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Growth performance of Spirulina under immobilised and suspended cultivation

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Abstract. Immobilised cultivation is a water- and harvest-efficient alternative to suspended growth for Spirulina (*Arthrospira platensis*), but side-by-side tests under controlled nutrients are scarce. In this study, we compared 6-day growth on agar plates versus suspension across Zarrouk 0.5×, 1×, and 2× in two semi-continuous cycles, measuring biomass, instantaneous specific growth rate, and plate coverage. In Cycle 1, immobilised cultures significantly outperformed suspension at 1× and 2× (μ = 0.312 vs. 0.263 day^{-1} and 0.328 vs. 0.254 day^{-1}), with no difference at 0.5×. After harvest and medium reuse (Cycle 2), the mean growth rate decreased by ~30–54% in all treatments; however, the immobilised culture remained superior at 2× (0.232 vs. 0.181 day^{-1}). Kinetically, the suspension showed a first-day peak in the specific growth rate (≈0.55–0.75 day^{-1}), followed by monotonic decay and early plateaus. In contrast, the immobilised culture maintained a steadier growth rate and continued to increase at 1×–2×. Plate coverage increased from ~20–23% to 55–74% by day 6 in Cycle 1, and to 53–67% in Cycle 2. Late-stage yellowing at treatments with ≤1× Zarrouk in Cycle 2 signaled nutrient depletion. These findings suggest that immobilised cultivation may be advantageous for high-density Spirulina production systems, supporting more sustainable and resource-efficient microalgae bioprocesses.

Keywords: Spirulina, *Arthrospira platensis*, immobilised culture, suspended culture, growth kinetics

1 Introduction

Spirulina (*Arthrospira platensis*), a well-known cyanobacterium commonly regarded as microalgae, contains high-quality proteins, vitamins, essential fatty acids, and pigments, notably phycocyanin, an antioxidant. This composition has established Spirulina as a leading "superfood" and a valuable ingredient in nutraceuticals, functional foods, and cosmetics [1, 2]. The escalating demand for these high-value products necessitates the development of highly efficient and sustainable cultivation systems that can maximise productivity while minimising resource consumption [3]. The conventional method for large-scale production is suspended cultivation, typically in open raceway ponds. However, this approach is constrained by inherent limitations, including high water and energy inputs, costly harvesting processes, and, most critically, a productivity ceiling imposed by light attenuation from mutual self-shading as cell density increases [4].

For overcoming these challenges, immobilised cultivation has emerged as a promising alternative technology. In this approach, microalgal cells are attached to or confined within a substratum, forming a dense biofilm [5]. This configuration offers significant operational advantages, including simplified and

low-cost harvesting, reduced water usage, and the ability to operate at much higher cell densities compared with suspended systems. By confining the biomass into a thin, optically transparent layer, immobilised systems can substantially improve light distribution to the cells, thereby mitigating the effects of self-shading and potentially enhancing photosynthetic efficiency and overall areal productivity [6–8].

Despite the well-documented theoretical benefits, a distinct knowledge gap persists in the literature. There is a scarcity of direct, head-to-head comparisons of Spirulina growth in immobilised versus suspended systems conducted under identical and systematically controlled nutrient conditions. Numerous studies on attached growth focus on wastewater treatment or non-standard nutrient media [9, 10], making direct comparisons of intrinsic growth potential difficult. Furthermore, the impact of semi-continuous operation—specifically, the effect of reusing the growth medium on performance and nutrient limitation across successive harvest cycles—remains unexplored, particularly for immobilised Spirulina cultures.

This study was designed to address this gap directly. Here, we compare the 6-day growth of Spirulina in agar-based immobilised plates versus conventional suspension across three Zarrouk dilutions (0.5×, 1×, 2×) over two semi-continuous cycles. This design allows us to (i) test whether immobilisation confers a growth advantage under nutrient-rich media, (ii) resolve mode-specific growth kinetics and their mechanistic signatures, and (iii) examine how medium reuse affects performance in each mode. By integrating kinetic and visual readouts under controlled nutrients, we aim to provide a rigorous side-by-side evaluation of immobilised versus suspended Spirulina cultivation.

