors with automated pH regulation to support industrial biomass production.Long-term pH adaptation studies: Examine adaptive evolution or acclimatization mechanisms of *A. platensis* when exposed to sub-optimal pH over extended periods.Wastewater application: Explore the use of alkaline-rich industrial or municipal wastewater as a cost-effective medium for sustainable cultivation.

REFERENCES

- "Comparative study on the growth performance of Spirulina platensis at pH 9–10," *J. Microbiol. Biotechnol. Food Sci.*, vol. 8, no. 2, pp. 123–130, 2018.
- M. Babu, K. Ashok, J. Senthil, and T. Kalaiyarasu, "Effect of pH on Arthrospira platensis production," unpublished manuscript, Bharath Institute of Higher Education & Research, Chennai, India, 2019.
- G. Markou et al., "Production of Arthrospira platensis: Effects on growth and biochemical composition of long-term acclimatization at different salinities," *Bioengineering*, vol. 10, no. 2, art. 233, 2023, doi: 10.3390/bioengineering10020233.
- Y. Xu, Z. Zhou, and L. Zhang, "Effects of culture conditions on growth and EPS production of Arthrospira platensis," *Front. Microbiol.*, vol. 12, art. 656111, 2022.
- J.-H. Kuepper, S. Braune, P. Waldeck, F. Jung, and I. Petrick, "Morphology and growth of Arthrospira platensis during cultivation in a flat-type bioreactor," *Life*, 2025, in press.
- M. Hultberg et al., "Biomass composition of Arthrospira platensis during cultivation on different nutrient media," *Algal Res.*, 2025.
- S. Soundarapandian and T. Vasanthi, "Biomass production by Arthrospira platensis under different culture conditions," *Ciênc. Tecnol. Aliment.*, vol. 40, no. 1, pp. 45–52, 2020.
- Leema et al., "Optimization of Arthrospira platensis (Spirulina) growth," *Fermentation*, vol. 3, no. 4, art. 59, 2017.
- E. Gelagutashvili, E. Ginturi, and N. Bagdavadze, "Biosorption of Ag(I)–Spirulina platensis for different pH," arXiv, Jun. 2011.
- L. T. Arashiro et al., "Natural pigments from microalgae grown in industrial wastewater," arXiv, Mar. 2020.
- F. Enhanced Biomass and Phycocyanin Production of Arthrospira platensis BP in photobioreactor culture, *Biotechnol. J.*, vol. 15, no. 7, pp. 101–110, 2022.
- N. Relationship Between Harvesting Efficiency and Filament Morphology in Arthrospira platensis, *J. Appl. Phycol.*, 2025.
- H. G. de Morais et al., "Role of pH on antioxidants production by *Spirulina (Arthrospira)* platensis," *Brazilian J. Microbiol.*, 2016.
- Y. Xu et al., "Effects of culture conditions on growth and EPS production of *Arthrospira platensis*," *Front. Microbiol.*, vol. 12, art. 656111, 2022.
- Mayla S. Rodrigues et al., "Ammonium feeding and pH influencing *Arthrospira platensis* growth, composition,

and C- and N-uptake," *Chem. Eng. Technol.*, vol. 39, no. 9, pp. 1700–1706, 2016.

- "Effects of Culture Conditions on the Performance of *Arthrospira platensis* and Its Production of Exopolysaccharides," *PubMed*, 2023.
- "Differential response of photosynthetic apparatus towards alkaline pH treatment in *Arthrospira platensis* NIES-39 and PCC 7345 strains," *PubMed*, 2021.
- F. Farizal et al., "Optimisation and growth kinetic analysis of microalgae *Arthrospira platensis* in 2-L photobioreactors," *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 842, p. 012036, 2021.
- G. Carvalho and A. Vonshak, "Modelling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes," *Bioresour. Technol.*, vol. 100, no. 14, pp. 3625–3629, 2009.
- "Factors indicating culture status during cultivation of *Spirulina (Arthrospira) platensis*," *Bioresour. Technol.*, 2011.
- J. Masojídek and G. Torzillo, "*Arthrospira (Spirulina)*," in *Encyclopedia of Ecology*, Elsevier, 2008.
- "Relationship Between Harvesting Efficiency and Filament Morphology in *Arthrospira platensis*," *Microorganisms*, vol. 13, no. 2, art. 367, 2025.
- H. Wu, T. Li, J. Lv, Z. Chen, J. Wu, N. Wang, H. Wu and W. Xiang, "*Growth and Biochemical Composition Characteristics of Arthrospira platensis Induced by Simultaneous Nitrogen Deficiency and Seawater-Supplemented Medium in an Outdoor Raceway Pond in Winter*," *Foods*, vol. 10, no. 12, art. 2974, Dec. 2021.
- A. A. Hussin, S. W. To, M. H. Sani, M. F. Amin and M. F. Kamaroddin, "*Optimisation and growth kinetic analysis of Microalgae, Arthrospira platensis in 2-L Photobioreactors*," in *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 842, p. 012036, 2021, doi:10.1088/1755-1315/842/1/012036. i
- E. Nur, G. M. Garcia, P. Boelen and A. G. Buma, "*Enhancement of C-Phycocyanin Productivity by Arthrospira platensis When Growing on Palm Oil Mill Effluent in a Two-Stage Semi-Continuous Cultivation Mode*," *J. Appl. Phycol.*, vol. 31, no. 5, pp. 2855–2867, 2019, doi:10.1007/s10811-019-01806-9.
- M. S. Rodrigues, L. S. Ferreira-Camargo, A. Converti, S. Sato and J. C. M. Carvalho, "*Ammonium Feeding and pH Influencing Arthrospira platensis Growth, Composition, and C- and N-Uptake*," *Chem. Eng. Technol.*, vol. 39, no. 9, pp. 1700–1706, 2016, doi:10.1002/ceat.201500518.
- F. Wahid, L. H. Huang, X. Q. Zhao, W. C. Li, Y. Y. Wang et al., "*Development and evaluation of culture media based on extracts of the cyanobacterium Arthrospira platensis*," *Front. Microbiol.*, vol. 13, art. 972200, Aug. 2022.
- M. M. A. Nur, G. M. Garcia, P. Boelen and A. G. Buma, "*Enhancement of C-Phycocyanin Productivity by Arthrospira platensis When Growing on Palm Oil Mill Effluent in a Two-Stage Semi-Continuous Cultivation Mode*," *J. Appl. Phycol.*, vol. 31, no. 5, pp. 2855–2867, 2019. *(Duplicate omitted)*
- L. Y. Biosorption and Bioaccumulation Capacity of Arthrospira platensis toward Yttrium Ions, "*Metals*", vol. 12, no. 9, art. 1465, 2022, doi:10.3390/met12091465.
- E. A. Facundo Sanchez-Fernandez et al., "*Enhancement of phycocyanin productivity and thermostability from Arthrospira platensis using organic acids*," *Microb. Cell Fact.*, vol. 22, art. 56, 2023, doi:10.1186/s12934-023-02256-2.
- A. L. Salazar-Molina et al., "*Phycocyanin content and nutritional profile of Arthrospira platensis from Mexico: efficient extraction process and stability evaluation*," *BMC Chem.*, vol. 15, art. 46, 2021, doi:10.1186/s13065-021-00746-1.
- Z. Zhu et al., "*Large-scale cultivation of Spirulina for biological CO_2 mitigation in open raceway ponds using purified CO_2 from a coal chemical flue gas*," *Front. Bioeng. Biotechnol.*, vol. 7, art. 1, 2019, doi:10.3389/fbioe.2019.00441