2 Materials and methods

2.1 Strain, medium, and culture maintenance

A unialgal strain of *Arthrospira platensis* was obtained from the Algal Technology Laboratory, Faculty of Biology – Agriculture – Environmental Science, University of Science and Education, The University of Danang. Stock cultures were maintained in Zarrouk medium (standard composition, Zarrouk) [11] at 25 °C under 2000 ± 200 lux white LED illumination with a 16/8 h light/dark photoperiod, with continuous aeration with ambient air. The canonical Zarrouk formulation was used as the 1× baseline and diluted or concentrated to prepare 0.5× and 2× media for experiments.

2.2 Experiment design

Two cultivation modes were compared across three nutrient levels (0.5×, 1×, and 2× Zarrouk): suspended (in liquid) and immobilised (on agar plates). The experiment consisted of two semi-continuous cycles, each lasting 6 days, with three biological replicates per mode × nutrient combination. The initial algal density was set to approximately 0.15 $mg \cdot mL^{-1}$. At the end of Cycle 1, cultures were harvested, and the same medium was reused for Cycle 2 (no fresh medium addition).

Suspended cultures were grown in 500 mL glass flasks with a working volume of 350 mL, aerated with ambient air (no CO_2 enrichment). Inocula were standardised across treatments. For immobilised cultures, Zarrouk media at 0.5×, 1×, and 2× were solidified with 12 $g \cdot L^{-1}$ agar and poured into 12 cm-diameter Petri dishes, with 120 mL per plate (constant gel thickness). Concentrated Spirulina inoculum (supernatant removed) was evenly spread over the agar surface. Plates were sealed with food-grade film to limit contamination and evaporative losses;

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two pin-holes (air-in/air-out) allowed gas exchange. Incubation conditions matched those of the suspended cultures.

2.3 Biomass quantification and percent surface coverage

For suspended cultures, optical density at 680 nm (OD_{680}) was measured daily with a UV-VIS spectrophotometer (Jasco V750, Japan, 1 cm path length) [12]. Biomass concentration y (mg·mL^{-1}) was calculated from our study-specific calibration. This calibration was constructed by preparing a dilution series of Spirulina suspensions with known dry weights, which were determined gravimetrically after drying at 60 °C to a constant weight. OD_{680} values were then plotted against biomass concentrations, yielding a linear regression (R^2 = 0.86). The resulting equation (Eq. (1)) was applied to convert OD measurements into biomass concentrations:

$$y = 0.485 \times OD_{680} - 0.0198 \quad (1)$$

For immobilised cultures, areal biomass density (mg·cm^{-2}) was estimated non-invasively from the mean green channel intensity (MGI) of daily plate images. Plates were imaged in a light-tight box with bottom white LED illumination at a fixed camera distance and exposure. Images were pre-processed (crop to plate ROI; rim removal), and MGI was computed for the ROI. Areal biomass y (mg·cm^{-2}) was obtained from Eq. (2), then converted to mg·mL^{-1} by multiplying by 1.058 for comparison purpose with suspended cultures:

$$y = -0.0139 \times MGI + 2.73 \quad (2)$$

Equation (2) was derived from a pre-established calibration curve, obtained by extracting MGI data from images of algal plates with known biomass densities (determined gravimetrically), yielding R^2 = 0.77. The conversion ratio was determined on the basis of the fact that each agar plate had an ROI area of 126.85 cm² containing 120 mL of medium. Besides, algae coverage (%) was computed from the same images by means of color-based segmentation (OpenCV). The green-dominant algal mat was segmented by using k-means clustering (k = 3) on colour features, followed by area-fraction measurement within the plate ROI.

2.4 Growth rate calculations

The mean specific growth rate (μ, day^{-1}) over a cultivation period was computed as:

$$\mu = \frac{lnX_t - lnX_{t0}}{t - t0}$$

where X_t and X_{t0} are the biomass density (mg·mL^{-1}) at day t and day t_0, respectively.

The instantaneous specific growth rate (μ_t, day^{-1}) was computed on a daily step:

$$\mu = lnX_t - lnX_{t-1}$$

2.5 Data analysis and statistics

All analyses were performed in Google Colab. Data are reported as mean ± SD (n = 3). For each cycle, the effects of cultivation mode (suspended vs. immobilised) and nutrient level (0.5×, 1×, and 2×) on mean μ were tested with two-way ANOVA, followed by Tukey's HSD for pairwise comparisons (α = 0.05). Assumptions of normality and homoscedasticity were evaluated prior to inference.

3 Results and discussion

3.1 Mean growth rate across cultivation modes and nutrient levels

Across two semi-continuous cycles, the mean specific growth rate depended on both cultivation mode and nutrient level (Fig. 1).

In Cycle 1, immobilised cultures outperformed suspended ones at treatment 1× and 2× Zarrouk with a mean growth rate of 0.312

± 0.015 day^{-1} and 0.328 ± 0.044 day^{-1}, respectively. These values marked increases of 18.6 and 29.1% compared with their suspended counterparts (0.263 ± 0.018 and 0.254 ± 0.014 day^{-1}, respectively, $p < 0.05$). Meanwhile, no difference was detected at treatment 0.5× (0.241 ± 0.010 vs. 0.235 ± 0.008 day^{-1}, $p = 0.490 > 0.05$). Within-mode analyses revealed a treatment effect for immobilised cultures ($p = 0.017$), with 0.5× significantly lower than both 1× and 2× ($p = 0.044$ and 0.019, <0.05) but not for suspended cultures ($p = 0.119 > 0.05$).

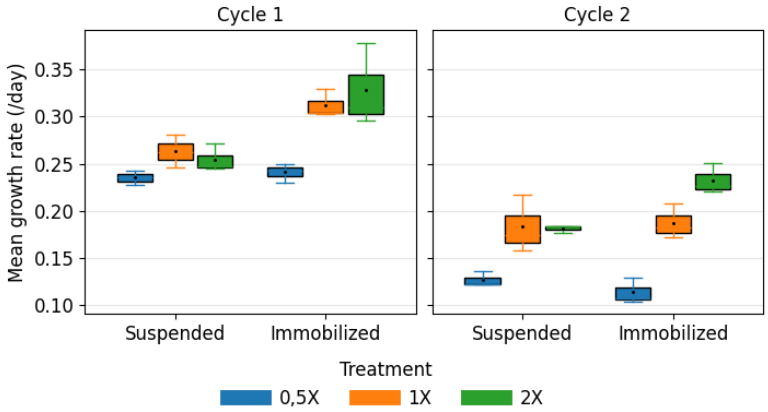

Fig. 1. Mean growth rate (day^{-1}) of Spirulina in suspended versus immobilised cultures across nutrient levels over two semi-continuous cycles. Boxplots indicate interquartile range with mean values shown as black dots ($n = 3$). Treatments 0.5×, 1×, and 2× correspond to Zarrouk medium concentration

After harvest and medium reuse (Cycle 2), the mean growth rate decreased by 30–54% across all treatments. The sharpest decrease occurred at 0.5× (–53% immobilised, –46% suspended). Despite this decrease, immobilised cultures remained superior to suspended mode at 2× (0.232 ± 0.016 vs. 0.181 ± 0.004 day^{-1}, +28.2%, $p = 0.006 < 0.05$), whereas cross-system differences at 0.5× and 1× were not significant ($p = 0.219$ and 0.844). Treatment effects in Cycle 2 were significant within both modes, all pairwise contrasts were significant for immobilised (0.5× < 1× < 2×), while the 1× – 2× difference was not significant in suspended cultures ($p = 0.990 > 0.05$).

The decrease in the mean growth rate from Cycle 1 to Cycle 2 was likely driven by residual nutrient depletion rather than intrinsic physiological fatigue. This finding emphasises the importance of nutrient management in sustaining high growth performance across consecutive cycles. Notably, despite this decrease, immobilised-2× in Cycle 2 (0.232 ± 0.016 day^{-1}) remained comparable with the Cycle-1 suspended benchmark at 1× (0.263 ± 0.018 day^{-1}).

Reported growth rates for Spirulina in suspension under replete laboratory conditions commonly fall in the ~0.2–0.4 day^{-1} range [13–15]. In contrast, attached systems are typically benchmarked by areal productivity and frequently outperform volumetric suspensions at equivalent light inputs. The mean growth rate values in our study (0.254–0.328 day^{-1} in Cycle 1; 0.181–0.232 day^{-1} in Cycle 2 at 2×) sit squarely within these published ranges and follow the expected rank order (immobilised ≥ suspended).

Our results indicate that surface immobilisation confers a clear growth advantage when nutrients are not limited (1× – 2×). This advantage could be driven by two coupled mechanisms: more effective light capture and enhanced CO_2 delivery. Thin, surface-attached layers shorten the optical path and reduce self-shading, increasing photon exposure, while direct

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headspace contact improves gas transfer and CO_2 availability at the air-biofilm boundary [6, 16, 17]. Numerous studies on attached and thin-layer cultivation of Spirulina report higher productivities under comparable irradiance and stable operation at high areal biomass [16, 18, 19]. Across publications, reported areal productivities range from ~0.04 to 20.7 $g \cdot m^{-2} \cdot d^{-1}$, with more than half between 0 and 5 $g \cdot m^{-2} \cdot d^{-1}$, as summarised by Zhuang et al. [6]. Our estimates, when converted, are 1.17–4.17 $g \cdot m^{-2} \cdot d^{-1}$. Productivity may vary across species, operating regimes, nutrient levels, light intensities, and other factors [6]; however, the integrated quantitative relationships between culture parameters and attached-culture productivity remain insufficiently constrained and warrant further study.

Overall, the results argue that immobilised cultivation is better suited to high-nutrient, high-density processes, with strong potential to sustain performance gains across consecutive harvests.

3.2 Growth kinetics of suspended and immobilised cultures

Biomass trajectories and instantaneous specific growth rates revealed distinct growth dynamics between cultivation modes (Figs. 2–3).

Across both cycles and nutrient regimes, immobilised cultivation consistently achieved higher or comparable final biomass compared with suspended cultivation, with the magnitude of the advantage increasing in nutrient-rich treatments (up to ~40% greater at 1× in Cycle 1 and ~18% greater at 2× in Cycle 2) (Fig. 2).

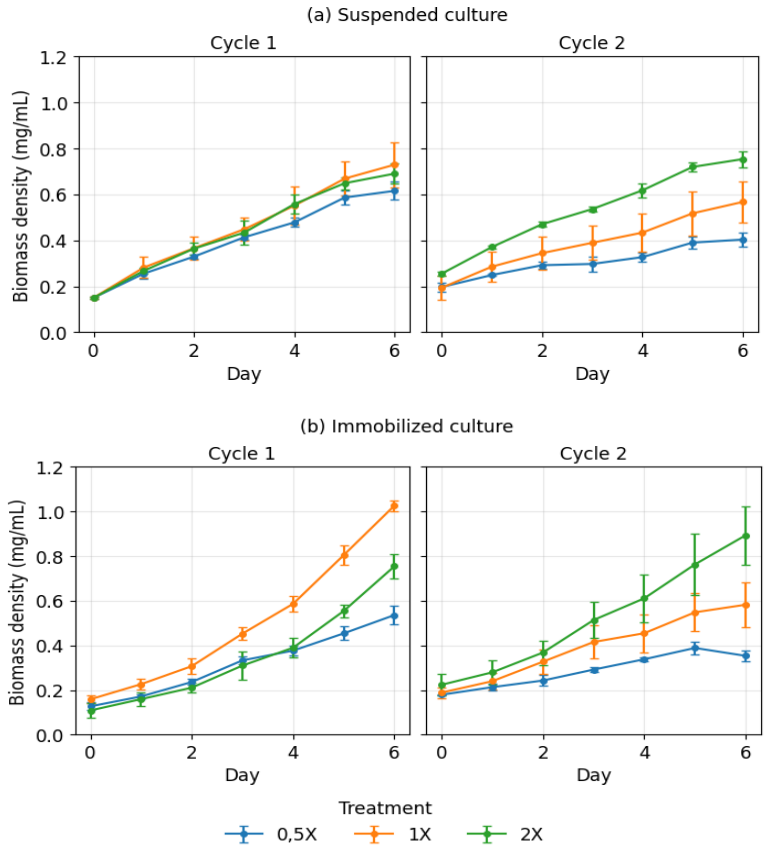

Fig. 2. Biomass growth curves ($mg \cdot mL^{-1}$) from day 0 to day 6 for suspended (a) and immobilised (b) cultures across nutrient levels over two semi-continuous cycles. Values represent mean ± SD (n = 3); treatment 0.5×, 1×, and 2× indicate Zarrouk medium concentrations

In the suspended system, biomass increased near-linearly from day 0 to day 5 in Cycle 1, then approached a plateau by day 6, reaching 0.615 ± 0.039 mg·mL^{-1} (0.5×), 0.729 ± 0.097 mg·mL^{-1} (1×), and 0.690 ± 0.045 mg·mL^{-1} (2×). In Cycle 2, growth was slower and stationarity occurred earlier; day-6 biomass reached 0.403 ± 0.029 mg·mL^{-1} in 0.5× treatment, 0.567 ± 0.090 mg·mL^{-1} in 1×, and 0.753 ± 0.035 mg·mL^{-1} in 2×. The instantaneous specific growth rate in the suspension peaked on day 1 (≈0.55–0.75 day^{-1} across treatments) in both cycles and decreased rapidly to ≤0.1 day^{-1} at the end of the experiments (Fig. 3a).

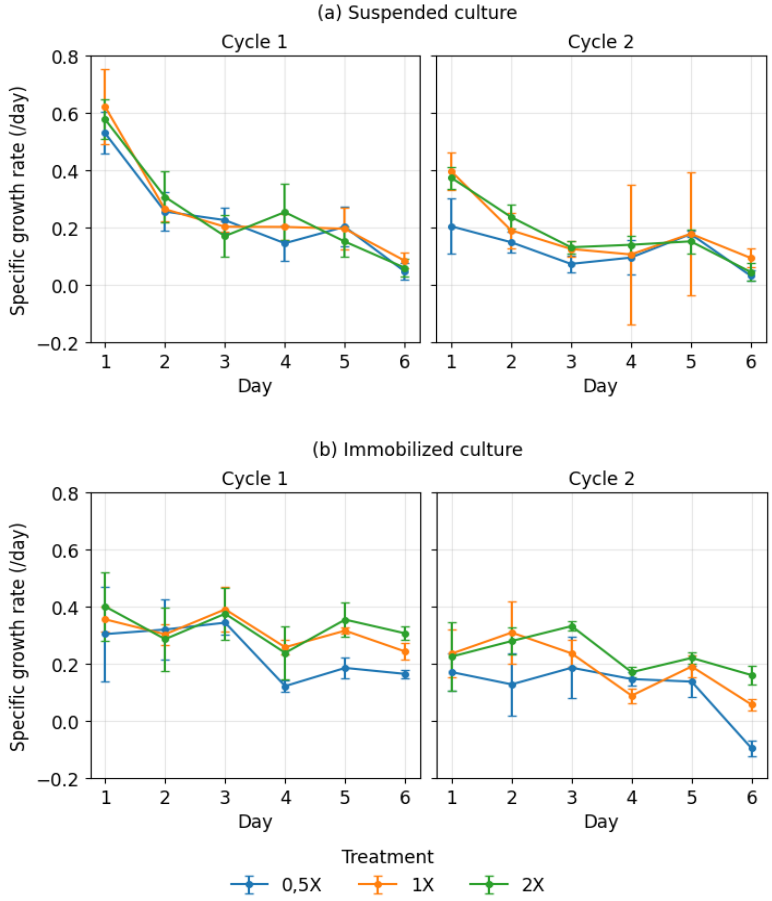

Fig. 3. Instantaneous specific growth rate (day^{-1}) from day 0 to day 6 for suspended (a) and immobilised (b) cultures across nutrient levels over two semi-continuous cycles. Values represent mean ± SD (n = 3); treatment 0.5×, 1×, 2× indicate Zarrouk medium concentrations

In the immobilised system, biomass increased monotonically without an evident plateau under nutrient-replete conditions (Fig. 2b). In Cycle 1, day-6 biomass reached 0.536 ± 0.043 mg·mL^{-1}, 1.026 ± 0.024 mg·mL^{-1}, and 0.754 ± 0.056 mg·mL^{-1} in 0.5×, 1× and 2×, respectively. The 1× and 2× trajectories tended to increase continuously at the end of the observation window. In Cycle 2, biomass again increased throughout, attaining 0.353 ± 0.023 mg·mL^{-1} (0.5×), 0.582 ± 0.100 mg·mL^{-1} (1×), and 0.893 ± 0.132 mg·mL^{-1} (2×) by day 6. In line with these patterns, the specific growth rate in immobilised cultures remained comparatively stable over time – typically at ~0.25–0.45 day^{-1} in Cycle 1 and ~0.10–0.35 day^{-1} in Cycle 2 (Fig. 3b). Notably, in the last

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day of the experiment (day 6 in Cycle 2), the specific growth rate dropped drastically below zero in treatment 0.5× and biomass slightly declined.

The difference in the growth curve between the two cultivation modes indicates different effects of limiting factors, primarily light. In suspension cultures, cells circulate through light-dark cycles. As the optical density increases, self-shading intensifies, resulting in growth that typically exhibits a brief lag, a logarithmic phase early on, and an early plateau as the time-averaged photon flux decreases [20]. In contrast, immobilised (thin, surface-attached) biomass shortens the optical path and reduces bulk self-shading, yielding near-constant specific growth under nutrient-replete conditions. The areal biomass then increases approximately linearly after a short lag until a plateau or sloughing occurs as the biofilm thickens [6, 21]. In essence, suspended systems are governed by density-dependent light limitation, which produces a log-shaped growth curve. In contrast, attached systems are constrained by depth-wise light penetration within a fixed layer, resulting in quasi-linear areal accumulation before levelling off.

The observed partial decrease in growth in the 0.5× treatment and towards the end of Cycle 2 suggests that nutrient depletion can override the inherent light-use benefits of immobilised cultivation. In contrast, the sustained upward trajectories in the 2× treatment across both cycles highlight that, under nutrient-replete conditions, light accessibility remains the dominant factor shaping productivity. Consistently, the maximum biomass recorded in immobilised cultures on day 6 reached 1.026 ± 0.024 mg·mL^{-1} (Cycle 1, 1×) and 0.893 ± 0.132 mg·mL^{-1} (Cycle 2, 2×), which are substantially higher than the corresponding maxima in suspended cultures under the same treatments (0.729 ± 0.097 and 0.753 ± 0.035 mg·mL^{-1}, respectively). These findings reinforce that immobilised systems, when adequately supplied with nutrients, can outperform suspended systems and sustain higher biomass accumulation over the cultivation period.

3.3 Visual assessment of immobilised biomass

Daily plate montages show progressive darkening and radial infilling of the algal mat across days and nutrient levels (Fig. 4). Image-based quantification of the percent covered area corroborates these trends (Fig. 5).

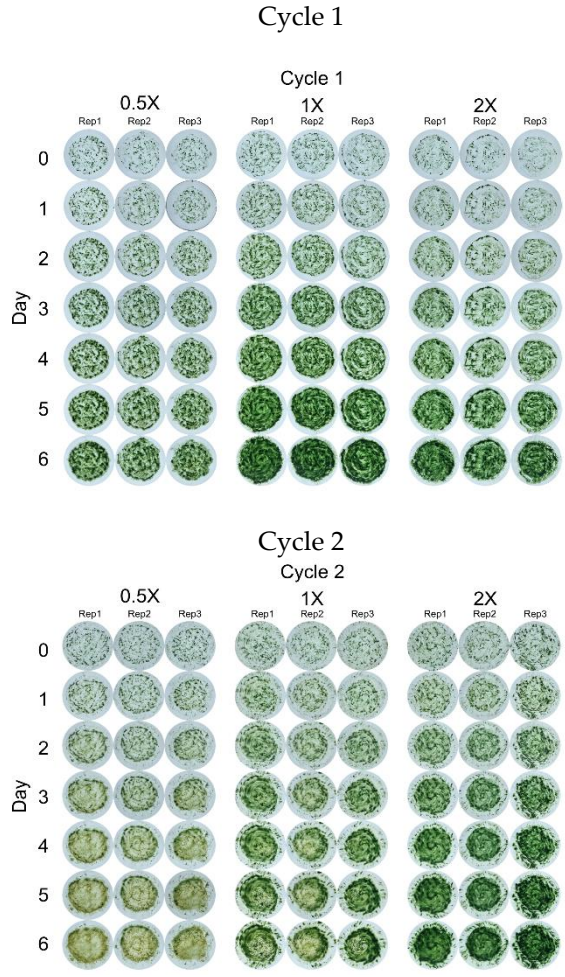

Fig. 4. Daily plate montage of immobilised cultures at different nutrient levels over two semi-continuous cycles

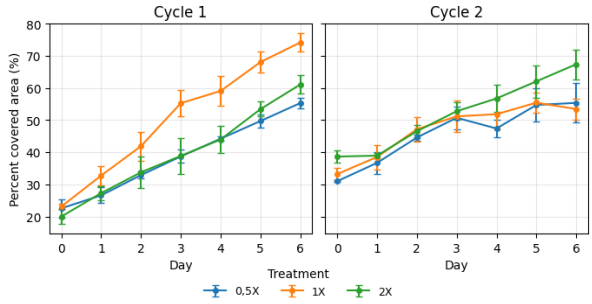

Fig. 5. Percent surface coverage of immobilised plates over time (day 0 to day 6) across nutrient levels over two semi-continuous cycles. Values represent mean ± SD (n = 3); Treatment 0.5×, 1×, 2× indicate Zarrouk medium concentrations

In Cycle 1, coverage increased from ~20–25% on day 0 to 55.32 ± 1.52% (0.5×), 74.18 ± 2.87% (1×), 61.11 ± 2.94% (2×) by day 6, gaining 32.77, 51.08, and 41.11 percentage points, respectively. In Cycle 2, plates started with higher initial coverage and increased by day 6 to 55.38 ± 6.08% (0.5×), 53.46 ± 3.28% (1×), and 67.40 ± 4.64% (2×) (n = 3), corresponding to percentage-point increases of 24.38, 20.24, and 28.73. Consistent with the panels, 0.5× and 1× in Cycle 2 showed a stagnation after peaking around day 5, whereas 2× increased continuously. Notably, late-stage yellowing (chlorosis) became evident in Cycle 2, most prominently at 0.5× and to a lesser extent at 1×, whereas Cycle 1 showed only incipient discolouration. This visual signature is consistent with nutrient exhaustion after medium reuse and aligns with the decrease in specific growth rate observed at the end of the cycle.

The results on image panels and coverage metrics reinforce the kinetic interpretation of an immobilisation advantage under nutrient-replete conditions. In Cycle 1, immobilised plates progressed from ~20–23% coverage at inoculation to 55–74% by day 6, with the most rapid infilling at 1×. After harvest and medium reuse (Cycle 2), microalgae on plates continued to expand to 53–67% by day 6. The closer tracking among replicates and the monotonic increase at 2× indicate that, when nutrients are abundant, the thin, surface-attached cultivation mode supports sustained mat formation and pigment accumulation across the entire observation window. The late-stage yellowing (chlorosis) observed mainly at 0.5× and, to a lesser extent, at 1× in Cycle 2 is a clear qualitative marker of emerging nutrient limitation following medium reuse. Chlorosis is a recognised response to nitrogen limitation in Spirulina and related cyanobacteria, reflecting degradation of phycobiliproteins and loss of chlorophyll. Recent studies report pigment reductions under nitrate depletion and describe the characteristic shift from blue-green to yellowish hues under nitrogen stress [22, 23].

This visual cue aligns with the concurrent decrease in the specific growth rate (including a negative day-6 value at 0.5×) and with the smaller coverage gains at sub-replete nutrients. Together, these observations suggest that (i) residual nutrients limited the second cycle at ≤1× Zarrouk medium, and (ii) maintaining nutrient sufficiency is necessary to preserve the immobilised system's advantage over time.

4 Conclusion

This study provides quantitative evidence that surface immobilisation enhances Spirulina growth when nutrients are sufficient. In Cycle 1, immobilised cultures exceeded suspension at 1× and 2× (μ = 0.312 ± 0.015 vs. 0.263 ± 0.018 day^{-1}; 0.328 ± 0.044 vs. 0.254 ± 0.014 day^{-1}), with no difference at 0.5×. After harvest and medium reuse, the mean growth rate decreased by 30–54% across treatments, yet the advantage at 2× persisted (0.232 ± 0.016 vs. 0.181 ± 0.004 day^{-1}; p = 0.006). Specific growth rate trajectories diverged between modes: suspension cultures exhibited an early peak (~0.55–0.75 day^{-1}) followed by monotonic decrease to ≤0.1 day^{-1} by day 6,

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whereas immobilised cultures maintained steadier rates (0.25–0.45 day^{-1} in Cycle 1; 0.10–0.35 day^{-1} in Cycle 2), with a transient negative value only at 0.5× late in Cycle 2. Image analysis corroborated these patterns as plate coverage increased from ~20–23% to 55–74% (Cycle 1) and to 53–67% (Cycle 2), with smaller gains at lower nutrient concentration and chlorosis appearing after medium reuse. Converted areal productivities for immobilised plates (1.17–4.17 $g \cdot m^{-2} \cdot d^{-1}$) fall within reported ranges for attached cultivation. Overall, maintaining nutrient sufficiency enables surface-attached cultivation to support sustained, high-density growth, and practical control of nutrient replenishment and biofilm thickness should preserve this advantage across successive harvests.

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References

1. Soni RA, Sudhakar K, Rana RS. *Spirulina* – From growth to nutritional product: A review. Trends in Food Science & Technology. 2017;69:157-71.
2. Sharoba AM. Spirulina: Functional Compounds and Health Benefits. In: Plant Secondary Metabolites, Volume One. Apple Academic Press; 2016.
3. Acién Fernández FG, Gómez-Serrano C, Fernández-Sevilla JM. Recovery of Nutrients From Wastewaters Using Microalgae. Front Sustain Food Syst. 2018;Volume 2 - 2018.
4. Brennan L, Owende P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev. 2010;14(2):557-77.
5. Gross M, Jarboe D, Wen Z. Biofilm-based algal cultivation systems. Appl Microbiol Biotechnol. 2015;99(14):5781-9.
6. Zhuang LL, Yu D, Zhang J, Liu FF, Wu YH, Zhang TY, et al. The characteristics and influencing factors of the attached microalgae cultivation: A review. Renewable and Sustainable Energy Reviews. 2018;94:1110-9.
7. Wang J, Liu W, Liu T. Biofilm based attached cultivation technology for microalgal biorefineries—A review. Bioresour Technol. 2017; 244:1245-53.
8. Singh G, Patidar SK. Development and applications of attached growth system for microalgae biomass production. BioEnergy Research. 2020;14(3):709-22.
9. Wang JH, Zhuang LL, Xu XQ, Deantes-Espinosa VM, Wang XX, Hu HY. Microalgal attachment and attached systems for biomass production and wastewater treatment. Renewable and Sustainable Energy Reviews. 2018;92:331-42.
10. Lee SH, Oh HM, Jo BH, Lee SA, Shin SY, Kim HS, et al. Higher biomass productivity of microalgae in an attached growth system, using wastewater. Journal of Microbiology and Biotechnology. 2014;24(11):1566-73.
11. Mógor ÁF, De Oliveira Amatussi J, Mógor G, De Lara GB. Bioactivity of cyanobacterial biomass related to amino acids induces growth and metabolic changes on seedlings and yield gains of organic red beet. American Journal of Plant Sciences. 2018;09(05):966-78.
12. Amos R. Handbook of Microalgal Mass Culture (1986). Boca Raton: CRC Press; 2017.
13. Chotchindakun K, Buddhasiri S, Kuntanawat P. Enhanced Growth and Productivity of Arthrospira platensis H53 in a Nature-like Alkalophilic Environment and Its Implementation in Sustainable Arthrospira Cultivation. Sustainability. 2024;16(19):8627.
14. De Souza DS, Valadão RC, Nascentes AL, Da Silva LDB, De Mendonça HV. Use of the cyanobacterium Spirulina platensis in cattle wastewater bioremediation. Acta Scientiarum Technology/Acta Scientiarum Technology. 2022; 44:e58806.
15. Gal JL, Cole NR, Eggett DL, Johnson SM. Growth comparison of Arthrospira platensis in different vessels: standard cylinder vs. enhanced surface area at low light. Applied Phycology. 2023;4(1):1-14.

16. Zhang L, Chen L, Wang J, Chen Y, Gao X, Zhang Z, et al. Attached cultivation for improving the biomass productivity of Spirulina platensis. Bioresour Technol. 2015;181:136-42.

17. Ji B, Zhang W, Zhang N, Liu T. Biofilm cultivation of the oleaginous microalgae Pseudochlorococcum sp. Bioprocess and Biosystems Engineering. 2014;37:1369-75.

18. Liu T, Wang J, Hu Q, Cheng P, Ji B, Liu J, et al. Attached cultivation technology of microalgae for efficient biomass feedstock production. Bioresource Technology. 2013;127:216-22.

19. Wang Y, Li L, Zhao D, Zhou W, Chen L, Su G, et al. Surface patterns of mortar plates influence Spirulina platensis biofilm attached cultivation: Experiment and modeling. Algal Research. 2023;71:103079.

20. Vera-Vives AM, Michelberger T, Morosinotto T, Perin G. Assessment of photosynthetic activity in dense microalgae cultures using oxygen production. Plant Physiology and Biochemistry. 2024;208:108510.

21. Saccardo A, Bezzo F, Sforza E. Microalgae growth in ultra-thin steady-state continuous photobioreactors: assessing self-shading effects. Front Bioeng Biotechnol. 2022;Volume 10 - 2022.

22. Shayesteh H, Laird DW, Hughes LJ, Nematollahi MA, Kakhki AM, Moheimani NR. Co-Producing Phycocyanin and Bioplastic in Arthrospira platensis Using Carbon-Rich Wastewater. BioTech. 2023;12(3):49.

23. Duangsri C, Mudtham NA, Incharoensakdi A, Raksajit W. Enhanced polyhydroxybutyrate (PHB) accumulation in heterotrophically grown Arthrospira platensis under nitrogen deprivation. Journal of Applied Phycology. 2020;32(6):3645-54.